ICAR PUBLICATIONS

D. K. M. A.

636 (05) CODEN : IJLAA4 70 (2) : 101-222 (1999) II ISSN 0367-8318

The Indian Journal of Animal Sciences

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THE INDIAN JOURNAL OF
ANIMAL SCIENCES
Previous Issue : Vol. 70, No. 1, pp. 1-100

Vol. 70, No. 2 February 2000

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ANIMAL HEALTH

Short Communications

Indian Journal of Animal Sciences **70** (2): 103-109, February 2000

Trends in bluetongue virus diagnostics: A review

GAYA PRASAD[1], MINAKSHI[2], YASHPAL MALIK[3] and SUSHILA MAAN[4]

CCS Haryana Agricultural University, Hisar, Haryana 125 004

Received: 11 March 1999; Accepted: 3 August 1999

ABSTRACT

Bluetongue (BLU) virus is widely prevalent in the world inflicting colossal economic losses due to mortality, morbidity, production losses and international trade barrier on animals and their germplasm from BLU endemic countries to BLU free regions. Twenty-four serotypes of bluetongue virus (BLUV) are known world over. Due to ds segmented RNA genome of the virus, it is prone to frequent mutations leading to emergence of new genetic variants in the natural mammalian host and vector cycle. Therefore, it is necessary to develop tools suitable for group specific diagnosis as well as detection of genetic and antigenic diversity in the BLUV circulating in nature. In the last over 2 decades, a wide array of molecular and traditional approaches have been developed for diverse situations to detect the presence of BLUV infection either by detecting the antibodies and antigens or the viral nucleic acids in the biological samples. A number of variations of enzyme immunoassays have been developed in several laboratories for detection of BLUV infection. Similarly molecular approaches such as RNA/DNA hybridization and RT-PCR have also to be evaluated for BLUV. This review is aimed at providing critical appraisal of the classical and biotechnological methods currently being used or evaluated for diagnosis of BLUV.

Key words: Bluetongue virus, Diagnostics, DNA probes, Dot- immunobinding assay, ELISA, Monoclonal antibodies, PCR, Recombinant antigens

Bluetongue (BLU), an economically very important disease affecting susceptible domestic and wild ruminants in tropical, semi-tropical and temperate regions of the world, is listed under category 'A' of OIE, thus imposing restrictions on movement of ruminants from BLU endemic regions to BLU free zones. The colossal economic losses due to bluetongue virus (BLUV) infection are mainly attributed to high morbidity, mortality, abortions, still-births, foetal abnormalities, milk, meat and fleece loss. In addition, the presence of BLUV in a country puts trade barrier on movement of ruminant animals, their germplasm, embryos and other animal products, thus disrupting international commerce. Twenty-four serotypes of BLUV have already been recognised and many more may be prevalent in the regions where no survey has been made so far. Similar diversity has been observed in the ability of a number of different species of *Culicoides* midges to transmit BLUV infection in different regions of the world. Since BLUV infection is insect-borne, ecological and environmental variables govern the infection in regional manner.

India is a geographically vast country having diverse geoclimatic zones. Major part of the country is tropical with high to moderate rainfall. The country has huge population of domestic and wild ruminants which are susceptible to BLUV infection. Over the past 3 decades, several exotic breeds of sheep and cattle have been introduced in the country for improvement of indigenous stock through crossbreeding. Thus more susceptible animals for BLUV infection are present now than early 60s when several outbreaks of BLU in indigenous and exotic sheep in Maharashtra were recorded for the first time. Since then outbreaks of BLU have been reported from several other states and 20 out of the 24 known serotypes of BLUV are prevalent in the country (Prasad *et al.* 1992). India has several outstanding breeds of cattle (*Bos indicus*) and buffaloes (*Bubalus bubalis*). In the recent years, several countries have shown interest in the germplasm of Indian cattle and buffaloes for genetic and molecular studies. But presence of BLUV in Indian ruminant livestock has resulted in strict quarantine measures and extensive costly testing. This emphasises the importance of BLU in a developing country like India having a vast and promising domestic and wild ruminant population.

Bluetongue virus belongs to *Orbivirus* genus of the family Reoviridae. The virus is architecturally complex and has ability to attach and replicate in a variety of vertebrate and invertebrate cells. Advances in molecular biology,

Present address: [1]Research Officer, [2]Assistant Scientist, [3]Senior Research Fellow, [4]Assistant Research Officer, Department of Veterinary Microbiology, College of Veterinary Sciences.

immunology and virology have provided unprecedented opportunities to develop innovative biotechnological approaches using molecular and immunological techniques such as recombinant-DNA and hybridoma for producing more effective, economical, thermostable, specific, sensitive and cost effective diagnostic reagents. The objective of this review is to describe briefly the traditional and molecular approaches being currently under investigation for development of diagnostics for BLUV.

Traditional diagnostic approaches

Bluetongue virus was first diagnosed as 'epizootic catarrh' (Hutchian 1881) based on clinical signs. Spreul (1905) named it 'bluetongue' due to appearance of cyanosed tongue. The field diagnosis of BLU is usually made by clinical signs characterised by pyrexia, swelling of muzzle, oral lesions, coronitis, stiffness of limbs and in some cases oedema of head and neck. However, field diagnosis of sub-clinical and in-apparent infection of BLUV present a difficult situation. Therefore, different laboratory methods are required to detect the virus, its antigens and antibodies, and the viral nucleic acid.

Isolation of the virus

The isolation of the virus from the clinical specimens is most reliable classical way of confirmatory diagnosis of BLUV. Virus can be isolated by inoculating the clinical material in susceptible sheep (Jain *et al.* 1986), suckling mice, embryonated chicken eggs (Foster and Luedke 1968) and a variety of vertebrate (Wechsler and McHolland 1988) and non-vertebrate cell lines (Bhat *et al.* 1996). Since the virus is transmitted by insect vector, susceptible sheep inoculation is normally not used for the diagnostic purposes because it requires costly midge-proof housing system and safety procedures. Chicken embryo is quite commonly used method for primary isolation of the virus. However, mammalian-cell line BHK_{21} is the most versatile system for isolation of the virus. In the recent years cell lines of insect origin were found better than the mammalian cells for the primary isolation of the virus. In our laboratory also we have successfully used *Aedes albopictus* (insect) cell line C6/36 for cultivation of the virus (Bhat *et al.* 1996).

Detection of the virus and its antigens

Since the virus isolation is very time consuming, direct detection of the virus and its antigens in clinical specimens provides a rapid means of diagnosis. The commonly used procedures are immunoperoxidase, immunofluorescence and enzyme immunoassays. Immunoperoxidase test has been used for detection of BLUV antigens in the specimens obtained from infected chicken embryo, cell culture, mononuclear cells and insect vector (Afshar *et al.* 1991, Garg and Prasad 1996). MacLachlan *et al.* (1990) used avidin-biotin complex immunoperoxidase procedure to detect BLUV antigens in the tissues of BLUV infected calves. Direct and indirect immunofluorescence tests were used for detection of BLUV antigens (Gould *et al.* 1989). Electron microscopy was used for detection of BLUV in cell culture and in infected blood samples of animals (Gould *et al.* 1989, Prasad *et al.* 1993).

Detection of anti-viral antibodies

Presumptive diagnosis of BLUV infection was made by serological tests rather than by isolation and identification of virus. Serological tests have played an important role in determining the distribution of BLUV infections and these are the primary methods used to certify animals for export.Traditional serological methods include agar gel immuno-precipitation test, counter current immuno-electrophoresis, etc.

The serological tests used to detect BLUV antibodies are of 2 types, viz. group specific and serotype specific. Serum neutralization test (SNT) and haemagglutination inhibition test are serotype specific. Of the 2, SNT is the most widely used test (Appelton and Lectchworth 1983). SNT requires cell culture facilities, thus haemagglutination inhibition was considered as an alternate test. As outer capsid protein VP_2 has a direct role in determination of serotype specificity, alternative tests using serotype specific reagents like antigens or monoclonal antibodies, could be developed. However, this would require development of MAb to all the 24 known serotypes.

Tests devised for the detection of group specific antibodies include agar gel precipitation test (Jochim and Chow 1969), complement fixation test, dot-immunobinding assay (DIA), enzyme-linked immunosorbent assay (ELISA) and competitive-ELISA (c-ELISA). All these tests are based on the detection of major inner core group specific protein VP_7 which is highly conserved and reacts with antisera against all the 24 known serotypes of BLUV. Complement fixation test suffers from many problems, especially anti-complementarity of sheep sera and lack of reproducibility (Della-Porta *et al.* 1983). The AGPT replaced complement fixation test as it is easy to perform and is reproducible. It remains most widely used test in spite of the fact that it lacks sensitivity and shows cross-reactions with related orbiviruses (Della-Porta *et al.* 1983). The AGPT replaced complement fixation test as it is easy to perform and is reproducible. It remains most widely used text in spite of the fact that it lacks sensitivity and shows cross reaction with related orbiviruses (Della-Porta *et al.* 1983). The reports that ID test failed to detect antibodies in a significant number of animals that were positive for BLUV (Osburn *et al.* 1981, Stott *et al.* 1982) and the problems of cross-reactivity suggested that the most likely serological test to replace AGPT is ELISA. In our laboratory dot-immunobinding assay was found quite sensitive compared to AGPT and SNT (Chander *et al.* 1991) and RNA- PAGE (Prasad and Minakshi 1999a).

It is now clear that the group specific antigens of BLUV may share minor epitopes with those of other orbiviruses, thus ID and ELISA may detect cross-reactive antibodies to

viruses belonging to serogroups other than BLUV. Therefore, Anderson (1984) showed blocking-ELISA as a specific and sensitive diagnostic test for detection of group specific antibodies to BLUV using monoclonal antibodies (MAb) against BLUV. The results of Anderson's blocking ELISA were confirmed by using MAb and a c-ELISA format (Afshar *et al.* 1987). Lunt *et al.* (1988) found that blocking-ELISA and c-ELISA were equally sensitive and specific showing no cross reaction with epizootic haemorrhagic disease virus (EHDV) of deer and superior to indirect-ELISA. Jeggo *et al.* (1992) defined c-ELISA protocol and set of reagents that could provide basis for an internationally accepted standard.

Biotechnological diagnostic approaches

Recombinant antigen and monoclonal antibodies based assays: The development of hybridoma technology and genetic engineering paved the way for production of well defined and highly specific non-cross reacting diagnostic monoclonal antibodies and a variety of recombinant diagnostic antigens. The BLUV group specific antigen VP_7 was expressed in yeast and *S. frugiperda* insect cells. The use of expressed VP_7 antigen as a diagnostic reagent in blocking-ELISA and c-ELISA for the detection of BLUV antibodies in sera collected from infected animals were reported by Eaton *et al.* (1990) and Martyn *et al.* (1990). Of the 2, yeast-expressed VP_7 was widely evaluated for c-ELISA. Naresh and Prasad (1995) also used c-ELISA based on yeast-expressed VP_7 and non-cross reacting monoclonal antibodies for detection of BLUV antibodies in all the 4 species of domestic ruminants. Naresh *et al.* (1996) also observed that recombinant VP_7 antigen produced by yeast and baculovirus expression systems was as good as the conventionally cell-culture produced groups specific antigen in dot-immunobinding assay. The advantages of recombinant antigens over conventional cell culture derived antigens will make them widely utilized antigens in coming years. The cost associated with the cultivation of virus in cell culture is more as compared to cost of production of recombinant antigens. The genetically engineered antigen is likely to be more consistent and uniform than cell culture derived. Recombinant antigens are non-infectious and can be used world-wide without the fear of infecting animals of BLU free regions.

Detection of viral nucleic acid

RNA electropherotyping: Over the past 1 decade there has been an explosion of information on the BLUV genome and this will serve as a basis for next generation of nucleic acid based diagnostic techniques that would be more rapid and sensitive. These techniques rely not on the isolation and identification of a live replicating virus, but on the detection of highly specific molecular subunits of the virus in infected tissues and clinical specimens. These subunits could be either gene sequences specific for BLUV or certain serotypes. Obviously, direct detection of these molecular subunits from infected cells or insect vector is more efficient diagnostic approach.

Separation of the genomic segments using RNA electrophoresis due to segmented nature of BLUV genome is possible (Gorman *et al.* 1981, Squire *et al.* 1983, Oberst *et al.* 1985). Squire *et al.* (1983) demonstrated that all the 10 segments of ds viral RNA can be separated by PAGE. Different serotypes of the virus have the distinct pattern of ds-RNA segments migration on PAGE (Gorman *et al.* 1978, Samal *et al.* 1987). Hence, the RNA-PAGE can be used as a preliminary diagnostic tool to identify the BLUV isolates from field samples (Sugiyama *et al.* 1981). In our laboratory, it was experienced that while attempting isolation of the virus either from blood of the suspected animals or insect vector, the virus is not detectable by DIA at early passage levels (Prasad, personal communication). Therefore, a comparison of DIA and RNA-PAGE was done to ascertain the sensitivity of these tests in our laboratory. Our results suggested that RNA-PAGE and DIA have comparable sensitivity and both tests can detect a minimum virus titre of 10^5 $TCID_{50}$/ml. The RNA-PAGE has advantage over immunological assays in that it identifies the RNA segment migration pattern of the virus, hence it is less likely to yield false positive results as some times may be the case in enzyme immunoassays. More over, RNA-PAGE also helps in detecting the different genotypes of the same serotype or different serotypes while group specific immunological assays can not give any idea about the difference in the viral genomic RNA diversity (Squire *et al.* 1983). The RNA-PAGE technique in addition to comparable sensitivity with enzyme immunoassays, also permits assessment of genetic relationships and provides a useful tool for the analysis of RNA segment reassortments in crosses between isolates of BLUV (Gorman *et al.* 1978, Samal *et al.* 1987). Migration pattern of genomic RNA segments based on molecular weight and presence of additional bands in the PAGE technique also provides an opportunity to identify mixed infections of the animal host and insect vector by more than 1serotype or more than 1 strain of the same serotype or reassortments which is not possible by enzyme immunoassays.

The RNA- PAGE method is reasonably rapid (less than 12 hr) and can be performed with small volumes (0.1-1ml) of BLUV infected cell culture debris (Squire *et al.* 1983, Sugiyama *et al* 1981, Waldvogel *et al.* 1986) or infected clinical material including blood and insect vector. Currently RNA-PAGE is being used in the authors laboratory to study genomic differences in different isolates of the virus. A preliminary study has indicated that 2 isolates of the same serotype of BLUV from different geographical locations have different viral RNA genome profile on RNA-PAGE (Prasad *et al.* 1998).

Nucleic acid probes

The nuclear acid (NA) hybridization technique offers several features including high sensitivity, specificity and

rapid screening of target sequences. The use of recombinant cDNA probes make standardization of test regulation easier than with serological assays based on polyclonal reagents in spite of the minor drawbacks like special processing of samples to make target NA available for hybridization and optimization of assay conditions. Different methods were used for labelling of nucleic acids to be used as probes with radioactive and non-radioactive labels. For radio-labelled probes the label is incorporated to the ends of NA fragments and the choice of method depends upon amount of NA available, its size in base pairs, type of NA (RNA or DNA) and whether it is single stranded or double stranded. The process of nucleic acid hybridization, involves the interaction between labelled cDNA/oligo probes and complementary target RNA or DNA to form a DNA-DNA or DNA-RNA hybrids. Generally such probes have ends which can be labelled. The 5'end of NA can be labelled with $\gamma\,^{32}P$ using T_4 polynucleotide kinase which catalyses the transfer of γ-phosphate group from ATP to 5' -OH terminus of DNA molecule. Using this method synthetic oligonucleotide or ds DNA fragments after dephosphorylation can be labelled. Normally 3' end of the DNA fragment can be labelled by incorporation of $\alpha\,^{32}P$ label using terminal deoxy nucleotidyle transferase (Tdt) which catalyses the repetitive edition of mono nucleotides from deoxynucleotide triphosphates (dNTP's) to the 3'-OH terminus of single stranded or ds DNA. Thus this enzyme provides a unique method for labelling probes with ^{32}P for use in hybridization assays. The DNA probes prepared by nick translation can be used for wide variety of hybridization technique like gel blot, colony and plaque lifts. Now-a-days random labelling system using random hexamers is commonly used to prime DNA synthesis *in vitro* from any linear ds DNA template to a high specific activity.

Recently greater emphasis has been given to the development of non-radio active label probes as these are safer, less health hazards, economical and reduce cost of storage and their disposal. Non-radio active labels can also be incorporated by random prime labelling, nick translation, using terminal transferase, and PCR amplification.

Two basic methods for detecting BLUV nucleic acid using hybridization technique were reported extensively, first one is based on the identification of viral nucleic acid sequences extracted bound to solid matrix like NCM or nylon paper. Now-a-days commercially available dot-blot, multifold vacuum filtration units allow multiple samples to be blotted directly, quickly and uniformly on to the solid matrix. Second method involves detection of nucleic acid within specially prepared, intact cells or tissues (*in situ* hybridization).

For the detection of the virus specific nucleic acids, cDNA/RNA blot and *in situ* hybridization techniques were developed using the knowledge of BLUV genome and recombinant-DNA technology. A number of nucleic acid probes have been evaluated in different laboratories for detection of the viral genetic sequences in tissues and other body fluids (Roy *et al.* 1985, Squire *et al.* 1985, Squire *et al.* 1986, Squire *et al.* 1987, Dangler *et al.* 1988, Unger *et al.* 1988, Schoepp *et al.* 1991). The asymmetric single stranded RNA probes were used to identify the relative amounts of positive and negative sense NA strands for studying the kinetics of viral nucleic acid products. These probes are used to distinguish viral mRNA and viral ds RNA components (Dangler *et al.* 1988). The problems of using a single genome segment as most suitable group specific probe is compounded by the fact that there is significant variation in conserved genes. Although VP_7 has group specific reactive epitopes and is the protein utilised in the group specific BLU serodiagnostics, yet gene encoding VP_7 shows greater sequence variation within serotypes and can not be recommended as target for group specific probe. The BLUV genome segments corresponding to VP_3 (segment 3) and NS1 (segment 6) showed greater sequence conservation and are being evaluated as group specific probes (Wilson 1994). $A^{32}P$ labelled cDNA probe was prepared from semi-nested PCR product of NS1 gene which has been evaluated for detection of the virus in spiked blood and semen samples. Our results suggested that DNA probes could be used for detection of BLUV infection in blood and semen of the carrier animals (unpublished observation). The authors laboratory is currently engaged in evaluation of sensitivity of PCR for detection of the virus in different clinical as well as spiked samples. The efforts are also underway towards development of Dig labelled cDNA probe using NS1 as target gene for diagnosis of BLUV.

Variations in DNA hybridization protocols were developed to suit to the specific requirements of different clinical specimens and the viruses. *In situ* hybridization method is particularly very suitable for detection of the virus in tissues and cells. The advantage of this method is that it allows identification of the viral RNA/DNA sequences within morphologically distinct cells in the infected tissue sections. In this procedure, the tissue sections fixed in methanol, acetone formaldehyde and glutaraldehyde can be used for detection of the desired nucleic acid. Frozen sections can also be used directly in this method.

Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR), discovered by Mullis (1983), revolutionized the whole field of molecular biology (Saiki *et al.* 1986). PCR has attained a paramount importance in diagnosis of infectious and non-infectious diseases due to its speed and sensitivity. Traditional methods of viral diagnosis generally involves cumbersome method of isolation in cell culture followed by confirmatory tests. The time required for identification of viral agent might take 7-10 days. The success of growth of a particular virus is also dependent on the quality of the sample submitted and to the time taken to reach to the diagnostic laboratory, as successful isolation of the virus requires the presence of viable organism in the clinical sample.

In contrast, use of PCR offers the possibility of same day diagnostic results and is much less dependent on the conditions in which the sample arrives. DNA has even been amplified using PCR from mammoths extinct for thousands of years (Binns 1993).

For PCR-based diagnosis, DNA sequence of the target viral pathogen must be available. The basic procedure is as follows: 2 short DNA primers based on the sequence of the target viral genome which bind to opposite strands of ds DNA or cDNA (in the case of RNA viruses) are added to the sample along with 4 bases for DNA synthesis and a thermostable DNA polymerase (Taq). The Taq polymerase is stable in high temperatures needed to denature the DNA between the cycles which circumvents having the need to add fresh enzyme after each denaturation stage. The mixture is briefly heated at 94°C to separate DNA strands in the target DNA. The mixture is rapidly cooled to a temperature at which the primers will bind to their target sequences and this intern allows DNA polymerase to synthesise new DNA between 2 primers. Heating and cooling is repeated for about 30 cycles with theoretical doubling of the target sequence with each cycle. The whole process is completed within a couple of hours. During this time the target sequence is amplified several million fold to make the detection of amplified target a simple task (Binns 1993).

In the specimens where small number of viral particles are suspected, PCR could be used to amplify the viral nucleic acid. The evaluation of PCR for detection of BLUV in cell cultures and in clinical specimens including blood and semen was done (Gould *et al.* 1989, Dangler *et al.* 1990). Wade Evans *et al.* (1990) have successfully used PCR to detect BLUV in blood samples of cattle experimentally infected with BLUV. PCR reproducibility allowed detection of as few as 6 molecules of ds RNA, after 60 reaction cycles.

Moreover, use of PCR for detection of BLUV eliminates detection of closely related EHDV. PCR could be used for detection of bluetongue virus in semen and embryos also. Various PCR protocols have been developed in the past 5 years. Recently VP_3 (segment 3) and NS1 (segment 6) genes of Avikanagar isolate of BLUV serotype 1 (Prasad *et al* 1999b) were amplified using full length and nested primers in cell culture and spiked blood and semen samples. In authors laboratory the sensitivity of the RT-PCR for detection of cell culture grown BLUV was determined as 10 virus particles using NS1 gene specific primers (Prasad *et al.* 1996). Different PCR primers used by various workers are listed in Table 1.

A number of variations including RT-PCR (Dangler *et al* 1990), nested PCR (Wilson 1994), multiplex PCR (Wilson and Chase 1993), immuno-PCR, random primer PCR, *in situ* PCR (McColl and Gould 1991), PCR-ELOSA (MacLachlan *et al.* 1994) in basic protocol have been developed in the past 10 years.

Nested PCR procedure provides greater specificity because it involves 2 rounds of amplification. In the first round, a pair of external primers is used to get a larger fragment which then acts as a template for second round of amplification with a second set of primers internal to the initial set. This process provides an additional specificity to the reaction and greatly enhances the efficiency of amplification. This nested PCR test was very sensitive detecting an equivalent to 1 plaque forming unit of BLUV viral RNA extracted from infected biting midges (Wilson and Chase 1993).

Table 1. Bluetongue virus PCR primers used by various workers

Primers 5' to 3'	Target gene segment	PCR product bp	Reference
5' GTTAAAATGCTTA 5' GTTAAAATGCTTA	1	Full length gene product	Wade-Evane *et al* (1990)
5' GTTTTAAAATAATAGCGGCG 5' GTAAGTCTAATAGTGCGCGGATCTG	2	Full length gene product	Wade-Evane *et al.* (1990)
5' GTTAAATTTCCGTA 5' GTAAGTGTGTT	3	Full length gene product	Wade-Evane *et al* (1990)
5' GTTAAAAAGTGCCCCCTTAGCG 5' GTAAGTGTAAGTGCTT	5	Full length gene product	Wade-Evane *et al* (1990)
5' GTTAAAAAAGTTCTCT 5' GTAAGTTGAAAAGTTCTAGT	6	Full length gene product	Wade-Evane *et al.* (1990)
5' GTTAAAAATCTATAGAG 5' GTAAGTGTAATCTAAGAGA	7	Full length gene product	Wade-Evane *et al.* (1990)
5' GTTAAAAAATCCTAGAGT 5' GTAAGTGTAAAATCCCCCC	8	Full length gene product	Wade-Evane *et al* (1990)
5' GTTAAAAAGTGTCGCTGCCATGCCATGCTATCC 5' GTAAGTGTGTAGCGCCGCATACCCT	10	Full length gene product	Wade-Evane *et al.* (1990)
5' GTTCTCTAGTTGGCAACCACC 5' AAGCCAGACTGTTTCCCGAT	6	274	Katz *et al* (1993)
5' CCA GCA TTT TGA GAG AGC GA 5' CCC GAT CAT ACA TTG CTT CCT	6	101	Katz *et al.* (1993)
5' CCT GAT GTT TCC AGG ACA AAT TAT ACT C	3	708	McColl and Gould (1991)

In situ PCR has 2 main advantages over conventional PCR -first, it is possible to correlate PCR results with specific cell type in tissue i.e. the cells with positive signal in a tissue specimen can be identified, and second that there are no chances for contamination by carry-over products. By *in situ* PCR the virus specific sequences can be amplified in morphologically intact cells or tissues. The basic procedure involves application of PCR reagents on fixed cells/tissue sections mounted on microscopic slides and then the amplification is done as for standard PCR. The amplified product can be detected by using target DNA specific radio-active or non-radioactive probes by *in situ* hybridisation assay. This procedure has been successfully used for quick detection of BLUV in peripheral blood leukocytes of the infected animals.

Multiplex PCR is based on the basic principle of PCR. In this procedure, a large number of samples can be subjected to PCR in a specifically designed plate. Using the multiplex PCR product detection kits, the result of the amplification can be read directly after completion of the PCR. In this process, running of gel for detection is not necessary. Hence, multiplex PCR appears to be very useful in processing a large number of samples at a time and can be used for detection of multiple pathogens. Multiplex PCR is being used in epidemiological investigations for a variety of bacterial and viral pathogens. The availability of commercial kits for conducting multiplex PCR will expand its application in future. The sensitivity of multiplex PCR is less sensitive then nested PCR but sufficient to detect virus in field samples (Wilson and Chase 1993).

Polymerase chain reaction (PCR) holds wider implications in molecular biology. In the specimens when small number of viral particles are suspected, PCR could be used to amplify the viral nucleic acid (Gould *et al.* 1989, Dangler *et al.* 1990). Wade Evans *et al.* (1990) successfully used PCR to detect BLUV in blood samples of cattle experimentally infected with BLUV. PCR reproducibility allowed detection of as few as 6 molecules of ds RNA, after 60 reaction cycles.

ACKNOWLEDGEMENTS

The authors thank the CCS Haryana Agricultural University and ICAR Centre of Advanced Studies, Department of Veterinary Microbiology, for providing financial and infrastructure support for some of the studies which are reported in this paper. We would like to acknowledge the support of Dr Abhay Kumar (USA) for PCR and nucleic acid probes studies. We also acknowledge the financial help received from Council of Scientific and Industrial Research in the form of Senior Research Fellowships to YP and Minakshi.

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Indian Journal of Animal Sciences 70 (2): 110-113, February 2000

Adaptation of infectious bursal disease virus in chicken embryo fibroblast cell culture and its fine structure

RAJESH KUMAR[1], R CHANDRA[2], V D P RAO[3] and S K SHUKLA[4]

Govind Ballabh Pant Krishi Evam Praudyogik Vishva Vidyalaya, Pantnagar, Uttar Pradesh 263 145

Received: 17 December 1998; Accepted: 10 August 1999

ABSTRACT

Two outbreaks of suspected infectious bursal disease (IBD) at university poultry farms were investigated. On the basis of epidemiological data, characteristic gross and microscopic changes in different organs including bursa of Fabricius (BF) and spleen, presence of IBD virus antigen in BF and demonstration of intracytoplasmic virus particles of 59.2 nm diameter in infected chicken embryo fibroblast cells by transmission electron microscopy confirmed the outbreak as IBD. The type and distribution of lesions and mortality up to 23.2% is highly suggestive of involvement of IBD virus of moderate virulence. Neutralization of cell culture grown virus with reference antisera revealed the identity of virus as IBDV serotype 1.

Key words: Chicken embryo fibroblast cells, Electron microscopy, Infectious bursal disease, Natural outbreaks

Infectious bursal disease (IBD), a highly contagious, immunosuppressive infection of chickens (McFerran 1993) has contributed significantly in overall losses to poultry industry because of its mortality and immunosuppressive effects resulting in other concurrent infections or vaccination failures. In India, the disease is present throughout the country (Krishna 1994) and during the recent past, outbreaks of acute IBD in chickens with high mortality rates have been reported. The present paper describes 2 outbreaks of IBD in University Poultry Farms at Pantnagar.

MATERIALS AND METHODS

Field outbreaks

Two outbreaks of IBD were recorded, one at Student's Poultry, College of Veterinary Sciences, and other at Poultry Research Centre, Nagla, Pantnagar. Epidemiological details are presented in Table 1. In the course of outbreak, clinical signs were recorded and gross lesions in various organs were observed after postmortem examination of dead birds. Tissue samples from bursa of Fabricius, spleen, liver and kidneys were collected in 10% formalin for histopathological examination. The bursa of Fabricius and spleen were also collected in 50% glycerol saline for virus isolation.

Present address: [1]Senior Research Fellow, [2]Associate Professor, Virology; [3]Professor, Virology, Department of Microbiology; [4]Associate Professor, Department of Medicine, College of Veterinary Sciences.

Histopathology

The fixed tissue samples were processed for routine haematoxylin and eosin (H&E) staining (Lillie 1965).

Preparation of sample for virus isolation

The bursal homogenates were processed separately as per the technique given by Ravi Kumar *et al.* (1996). After centrifugation, the supernatants were passed through membrane filters of 0.45, and 0.22 μm average pore diameter (APD) and the filtrates were subjected to AGPT against known positive antisera obtained from IVRI, Izatnagar. The positive samples served as source of virus for isolation attempts.

Isolation of virus in chicken embryo fibroblast (CEF) cell culture

Primary CEF cell culture was prepared using medium-199 supplemented with 5% and 1% calf serum as growth and maintenance medium. The confluent monolayers in tissue culture bottles and Leighton tubes were inoculated with samples prepared from BF @ 1.0 ml and 0.2 ml inocula respectively. After 4-5 days post infection (PI), the virus was harvested by freezing and thawing for 3 times followed by centrifugation at 4 000 rpm for 30 min. The supernatants of the samples were filtered using 0.22 μm APD filter and stored at —20°C to serve as source of virus for further successive passages.

Study of cytopathic effects

The cytopathic effects (CPE) produced by isolates from both the outbreaks were observed in unstained bottles and in

infected cover-slip cultures stained with May-Grunwald and Giemsa stain at 36, 48, 72, 96 and 120 hr post PI (Merchant *et al.* 1960). Only 2 samples out of 5; 1 from student's poultry (S1) and other from PRC, Nagla (S3) yielded virus.

Electron microscopy

Cells from infected tissue culture bottles showing characteristic CPE were harvested by gentle pipetting without freezing and thawing. The cell suspension infected with isolate S_1 was centrifuged at 3 000 rpm for 30 min at 4°C and pellet was fixed in 2.5% glutaraldehyde solution in PBS for transmission electron microscopy (Doane and Anderson 1987). The pellet was post fixed in 1.0% osmium tetraoxide and after dehydration, the pellet was embedded in araldite. The ultra thin sections stained with uranyl acetate and lead citrate, were examined by transmission electron microscope at the AIIMS, New Delhi.

Serological tests

The antigen prepared by concentrating the cell culture harvest to 1/10th of original volume by dialysis against saturated sucrose solution was tested against standard IBD serum in AGPT as per Cullen and Wyeth (1975). After charging the wells, the slides were kept in moist chamber at 37°C for 24 hr and examined for the presence of precipitin lines and then transferred to 4°C for further development of lines.

Neutralization of cell culture grown virus was done with hyperimmune sera raised against vaccine strain of IBD virus and assayed in CEF cell monolayers. The neutralization index was calculated as per the method of Reed and Muench (1938).

RESULTS AND DISCUSSION

Epidemiological observations

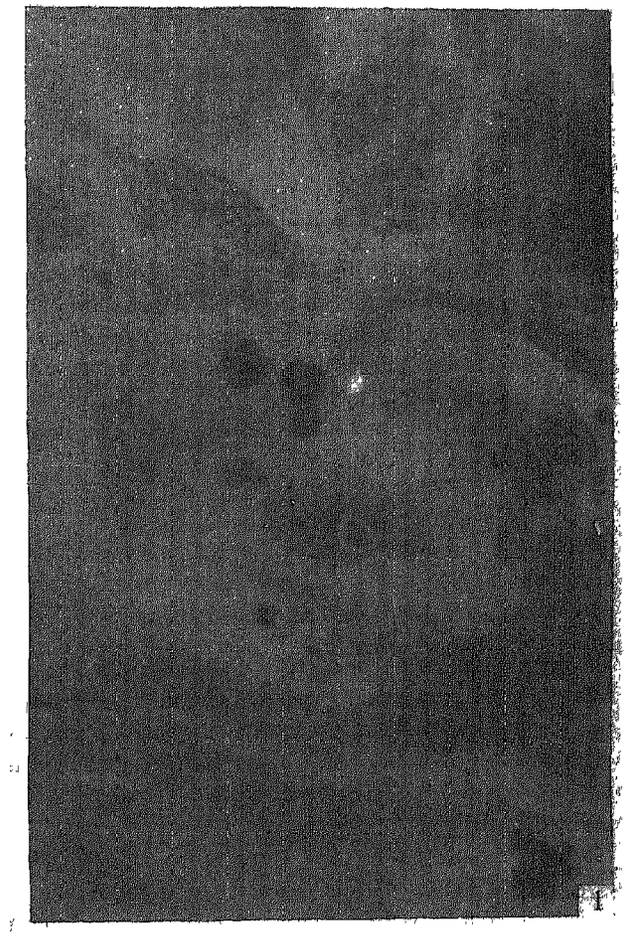
The outbreak of IBD at Student's Poultry Farm resulted in 11.1% mortality while mortality at the PRC, Nagla, was 23.2%. The duration of the disease was for 6-8 days.

Clinical signs and gross lesions

Clinical signs in both the outbreaks included depression, unwillingness to move, pecking at their vent, reduced feed intake, ruffled feathers, whitish diarrhoea and dehydration. Postmortem examination of the birds revealed dehydration of subcutaneous fascia and pectoral muscles, hemorrhages in the thigh muscles, inguinal region and in the pectoral area. Kidneys showed enlargement and liver was congested and showed necrotic foci on the surface. Bursa of Fabricius was invariably enlarged with occasional hemorrhages in some birds. Splenomegaly, mucous in the lumen of intestine and in some birds haemorrhages at the junction of proventriculus and gizzard were observed.

Histopathology

The bursa of Fabricius showed depletion of lymphoid follicles along with degenerative and necrotic changes in the



Figs 1-2. 1. Photomicrograph of CEF monolayer infected with S1 isolate showing moderate level of CPE with increase in cytoplasmic granularity and presence of occasional intracytoplasmic inclusion bodies. MGG × 600. 2. Electronmicrograph showing paracrystalline arrangement of virus particles and inclusions containing virus particles surrounded by a "halo". TEM×8200.

Table 1. Epidemiological data of 2 outbreaks of IBD in university poultry farms

Farm	Poultry strain	Status of birds	Age (week)	Total strength	Mortality	Per cent mortality
Student's poultry	Hubbard (cross strain of broiler)	Unvaccinated	4	340	38	11.1
PRC, Nagla	Babcock (WLH)	Vaccinated with Georgia strain	10	3000	696	23.2

lymphoid cells. Congestion the in red and white pulp, rarefaction of lymphoid tissue in some follicles of spleen and necrosis of tubular epithelium of kidneys with deposition of hyaline mass in the lumen of some of the tubules were observed. Some tubules also showed deposition of urates and mild infiltration of mononuclear cells.Liver showed only congestion and mild necrotic changes.

Demonstration of IBD antigen in bursa of Fabricius

Only 2 out of 5 bursal tissue homogenates showed 1 or 2 precipitin lines against standard IBDV antisera by AGPT. The isolates were named as S_1 and S_3.

Isolation and adaptation of IBDV in CEF cell culture

The CEF cell culture inoculated with bursal homogenates found positive for IBDV antigen by AGPT (S_1 and S_3) resulted in the isolation of virus. The isolates were adapted to cell culture after 5 successive passages as evidenced by stabilization of CPE.

Both the isolates showed detectable CPE in CEF monolayers at fourth passage characterized by rounding of few cells at 60 hr PI, which progressed to clumping followed by increase in granularity by 120 hr PI. At fifth passage, cellular degeneration was recorded by 96 hr. At sixth and seventh passage, the CPE started at 36 hr PI and by 72 hr, 70-90% of cells were involved. The infectivity titre of virus at sixth passage was $\log_{10}$ 4.3 EID50/ml. Uninfected control CEF monolayer did not exhibit any change.

May-Grunwald and Giemsa staining of CEF cells infected with both the isolates at fifth passage level revealed CPE with increase in cytoplasmic granularity and presence of occasional intracytoplasmic inclusion bodies at 48 hr PI (Fig. 1). Severe degenerative changes with cytoplasmic granulation, nuclear degeneration with intracytoplasmic eosinophilic inclusion bodies surrounded with a clear 'halo' in several cells were observed by 72 hr PI.

Electron microscopy

The electron microscopy of chicken embryo fibroblast cells infected with S1 isolate showed virus particles arranged in the form of paracrystalline array and membrane bound inclusion bodies containing moderately dense spherical structure surrounded by an electronlucent 'halo' containing few virus particles (Fig.2). The infected cells also showed pronounced aggregations of filaments within cisternae of rough endoplasmic reticulum. These granulated filaments were frequently observed in the vicinity of virus particles. The size of mature virus particles was 59.2 nm in diameter. The nucleus showed mild degenerative changes and were also devoid of virus particles. Numerous electronlucent inclusions containing immature virus particles in the cytoplasm of the cell were also seen.

Serological tests

By double immunodiffusion technique one precipitin line was seen with infected bursal homogenate in 24 to 36 hr of incubation and sharpness of precipitin lines increased after 24 hr at 4°C. The concentrated CEF cell culture antigen showed 2 precipitin lines as early as 12 hr of incubation at 37°C, however, the precipitin line was of comparatively low density compared to IBDV infected chicken bursa.

Hyperimmune serum raised against IBD live vaccine (IBD 228E) neutralized CEF grown virus with a serum neutralization index of 3.25.

In this study, echymotic haemorrhages in the thigh and pectoral muscles and haemorrhages in the mucosa at the junction of proventriculus and gizzard in some birds were also seen earlier (Lukert and Saif 1991). These observations along with microscopic changes such as degeneration and necrosis of lymphocytes in BF and rarefaction of lymphoid follicles of spleen are suggestive of extremely lymphocidal nature of virus. Similar observations were also reported by various workers in the birds infected with IBDV during the first 3 days of infection (Verma *et al.* 1987, Dash *et al.* 1991).

The bursal homogenates prepared from infected birds from both the outbreaks were positive for IBD antigen against standard reference antisera, which confirms the outbreak as IBD. However, only 2 out of 5 samples were positive for IBD antigens.

The virus isolates adapted well in CEF cells after 5 successive passages and showed characteristic CPE. Similar results were also obtained by (Casnocha 1980, Verma *et al.* 1981, Dash *et al.*1991). The CEF cell culture grown virus also reacted with standard reference antisera, which is indicative of growth of IBD virus in cell culture. These observations were further supported by demonstration of intracytoplasmic virus particles in the infected cells by electron microscopy and neutralization of virus by specific antisera.The latter also confirms the involvement of IBD virus serotype 1. Transmission electron microscopy of infected CEF cells have not been done earlier. Electronlucent inclusion bodies containing mature/immature virus particles of 59.2 nm in diameter with hexagonal shape were seen in the infected

CEF cells.A similar observation was recorded by Almeida and Morris (1973) in BF.

The present investigation is suggestive of widespread circulation of strains of IBDV of moderate to high virulence. The vaccination of birds with isolates prevalent in the area/ region may, therefore, be recommended to control the losses due to IBD.

ACKNOWLEDGEMENTS

The facilities provided by Dean, College of Veterinary Sciences and Director, Experiment Station are gratefully acknowledged. The senior author is thankful to Uttar Pradesh Council of Agricultural Research (UPCAR) for providing financial assistance in the form of Junior Research Fellowship. The authors are also grateful for facilities and technical assistance provided by Electron Microscopy Lab., AIIMS, New Delhi for carrying out electron microscopic study in the present communication.

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Indian Journal of Animal Sciences 70 (2): 114-117, February 2000

Efficacy of vaccines prepared from *Pasteurella multocida* cells grown in chemically defined media

S K SRIVASTAVA[1]

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122

Received: 15 January 1999; Accepted: 6 August 1999

ABSTRACT

Pasteurella multocida serotype B: 2 was grown in chemically defined medium (CDM), CDM supplemented with iron (CDM.Fe) and brain heart infusion broth (BHIB) and studied for their immunogenicity. Cells grown in CDM expressed maximum outer membrane protein (OMP) as demonstrated by congo red binding property of the bacterial cells. These cells, when used as vaccine, afforded maximum protection (100%) in mice than cells grown in the CDM.Fe (78%) or BHIB (72%). In buffalo calves the CDM.Fe and BHIB grown cell vaccine elicited higher anti-protein passive haemagglutination antibody responses than the CDM vaccine. Cells grown in CDM.Fe were more agglutinogenic than the CDM or BHIB grown cells. The study suggested that *P.multocida* cells grown in CDM or CDM.Fe media possessed different antigens resulting in higher seroreactivity.

Key words: Heamorrhagic septicaemia, Outer membrane protein, *Pasteurella multocida* vaccine

Pateurella multocida serotype B: 2 is the causative agent of haemorrhagic septicaemia in cattle and buffaloes. Several vaccines are currently available for control of the disease, mainly, an alum precipitated and an oil adjuvant whole cell vaccine (Iyer *et al.* 1955, Bain 1963). Recently subunit vaccines have been tested incorporating purified or semi-purified immunogens of *P.multocida,* such as glycoprotein (Mukkur and Nilakantan 1972), outer membrane protein (Pati *et al.* 1996) and total cellular proteins (Srivastava 1998). Of these, the OMP vaccine appears to be the most promising, however, difficulty involved in the extraction of OMP may be one hindrance in attempting this preparation as a commercial vaccine. *P. multocida* cells grown under iron restriction conditions express novel iron binding outer membrane proteins (Choi-kim *et al.* 1991, Veken *et al.* 1996). In the present study, *P.multocida* serotype B: 2 was grown in an iron-restricted and iron-sufficient media and studied for their immunogenicity. For comparison cells grown in a conventional medium were included with the present study. The ultimate objective of the study was to improve the immunogenicity of the *P.multocida* vaccine for controlling the disease in animals.

MATERIALS AND METHODS

Pasteurella multocida *strain and the media used*

Present address: [1]Principal Scientist, National Research Centre on Equines, Sirsa Road, Hisar, Haryana 125 001.

P.multocida strain P52 being maintained in the division was used. The organisms were passaged in mice 5-6 times and grown in brain heart infusion broth (BHIB) or a chemically defined medium (CDM) (Hu *et al.* 1986). The organisms were also grown in CDM containing 50 μm of $FeCl_3$ (CDM.Fe).Antiserum against the strain was prepared in rabbits (Pati *et al.* 1996).

Preparation of antigens

Total protein (PSAP) from sonicated *P.multocida* cells was precipitated at 45% saturation of ammonium sulphate (Knights *et al.* 1990). For extracting outer membrance protein (OMP) method of Pati *et al.* (1996) was used and for lipopolysaccharide (LPS) technique described by Westphal and Jaan (1963) was adapted. Proteins and soluble sugars in the antigens were measured (Lowry *et al.* 1951, Dubois *et al.* 1956).

Congo red binding assay

The ability of *P.multocida* cells to bind congo red dye was studied (Choi-Kim *et al.* 1991, Srivastava 1998).

Serology

Passive haemagglutination assay (PHA) was carried out based on the technique of Srivastava *et al.* (1985). Various antigens, viz. sonicated cells, PSAP, OMP (all 10 mg protein/ml) and LPS (10mg carbohydrate/ml) were separately adsorbed on tanned sheep red blood cells for 4 hr at 37°C followed by over night storage at 4°C. A 2% suspension of

each of the adsorbed antigens was used for titration of sera.

Slide agglutination test (SAT) was performed by mixing 50 µl of test sera with an equal volume of freshly cultured live *P.multocida* cells (opacity to Brown's tube No. 8) grown previously in BHIB, CDM or CDM.Fe, over a clean glass slide and recording of reaction after 2 min of mixing.

Vaccination and challenge studies

Three vaccines, viz. an 18 hr-old formalin-killed culture of *P.multocida* grown in BHIB, CDM or CDM.Fe were used. The concentration of cells was brought to Brown's tube No. 6, and 15 parts of this was emulsified with 10 parts of liquid paraffin and 1 part of lanolin and used fresh.

Animals vaccinated with each of these vaccines comprised mice (18-20 g body weight) and male buffalo calves, 8-10 months of age. Mice (10) in a group were given each with 0.2 ml of either of the 3 vaccines, intramuscularly. At day 28-post vaccination (PV) all the mice were challenged with 200 colony forming units (CFU) of *P.multocida*, intraperitoneally. Simultaneously, 3 buffalo calves in a group were given each with 3.0 ml of each of the vaccine and bled at days 7, 14, 21, 28 PV and sera subjected to antibody titration using PHA and SAT. Mean antibody levels were calculated and compared between the groups and within the group employing a student's 't' test.

RESULTS AND DISCUSSION

P.multocida cells when grown in CDM and CDM.Fe showed a poor growth compared to BHIB during the early passages but later on the growth improved after 6-7 passages. Morphologically, the CDM grown bacterial cells appeared to be longer in size than the cells grown in BHIB, and also lost the bipolar appearance.Congo red binding assay revealed that the cells grown in CDM possessed maximum OMP concentration (Fig. 1). In this medium the mouse passage of cells had no effect on the OMP production compared to the cells grown in CDM.Fe. Mouse passage of *P.multocida* cells enhances OMP production (Choi-Kim *et al.* 1991). Therefore, the CDM was considered to be enhancing the OMP production by *P.multocida* strain under study.

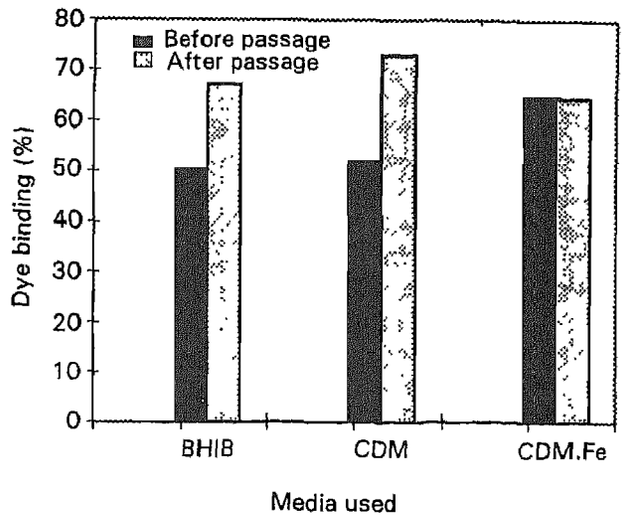

Fig. 1. Congo red binding by *P multocida* grown in different media before and after mouse passage.

The immunogenicity of *P.multocida* grown in 3 different media was studied in mice and buffalo calves. The protection against the challenge in the mice vaccinated with the BHIB, CDM and CDM.Fe grown cells was 72, 100 and 78% respectively. The results suggested that, although, all the

Table 1 Passive haemagglutination titres ($10\times\log_2$) observed in sera from vaccinated buffalo calves using various *P.multocida* antigens

Animal group	Vaccine containing cells grown in	Days P.V.	*P.multocida* antigen used			
			Sonicated	OMP	Total protein	LPS
1	BHIB	7	4.3±0.5	2.50±0.5	5.3±0	4.5±0.5
		14	6.7±0.6	4.37±0.3	7 67±0.4	5 5±0 3
		21	8.67±0.5	4.67±0.2	8.30±0	6.3±0.5
		28	7.67±0.6	4.00±0.3	8.5±0.5	6.0±0.3
2	CDM	7	4.37±0.3	3.00±0	6.0±0.6	5.0±0.5
		14	8.60±0.4	5.00±0.4	7.81±0.3	6.0±0
		21	8.67±0.5	4.67±0.4	7.5±0 3	6.0±0.5
		28	8.34±0.0	3.30±0.5	6.0±0.5	6.8±0.3
3	CDM.Fe	7	4.50±0.5	3 8±0.6	7.6±0	5.5±0.5
		14	6.55±0.5	6.8±0	8.0±0.6	6.67±0 6
		21	6.67±0.5	6.6±0.5	9.0±0*	7.0±0.4
		28	8.30±0.0	6.7±0*	9 67±0.5*	5.5±0.3**

*Significantly higher (P>0.025) than CDM vaccine at the same day PV; **significantly different (P>0.01) than CDM or BHIB vaccines at the same day P.V.

vaccines conferred acceptable levels of protection, maximum protection was seen with the CDM grown cell vaccine. The CDM vaccine has this property may be due to the increased production of OMP by the cells as evident by the congo red assay. Daneer and Potter (1989) have demonstrated an increased binding of congo red dye by *P.multocida* cells with a simultaneous increase in OMP production.

To elucidate the role of various immunogens, including OMP, of *P.multocida* in seroconversion in the vaccinated animals, buffalo calves were used. Four immunogens were used to monitor the immune response in the vaccinated animals. PHA antibody titres detected in these animals using either of the 4 antigens started increasing at day 7 PV which further increased at day 14 PV and then did not change throughout the study. The responses were higher against the sonicated cells and the total cell protein than the OMP or LPS. The 2 latter antigens were the purified preparations and the lower titres against these antigens was quite expected as the immune response in animals is directed against an array of antigens present on the surface of the bacterial cells (Tizard 1976). When the titres generated in the animals given different vaccines were compared from each other, no statistical difference was observed between BHIB and CDM vaccines at any day post-vaccination. With the CDM.Fe grown cell vaccine, the titres were higher than the CDM vaccine either at day 21 or 28 using OMP or total cell protein antigens. CDM.Fe vaccine did not differ from BHIB grown cell vaccine. These results indicated that using the sonicated antigen, i.e, a mixture of all the antigens the efficacy of all the vaccines was comparable. However, use of purified or semi-purified proteins as antigens revealed that the CDM.Fe vaccine generated more of the anti-protein (whole protein and OMP) immune response. Though in the congo red binding assay this vaccine did not possess higher OMP. Probably, the arrangement of immunogenic epitopes on the cell surface was such that it induced higher antibody response. Most probably the total protein concentration of the CDM.Fe grown cells was higher than that of the other cells. This requires further investigation.

Table 2. Agglutinin titres observed in buffalo calves administered vaccines prepared using *P.multocida* cells cultured in various media

Animal group	Medium used	Days PV	Mean ($10 \times \log^2$) titres detected using *P.multocida* cells as antigen grown in		
			BHIB	CDM	CDM(Fe)
1.	BHIB	14	5.6±0.4	3.6±0.4	4.6±0.4
		21	4.6±0.5	4.6±0.7	5.0±0.5
		28	3.6±0.5	3.6±0.5	4.6±0.4
2.	CDM	14	4.3±0.4	3.6±0.5	4.3±0.3
		21	4.3±0.4	4.3±0.4	4.6±0.7
		28	4.6±0.3	3.5±0.3	3.3±0.5
3	CDM.Fe	14	4.3±0.6	5.0±0*	6.6±0.5**
		21	4.3±1.0	5.6±0.3*	6.6±0.5**
		28	2.3±0.4	4.0±1.0	4.6±1.5***

*Significantly higher (P>0.05) than values obtained using CDM or BHIB grown vaccine at the same day, PV; **significantly higher (P>0.025) than titres detected using BHIB or CDM grown cell antigen at the same day PV; ***significantly higher (P>0.01) than values obtained using BHIB antigen.

The antigenic and immunogenic properties of *P.multocida* were also assessed using SAT. Using BHIB grown cells as antigen no difference in the SAT titres generated by the 3 vaccines was observed (Table 2). When CDM or CDM.Fe antigens were used, highest titres were recorded with CDM.Fe vaccine. These results indicated a better agglutinogenicity of these cells as compared to the BHIB grown cells. As suggested above, higher response by the CDM and CDM.Fe grown cells could be possibly due to enhanced protein production. The present study, therefore, suggested that use of a chemically defined medium have an advantage over the conventional BHIB medium in the preparation of *P. multocida* vaccine. However, the role of iron in the medium in enhancing the immunogenicity of *P.multocida* cells as related to OMP concentration remained inconclusive. This warrants further studies.

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Indian Journal of Animal Sciences 70 (2): 118-120, February 2000

A histomorphological and immunohistochemical study on the lactotrophs in pars distalis adenohypophysis of buffalo

MASSARAT KHAN[1], R P SAIGAL[2] and R S SETHI[3]

Punjab Agricultural University, Ludhiana, Punjab 141 001

Received: 1 July 1999; Accepted: 17 August 1999

ABSTRACT

In order to localize and characterize the lactotrophs in hypophysis cerebri of 53 buffalo of either sex of varying age were used. The lactotrophs were round, irregularly polygonal or rarely elongated in shape. The average diameter of these cells was 7.6 to 8.0 μm in males up to 4 months of age, and 9.8 μm in adult males and in female during follicular phase. These cells were larger in luteal phase and in pregnancy. The regional variations in the size of the lactotrophs evidenced the smallest cells in zona tuberalis (8.8 μm) and the largest in the ventral region (9.6 μm). Of the chromophils the lactotrophs were distributed in maximum proportions (29.46%) in the ventral aspects of the pars distalis and minimum in the central region and zona tuberalis. In males, the lactotrophs constituted 15.48% of the chromophils in one week calves, decreased to 8.68% by 4 months, whereafter increased to 14.08% in adults. The lactotrophs were in higher proportions in cyclic/pregnant females. They decreased during early pregnancy (18.76%) and were in largest in later stage of pregnancy (27.60%).

Key words: Adenohypophysis, Buffalo, Immunohistochemistry, Histomorphology, Lactotrophs, Pars distalis

A little information is available on the histomorphology and immunohistochemical aspects of the lactotrophs in pars distalis adenohypophysis buffalo. Keeping in view the significance of lactotrophs (PRL) in relation to the production, the present investigation is envisaged to work out details that are important and not reported so far in buffalo.

MATERIALS AND METHODS

The hypophysis cerebri from 52 buffaloes (belonging to different age groups of either sex) fixed in Bouin's Holland sublimate solution were used for differential staining of acidophils cell types. Serial paraffin sections of 5 μm were collected and stained with following methods: 1. Haematoxylin and eosin staining for routine examination (Luna 1968). 2. Elftman's paraldehyde fuchsin-PAS-orange G stain after Girod (1976) except that the chromatin of the sections was performed for 3 days and staining was performed with previous oxidation, 3. Herlants alcian blue-PAS-orange G stain (Girod 1976). 4. Modified trichrome stain of Cleveland and Wolfe (1932) with the introduction of luxol fast blue as an additional counter staining after Das (1972) 5. Luxol fast blue- PAS- orange G staining (Dubois and Herlant's 1968). The stained sections were examined histologically and quantitative observations were recorded in 7 different regions of pars distalis, viz. zona tuberalis, rostral, dorsal, ventral, central, caudal and lateral regions of the pars distalis. The relative proportions and size of GH cells were averaged region-wise. and group-wise separately and the data was subjected to statistical analysis using analysis of variance (ANOVA) to find out regional and or group differences, if any (Snedecor and Cochran 1968).

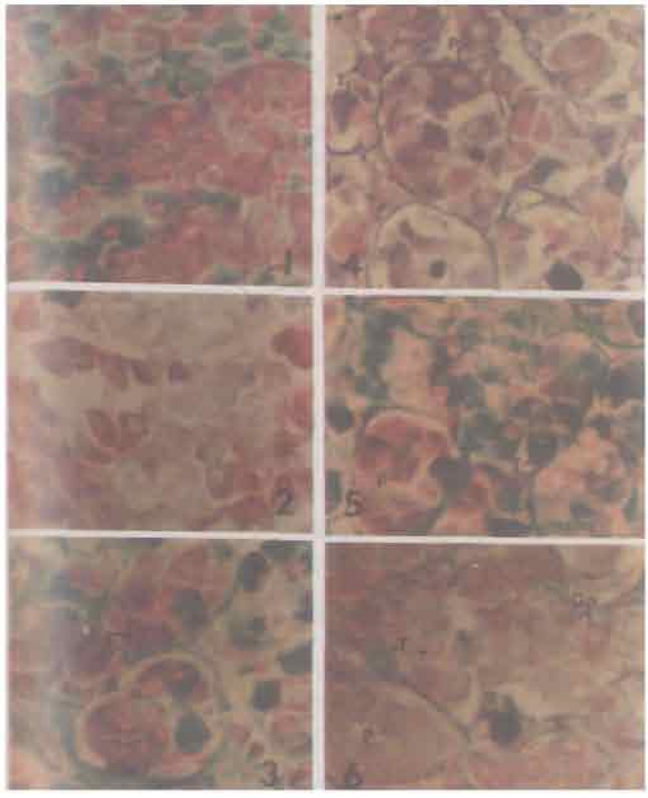
For immunohistochemical demonstration of cell type the paraffin sections were deparaffinized and hydrated and were further treated by labelled avidin biotin method using rabbit-anti-human GH serum with a working dilution of 1: 1 000 and incubation period of 4 hr at room temperature. The primary antibodies were procured commercially and protocol followed was that of Sigma diagnostics St. Louis, USA.

RESULTS AND DISCUSSION

PRL cells were distributed singly as well as in groups (Fig. 2). The PRL cells were demonstrated as staining brick red with luxol fast blue- erythrosin-orange G-aniline blue method (Figs 1, 3, 5) and purplish magenta with aldehyde fuchsin-PAS-orange G method (Fig.4). With alcian blue-PAS-orange G (Fig.6), the PRL cells showed weak to strong orangeophilia but with luxol fast blue-PAS-orange G they could not be distinguished as reported by Mohammad and Khan (1984).

The PRL cells were round, pyramidal, irregularly

Present address: [1,3]Assistant Professor, [2]Professor-cum-Head, Department of Veterinary Anatomy and Histology.



Figs 1-6. Adenohypophysis pars distalis showing: **1**. Brick red PRL cell from 12 days old male buffalo calf, luxol fast blue-erythrocin- orange G-aniline blue stain.× 800; **2**. Immunoreactive PRL cells from one week old male buffalo calf, avidin-biotin stain. × 800; **3**. Brick red PRL (P) cells along with green colloid (Co) from a pregnant buffalo, luxol fast blue-erythrocin-orange G-aniline blue stain. × 800; **4**. Purplish magenta PRL (P) cells from a buffalo in follicular phase, purplish blue are TSH (T) cells, paraldehyde fuchsin-PAS-orange G stain. × 800; **5**. Brick red PRL (P) cell from a heifer, dark blue are TSH (T) cells, luxol fast blue-erythrocin-orange G-aniline blue stain. × 800; **6**. Orangeophilic PRL (P) cells from a buffalo in luteal phase, also seen are gonadotrophs (Gn) and TSH (T) cells, alcian blue-PAS-orange G stain. × 800.

polygonal and rarely elongated in shape. The shape of the PRL cells in buffalo was reported as irregular (Pathak and Janakiraman 1988). They were found as round, irregular polygonal and rarely elongated or pyramidal. Their nuclei were round or oval and eccentric in location. The nucleus of PRL cells in buffalo was usually round or oval generally placed eccentric. Chakravarthy and Mariappa (1975), however, reported them round and placed centrally. The chromatin substance was coarse granular and irregularly distributed in the nuclei of young buffalo (Fig.1) and in the adults it appeared finer with prominent nucleolus.

The average diameter of PRL cells was shortest (8.8 μm) in zona tuberalis and largest (9.6 μm) in the ventral region, with mean values of 9.2 ± 0.09 μm in the pars distalis having no significant ($P > 0.05$) regional differences.

Group-wise comparison of size of PRL cells revealed that the average diameter of these cells was shortest between 7.6 to 8.0 μm in the male buffalo calves up to 4 months of age which increased to 9.8 μm in adult males and females in follicular phase respectively. During luteal phase it further increased to 10.5 μm. During early pregnancy the size of PRL cells was increased to 11.0 μm showing a gradual decrease with advancement of pregnancy to 10.2 μm in the pregnancy of above 3 months.

The PRL cells were distributed throughout the gland but majority of them were localized within the developing (in 1-week-old male calf) and developed (in all other older animals) follicles/acini. The PRL cell proportion was maximum in the ventral (29.46%) aspect of pars distalis which decreased in caudal, lateral, dorsal and rostral directions. Comprising very low proportions in the central region 18% and zona tuberalis 17.24%. The PRL cells in males at 1 week of age accounted for 15.48% which gradually decreased to 8.6% by 4 months of age, were after it increased to 14.08% in the adults. The PRL cells in the cyclic animals comprised a higher value (21.66 and 22.73% in follicular and luteal phase respectively) than the average value (17.65 ± 1.84%) which decreased during early pregnancy (18.96%) close to the average value but further increased to higher values during later stages of pregnancy (27.60%) than those of cyclic animals.

The size of the PRL cells in buffalo whereas showed no significant regional differences, they varied significantly ($P > 0.05$) with age and cyclic changes. The smallest cells (7.6 μm) were recorded at 4 months of age in male buffalo calf and the largest (11.0 μm) in the pregnant animals, the mean diameter being 9.4 ± 0.36 μm. Present data shows relatively lower values than those (11.73 ± 10.5 μm) recorded in the same species by Pathak and Janakiraman (1988).

Although the PRL cells in buffalo were distributed singly throughout the gland, the developing (in 1-week-old male calf) and developed (in older animals) follicles/acini were mainly comprised of the PRL cells.

As for the relative proportions of the cell types, the PRL cells comprised very low proportions in buffalo (17.65 ±1.84%) than that reported earlier by Hashimoto *et al.* (1987) in cow (53.8%).

The regional distribution of PRL cells in buffalo was in agreement with the observations of Pathak and Janakiraman (1988) in that the central region of the pars distalis had relatively scattered PRL cells than in other regions. However, they constituted least percentage in the zona tuberalis region.

The highest concentration of PRL cells in cycling female buffaloes was found during luteal phase. This was in agreement with the observations of Abul-Ela *et al.* (1987). They further increased during pregnancy above 3 months. In goat the PRL cells are reported more in number during pregnancy (Singh and Dhingra 1979).

Thus it appears that at least in buffalo the PRL cell proportions vary with regions, age or with phases of reproductive cycle but not significantly ($P > 0.05$).

The higher values by immunohistochemical methods for the cell size and the relative proportions of PRL cells were 11.6 ± 0.46 μm and 9.2 ± 0.51 μm by differential staining, however, relative proportion of PRL cells were 27.39 ± 3.45% by immunohistochemical method and 19.49 ± 2.60% by differential staining might be due to the cell boundaries being not so distinct in the differential stainings.

ACKNOWLEDGEMENTS

Authors thank the Dean, College of Veterinary Sciences, for providing facilities. The antisera for immunohistochemical work supplied by Dr Terry Taylor, Director, NIDDK, USA, is duly acknowledged.

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Indian Journal of Animal Sciences 70 (2): 121-125, February 2000

Morphohistogenesis of oesophagus in fowl (*Gallus domesticus*)

D VERMA[1], M R MALIK[2], M L PARMAR[3] and J S TALUJA[4]

Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh 482 001

Received: 27 September 1998; Accepted: 10 August 1999

ABSTRACT

Oesophagus extended from pharynx to pro-ventriculus in right side of the neck. It had 2 parts, pre-crop and post-crop. The pre-crop part was longer (9.80-65.00 mm) and wider (1.72-14.00 mm) than the post-crop (7.70-50.00 mm) and (1.68-13.20 mm) in all the age groups. The CS area pre-crop was more than post-crop in all the age groups except groups 8 and 10 where it was more in post-crop. The growth spurt of length and width of both the parts revealed maximum (85.18 and 46.66%, 132.55 and 90.40%) increase in groups 3-4 and 2-3 respectively. However, CS area revealed maximum and minimum values of growth spurt in groups 4 onward.

The overall mean thickness of the wall of post-crop part (1201.40 µm) was more than that of the pre-crop (1172.80 µm). The tunica mucosa of both the parts of oesophagus on an average contributed approximately double (46.40-67.49%) than tunica muscularis (29.00-44.59%). The epithelium changed from simple cuboidal (group 2) to stratified squamous (group 4). The villi and oesophageal glands appeared in group 3. In groups 4 onward, the cells of the superficial layer of epithelium in post-crop part were keratinized. However, keratinization was not conspicuous in pre-crop. The tubuloalveolar mucous glands in post-crop were profuse in the mucosal folds in contrast to uniformly distributed in the pre-crop.

Key words: Fowl, Growth spurt, Histogenesis, Post-crop oesophagus, Pre-crop oesophagus

There is paucity of detailed information on histological and histomorphological aspects of oesophagus in pre-and early post-hatch period of the fowl. Hence, the present experiment was designed in poultry to explore the developmental, gross and histological changes in pre-and post-crop oesophagus during pre-and post-natal development.

MATERIALS AND METHODS

The gross, histological and histochemical study of the oesophagus was conducted in 60-embryo/chick of normal White Leghorn (WLH) birds comprising 18 pre-hatch and 42 post-hatch chicks. Birds were divided into 10 groups of 6 birds each. The birds were procured from the Livestock Poultry Farm, Krishi Nagar, Adhartal, Jabalpur. Fertilized eggs were incubated at 38°C in co-operate automatic incubator. The digestive system of the chicks were harvested at specified days falling on days 7, 12 and 19 of incubation. Chicks up to 8 weeks were reared in brooder house, up to 18 weeks in grower house, and those from 18 to 32 weeks in layer cages under standard management, nutritional and disease control practices of the poultry farm. The post-hatch stage comprised 7 groups day-old and 1, 2, 4, 8, 16 and 32 weeks of age. Greatest length and width of the pre-and post-crop oesophagus were measured with the help of vernier callipers, meter scale/thread and compass in formalin fixed specimen of different age groups. For cross sectional area (groups 4-10), each part of the organs was transacted in the middle and stamp pad ink impressions of the cut surface were made on graph paper. The area was calculated in mm^2 by graph method. For histological studies samples from pre-and post-crop parts of oesophagus were collected and fixed in 10% buffered formalin for 48 hr. The tissue samples were processed in acetone-benzene and alcohol-cedarwood oil sequence, embedded and blocked in paraffin wax of 58-60°C melting point (Lillie and Fullmer 1976). Thick sections (5-8 mm) were cut and stained in H & E. Van Gieson, Wilder's reticular, Verhoeffs, Periodic-acid schiffs and calcium, cobalt stain for normal histological structure, collagen, reticulin, elastin, PAS-positive material and alkaline phosphatase activity respectively. Tunica mucosa, tunica muscularis and total wall of the pre-and post-crop oesophagus were measured with the help of ocular micrometer which was standardized with stage micrometer. The data was analyzed statistically (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

Gross anatomy

The oesophagus extended from pharynx to the

Present address: [1]M.V.Sc. Student, [2]Professor and Head, [3,4]Associate Professor, Department of Anatomy, College of Veterinary Science and Animal Husbandry.

Table 1. Mean and S E of biometrical and micrometrical parameters of pre-crop oesophagus in pre-and post-hatch fowl

Group		Biometrical parameter			Micrometrical parameter (thickness in μm)			Percentage thickness of tunica mucosa of the total wall thickness	Percentage thickness of tunica muscularis of the total wall thickness
		Length (mm)	Width (mm)	CS area (mm^2)	Tunica mucosa	Tunica muscularis	Total wall		
1	Mean	–	–	–	–	–	–	–	–
	S E								
2	Mean	9.80	1.72	–	64.60	–	180.20	35.84	–
	S E	1.11	0.19		6.36		10.20		
3	Mean	10.80	4.00	–	195.50	198.50	400.00	48.87	49.62
	S E	1.80	0.16		28.24	10.44	35.25		
4	Mean	20.00	4.30	6.20	462.40	309.40	816.00	56.66	37.00
	S E	4.30	0.30	0.37	63.24	18.38	124.90		
5	Mean	21.20	5.10	7.00	482.80	370.60	997.00	48.42	37.17
	S E	5.10	0.64	0.54	60.24	22.42	133.35		
6	Mean	26.80	5.80	10.20	595.00	377.40	1275.00	46.66	29.60
	S E	5.80	0.37	1.15	142.48	19.60	139.66		
7	Mean	35.60	6.20	14.00	867.00	435.20	1309.00	66.23	33.24
	S E	1.29	0.47	1.41	127.21	37.08	124.34		
8	Mean	45.40	6.80	15.40	935.00	476.00	1462.00	63.95	32.55
	S E	1.29	0.37	0.80	126.07	20.82	198.50		
9	Mean	54.00	7.40	39.80	1054.00	572.40	1634.00	64.50	35.00
	S E	1.87	0.24	3.20	187.38	19.82	213.79		
10	Mean	65.00	14.00	56.80	1445.00	782.00	3482.00	55.21	31.50
	S E	1.58	0.63	1.80	144.74	31.32	148.20		
Overall Mean thickness (μ m)		–	–	–	677.92± 145.86	440.18± 62.66	1172.80± 229.12	–	–

proventriculus in right side of the neck.It has 2 parts-pre-crop and post-crop. The pre-crop part was longer than post-crop in all the age-groups. The length of the pre-and post-crop parts ranged from 9.80 to 65.00 mm and 7.70 to 50.00 mm, respectively, in groups 2-10 (Tables 1, 2). The greater length of pre-crop part of oesophagus in all the age groups is in accordance with the observations of McLelland (1975) who reported shorter thoracic oesophagus than that of cervical in chick.

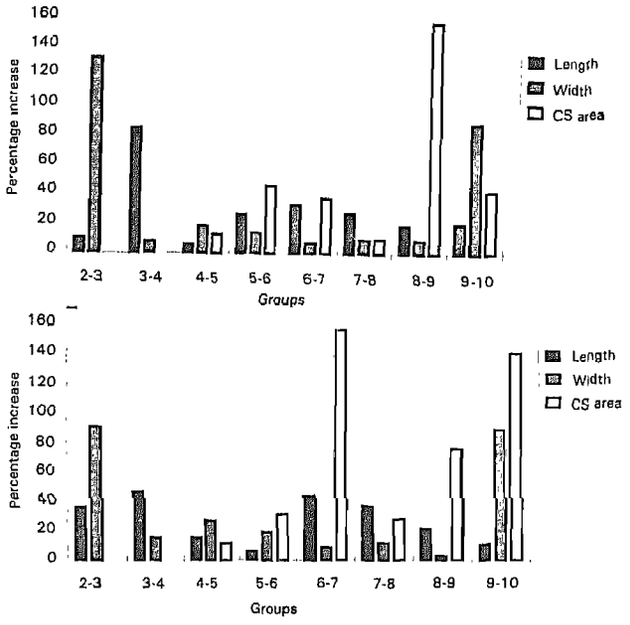
The width of the pre-crop oesophagus was more than that of post-crop and ranged from 1.72 to 14.00 mm and 1.68 to 13.20 mm, respectively, in different age groups. However, mean width of pre-and post-crop oesophagus was 13.60 mm in group 10. The difference in the width of the 2 parts appears on account of nature and amount of the food intake. The cross sectional area (Tables 1, 2) of pre-and post-crop oesophagus ranged from 6.20 to 56.80 mm^2 and 3.40 to 71.80 mm^2, respectively, in groups 4 – 10. However, mean CS area of pre-and post-crop parts of oesophagus was 64.30 mm^2 in group 10. Probably, the difference in the movements of ingesta from pharynx into pre-crop oesophagus and from crop into post-crop oesophagus may vary to affect CS area in the 2 parts of the oesophagus.

Growth spurt

The growth spurts of length, width and CS area of the oesophagus ranged in different age groups (Figs 1-2). However, growth spurt of the width of pre-and post-crop oesophagus was maximum (132.55 and 90.47%) in pre-hatch period (groups 2 – 3) and minimum (6.80 and 2.94%) in groups 6-7 and 8-9 respectively. The length of pre-and post-crop parts of oesophagus revealed maximum (85.18 and 46.66%) increase in groups 3-4. However, it was minimum (6.00%) in groups 4-5 and 5-6. The growth spurt of CS area of pre-and post-crop oesophagus revealed maximum values in groups 8-9 and 6-7 and minimum in groups 7-8 and 4-5 respectively (Figs 1, 2). However, time difference in the growth spurts of length, width and CS area probably is on account of difference in the growth spurt of wall and lumen of the oesophagus and nature and quantity of food intake.

Histology

The wall of oesophagus consisted of usual 4 tunics – tunica mucosa, tunica sub-mucosa, tunica muscularis and tunica serosa/adventitia as described by Stinson and Calhoun (1993). However, tunica submucosa was thin and not regularly conspicuous (Figs 5, 6) as also reported by Calhoun (1954). The tunica mucosa formed the vital core of the oesophagus and harboured the glandular tissue in the lamina propria (Figs 7, 8). The measurements on the thickness of the tunica mucosa, tunica muscularis and total wall of pre-and post-crop oesophagus have been given in Tables 1 and 2 respectively.



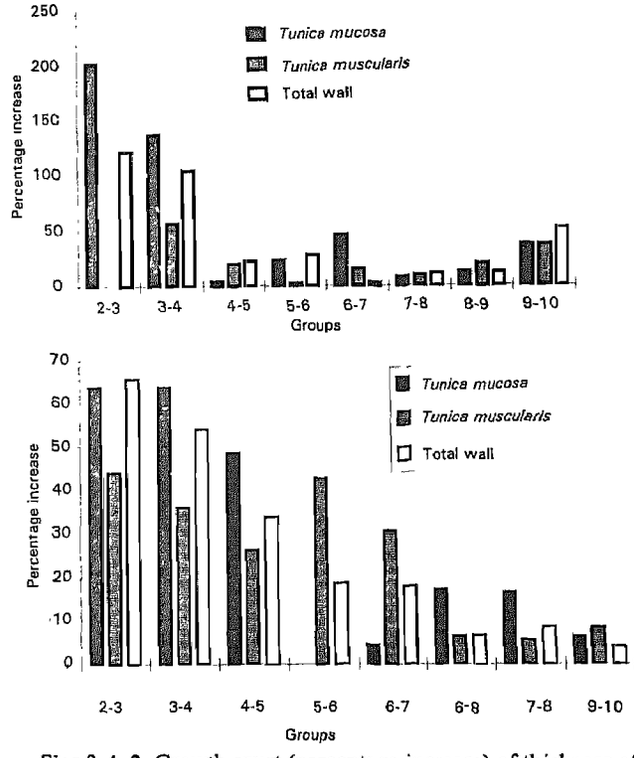
Figs 1-2. **1.** Growth spurt (percentage increase) of length, width and CS area of pre-crop oesophagus in pre-and post-hatch fowl. **2.** Growth spurt (percentage increase) of length, width and CS area of post-crop oesophagus in pre-and post-hatch fowl.

The overall mean thickness of the wall of post-crop oesophagus (1201.40 μm) was more than pre-crop part (1172.80 μm). (Tables 1, 2). The tunica mucosa contributed 35.84 – 66.23% in pre-crop and 56.25 – 68.75% in post-crop part, against tunica muscularis (29.60 – 49.62% and 28.40 – 39.53%) respectively. The tunica mucosa was lined by simple cuboidal, 3-layered epithelium-basal, middle and superficial, and stratified type of epithelium in group 2, 3 and 4 respectively. Van Alten and Fennel (1957) observed pseudostratified columnar ciliated epithelium on 11th day of incubation in contrast to stratified cuboidal reported by Ivey and Edgar (1952) at this stage of development. The villi that appeared in group 3, increased with the advancement of age. The glands in the lamina propria were absent in group 2. These appeared in group 3 and were well developed in groups 4 onward. The long and short diameters of the gland increased from 170.00 and 125.00 μm in group 4 to 561.00 and 395.00 μm in group 10 in pre-and post-crop oesophagus respectively. The appearance of tubuloalveolar glands in the lamina propria in the present study is in support of Hamilton (1952) who reported that glands of oesophagus develop on 16th day of incubation, by 18th day glands get branched and open in the lumen on 19th day. In group 2, the visceral wall consisted of epithelium and subepithelium connective tissue. The lamina muscularis mucosae and tunica muscularis were not conspicuous. The tunica muscularis was relatively thin and consisted of inner-circular and outer longitudinal smooth muscle fibres. The inner layer was about 3 times thicker than outer muscular layer. All the oesophageal layers thickened and developed with the advancement of age (Tables 1, 2).

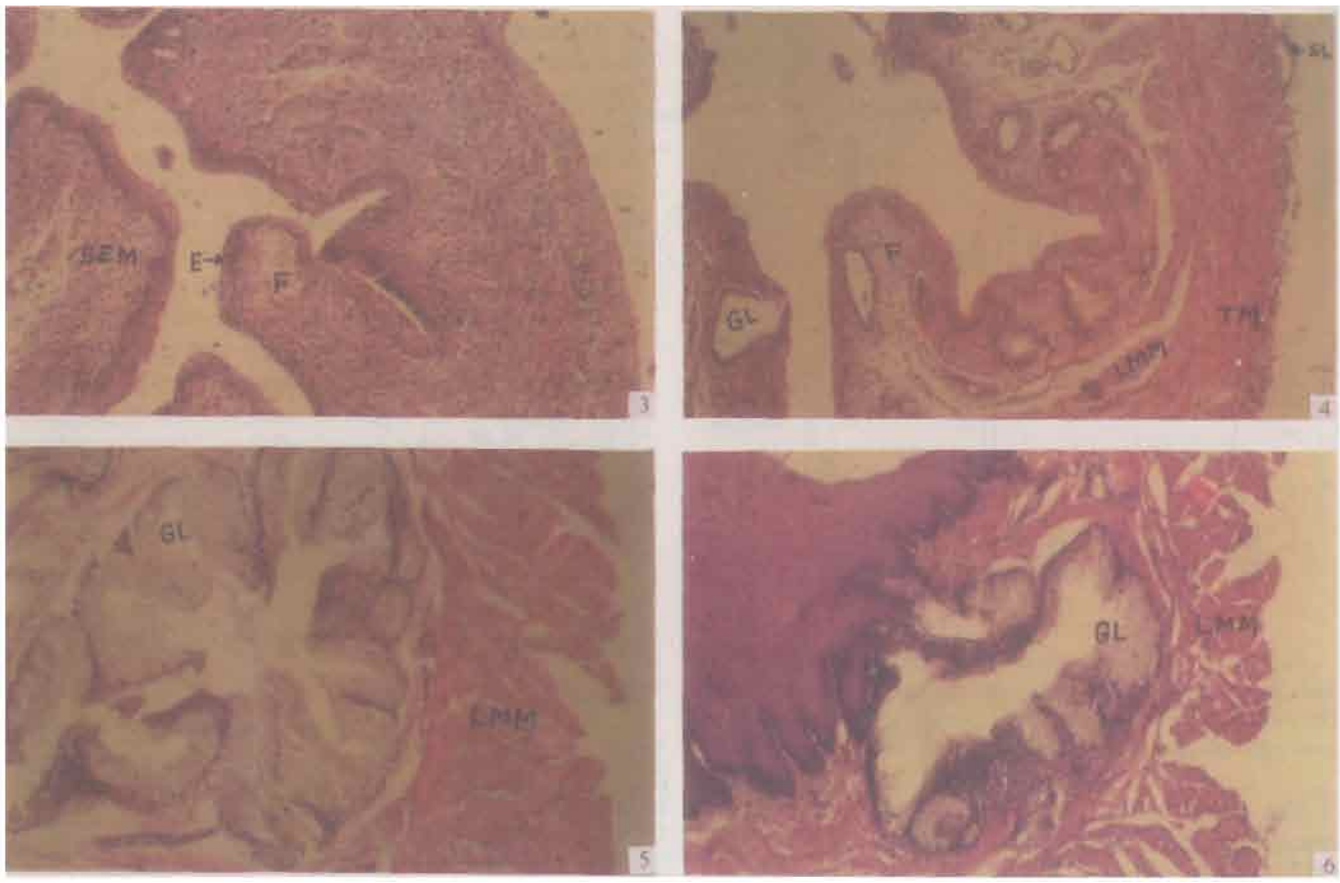
The histological structure of post-crop part of oesophagus was similar to of pre-crop. However, in groups 4 onward the superficial layers of epithelium in post-crop part were keratinized while non-keratinization has been observed in pre-crop part. However, Bradley and Grahame (1960), and Stinson and Calhoun (1993) reported throughout keratinized mucosa in contrast to Das and Biswal (1967) who observed no keratinization of stratified squamous epithelium in duck. The tubuloalveolar mucous glands in post-crop oesophagus were profuse in the mucosal folds close to the surface epithelium (Fig. 6) but in pre-crop part these were uniformly distributed adjoining the lamina muscularis mucosae (Fig. 5). This finding is at variance to the report of Batt (1925) who contended numerous glands in the upper oesophagus. However, Calhoun (1954), Bradley and Grahame (1960) and Banks (1981) reported similar structure of both the parts of oesophagus. The connective tissue in the lamina propria of the post-crop part was dense and cellular to the pre-crop part. The lamina muscularis mucosae in post-crop part was unevenly thin against the regular and thick layer in pre-crop part.

Growth spurt

The growth spurt of the thickness of tunica mucosa, tunica muscularis and total wall in pre-and post-crop oesophagus revealed variable values in different age groups (Figs 3, 4).



Figs 3-4. **3.** Growth spurt (percentage increase) of thickness of tunica mucosa, tunica muscularis and total wall of pre-crop oesophagus in pre-and post-hatch fowl. **4.** Growth spurt (percentage increase) of thickness of tunica mucosa, tunica muscularis and total wall of post-crop oesophagus in pre-and post-hatch fowl.



Figs 5-8 **5.** Photomicrograph of cross section of pre-crop oesophagus (group 3). H & E stain. ×100 showing epithelium (E), subepithelial connective tissue matrix (SE M) and mucosal folds (F). **6.** Post-crop oesophagus (group 3). H & E stain.×100 showing mucosal folds (F). glands in lamina propria (GL), lamina muscularis mucosae (LMM), tunica muscularis externa (TM) and serous layer (SL). **7.** Pre-crop oesophagus (group 10). H & E stain. × 100 showing tubuloalveolar glands (GL) in lamina propria and thick lamina muscularis mucosae (L M M). **8.** Post-crop oesophagus (group 10). H & E stain. ×100 showing tubuloalveolar glands (GL) in lamina propria and thin lamina muscularis mucosae (L M M).

However, thickness of all these layers showed maximum values of increase in groups 2-4 i.e. in pre-hatch and early post-hatch period. The minimum value for different layers and total wall followed variably in groups 4-10. Among all these 3, the tunica mucosa revealed maximum overall mean increase (58.63% and 27.67%) in pre-and post-crop oesophagus, the total wall and tunica muscularis were next in order.

The connective tissue fibres were less thin and showed mild colour intensity in early groups in comparison to late. The collagen fibres appeared more in comparison to elastic and reticular fibres. The abundant collagen in subepithelium and submucosa of oesophagus in the present study is in support of Trautmann and Fiebiger (1957) and Stinson and Calhoun (1993). The distribution of elastic fibres observed in this study and increase in their number and thickness from early to later groups in different locations is in support of Calhoun (1954) who reported the thickening and increase of elastic fibres in the subepithelium and submucosa of oesophagus. The high alkaline phosphatase activity in the glandular epithelial cells of oesophagus and mild to moderate PAS-positivity in the basement membrane and glandular epithelium in the chicks of different age groups is in support of Ushakumary (1996) and Van Altan and Fennel (1957) who reported similar distribution in different organs of digestive system in neonatal bovine calves and chick respectively.

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Table 2. Mean and S E of biometrical and micrometrical parameters of post-crop oesophagus in pre- and post-hatch fowl

Group		Biometrical parameter			Micrometrical parameter (thickness in (μm) total wall thickness			Percentage thickness of tunica mucosa of the total wall thickness	Percentage thickness of tunica muscularis of the
		Length (mm)	Width (mm)	CS area (mm^2)	Tunica mucosa	Tunica muscularis	Total wall		
1	Mean	–	–	–	–	–	–	–	–
	S E								
2	Mean	7.70	1.68	–	187.00	125.80	318.20	58.70	39.53
	S E	0.62	0.13		23.43	4.16	33.65		
3	Mean	10.50	3.20	–	306.00	180.20	527.00	58.06	34.19
	S E	0.92	0.49		71.52	19.82	90.75		
4	Mean	15.40	3.70	3.40	503.20	244.80	812.60	61.92	30.12
	S E	1.29	0.20	0.24	55.24	32.96	68.92		
5	Mean	17.80	4.70	3.80	748.00	309.40	1088.00	68.75	28.40
	S E	1.36	0.44	0.37	108.85	29.86	135.46		
6	Mean	18.80	5.60	5.00	748.00	442.00	1292.00	57.89	34.21
	S E	2.01	0.68	0.44	62.46	31.80	112.20		
7	Mean	27.00	6.10	12.80	782.00	578.00	1527.00	59.21	37.85
	S E	3.60	0.40	0.73	31.80	31.80	31.80		
8	Mean	37.00	6.80	16.40	918.00	616.00	1632.00	56.25	37.74
	S E	3.60	0.58	0.70	135.46	23.72	175.43		
9	Mean	45.00	7.00	28.80	10.71.00	650.00	1772.00	60.44	36.68
	S E	1.58	0.32	1.88	162.61	17.46	112.32		
10.	Mean	50.00	13.20	71.80	1139.00	703.80	1844.00	61.76	38.16
	S E	3.50	1.62	3.50	166.99	31.62	127.78		
Overall Mean thickness (μ m)		–	–	–	711.35± 108.34	427.77± 73.00	1201.40± 246.84	–	–

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Indian Journal of Animal Sciences 70 (2): 126-128; February 2000

Histological studies on the exocrine portion of pancreas of the domestic duck (*Anas boschas domesticus*)*

G MADHAVI[1], T S CHANDRASEKHARA RAO[2], D PRAMOD KUMAR[3], Y NAGAMALLESWARI[4] and M SREENU[5]

Acharya N G Ranga Agricultural University, Tirupati, Andhra Pradesh 517 502

Received: 8 February 1999; Accepted: 13 August 1999

Key words: Duck; Exocrine pancreas; Histology

ABSTRACT

Lobulation was not evident in pancreas of the duck. Three types of acinar cells i.e resting, active and exhausted were identified in a section of pancreas based on the nuclear and cytoplasmic appearance. The nucleus increased in size while the chromatin material diminished and the nucleolus became larger and more deeply stained from the resting state through active state to exhausted state. The zymogen granules were abundant in resting cells, moderate in active cells and least in exhausted cells. The acini emptied into intermediate tubules through intercalated ducts both of which were lined by simple squamous epithelium. The larger ducts were lined by simple cuboidal epithelium while the extraglandular ducts showed tunica mucosa, tunica muscularis and tunica adventitia. Their folded mucosa was lined by simple columnar epithelium.

Information on histology of the exocrine pancreas of the duck is limited. Hence the present study was undertaken.

MATERIALS AND METHODS

Adult ducks (15) irrespective of the sex were utilized for the present study. Tissue pieces from dorsal; ventral and splenic lobes of the pancreas and its ducts were collected after sacrificing the birds. The tissues were processed for paraffin sections which were subjected to routine H&E and also special histological staining methods like Van Gieson's method; Gomori's method; Weigert's method and Crossman's modification of Mallory's trichrome method (Singh and Sulochana 1997). Micrometry was done wherever necessary and the data were analyzed statistically (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

The pancreas of duck was made up of an exocrine and an endocrine portion. The bulk of it was formed by exocrine tissue consisted of serous compound tubuloalveolar acini arranged somewhat concentrically around the islets of Langerhans (Fig. 1) as in fowl (Calhoun 1954). The gland was enclosed in a layer of peritoneum underneath which was a thin fibrous capsule as reported by the Hodges (1974) in fowl and Malewitz and Calhoun (1957) in turkey. The latter showed collagen and reticular fibres and a few elastic fibres as in Japanese quail (Shivakumar 1997). The gland was compact in appearance and was not divided into lobules as there were no connective tissue septa (Figs 1, 2, 3). The absence of lobulation of the pancreas observed in the present study is contrary to the findings of Sturkie (1954), Mc Lelland (1979) and Nickel *et al.* (1977) in birds wherein they stated that lobulation was observed in avian pancreas. Kendall (1940) and Nomidez and Windle (1953) stated that the capsular elements continued into the gland to form the supporting fibroelastic and reticular tissue about the end pieces. But in contrast to their observations there was very little interacinar connective tissue composed of only reticular fibres around the acini in duck. The collagenous tissue was confined to the capsule, ducts, blood vessels and their vicinity. Lymphocytic granulocytes and undifferentiated cells were observed beneath the capsule in accordance with the findings of Fancist (1972). The undifferentiated cells may be stem cells for replacement of exocrine cells as suggested by Williams *et al.* (1989) in human beings.

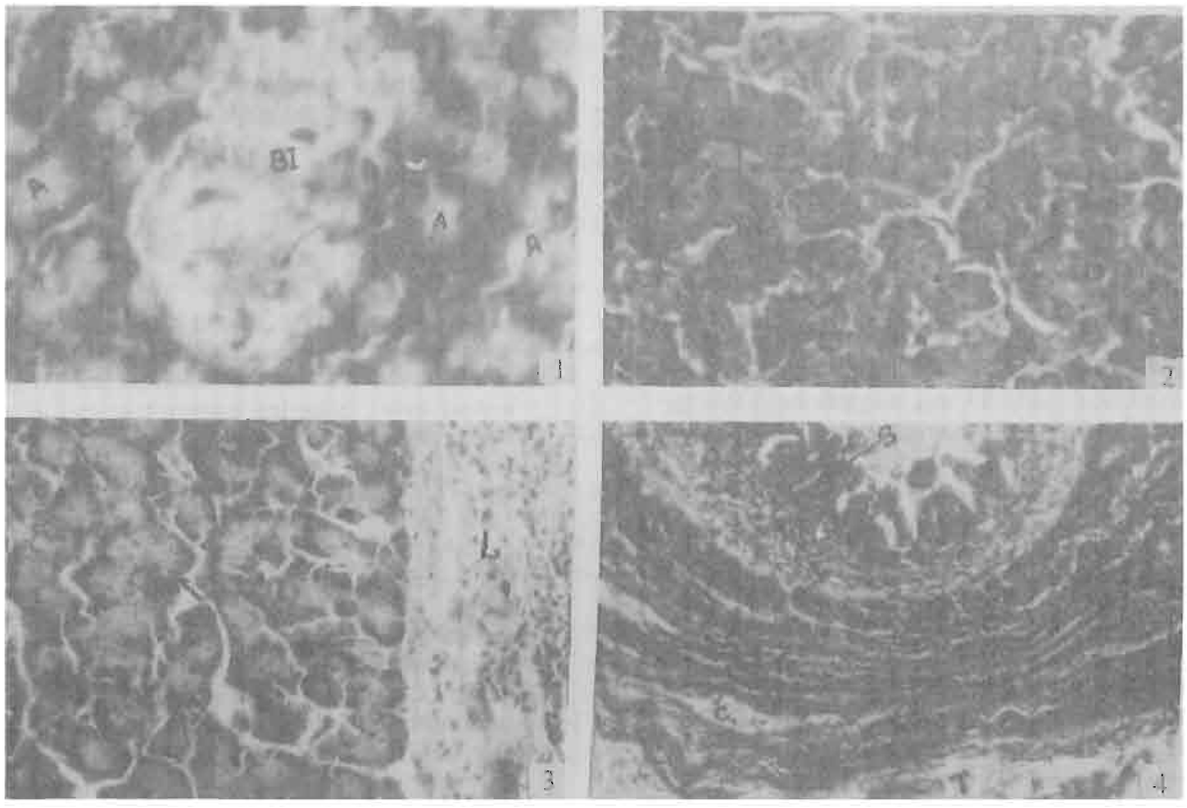
The acinar cells were pyramidal in shape being wider at

*Part of the M.V.Sc., Thesis submitted by first author to ANGRAU in 1998.

Present address: [1] Veterinary Assistant Surgeon, Racherla, Dhone Mandal, Kurnool District, Andhra Pradesh.

[2]Associate Professor and Head, Department of Anatomy; [5]Assistant Professor, Department of Surgery, College of Veterinary Science, Tirupati 517 502.

[3, 4]Assistant Professors, Department of Anatomy, College of Veterinary Science, Rajendranagar, Hyderabad 500 030.



Figs 1-4. 1. Photomicrograph of the pancreas showing exocrine and endocrine portions. BI, b islet; A, exocrine exhausted acinar cells with spherical euchromatic nucleus; least amount of zymogen granules and more intensely basophilic basal cytoplasm. H & E × 630 2. Photomicrograph of the pancreas showing resting acinar cells (arrows) with oval heterochromatic nucleus and abundant zymogen granules. H & E.× 1000. 3. Photomicrograph of the pancreas showing active acinar cells (arrows) with spherical to oval nucleus (chromatin intermediate in appearance) and moderate amount of zymogen granules L, lymphoid tissue in the vicinity of blood vessels and ducts. H & E. × 630. 4. Photomicrograph of the extraglandular pancreatic duct showing different layers.S, Simple columnar epithelium; LP, lamina propria; L, longitudinal layer of smooth muscle fibres; C, circular layer of smooth muscle fibres; T, tunica adventitia. H & E.× 630.

their base than near the lumen as reported in turkey (Malewitz and Calhoun 1957). The acinar lumen was narrow and the cell boundaries were indistinct. Often cleft like intercellular spaces were observed between acinar cells within the acini as reported earlier in duck (Das and Biswal 1967) and Japanese quail (Shivakumar 1997). A distinct basement membrane around acini could not be recognized contrary to the findings of Shivakumar (1997) in Japanese quail. Absence of basket cells around acini is in accordance with the findings of Fitzerald (1969) in Japanese quail. The nucleus of the acinar cells was located in the basal half of the cells as reported by Calhoun (1954) and Hodges (1974) in fowl; Malewitz and Calhoun (1957) in turkey and Shivakumar (1997) in Japanese quail. The cytoplasm in the basal one third of acinar cells was dense; finely granular and basophilic; while in apical two-thirds normally filled with coarse eosinophilic granules (Figs 1, 2, 3). These findings concur with the reports of Hodges (1974) in fowl; Das and Biswal (1967) in duck and Shivakumar (1997) in Japanese quail.

Three types of acinar cells i.e resting; active and exhausted were identified in a section of pancreas basing on the nuclear and cytoplasmic appearance. However; all the cells in a single acinus were in a similar state of activity. In resting cells the nucleus was oval and heterochromatic. The zymogen granules in the cytoplasm were abundant and filled a large proportion of the cells (Fig. 2). In active cells the nucleus was spherical to oval and the chromatin was intermediate in appearance. The zymogen granules were moderate in amount (Fig. 3). In exhausted cells the nucleus was spherical; largest and euchromatic. The nucleolus was more distinct. The zymogen granules were least in amount and the basal cytoplasm was more intensely basophilic (Fig. 1). These observations concur with the findings of Bloom and Fawcett (1968) in human beings, while Singh (1980) also reported similar types of acinar cells in fowl but basing on nuclear appearance only. The mean diameter (mm) of the nucleus in resting; active and exhausted cells was 2.7 ± 0.30, 3.0 ± 0.44 and 3.2 ± 0.42 respectively. Highly significant difference in mean nuclear diameter between resting and active cells and between resting and exhausted cells was reported by Singh (1980) in fowl. But in the present study significant and highly significant differences were observed in nuclear diameter between resting

Table 1. Showing difference in mean nuclear diameter (mm) between different types of acinar cells category

	Category	Mean diameter	t value
Resting vs active	2.7±0.30	3.0±0.44	1.98*
Resting vs exhausted	2.7±0.30	3.2±0.42	5.4**
Active vs exhausted	3.0±0.44	3.2±0.42	-1.6NS

*Significant difference, **highly significant difference, NS no significant difference.

and active cells and between resting and exhausted cells respectively. The difference in mean nuclear diameter between active and exhausted cells was insignificant (Table 1).

The centroacinar cells were observed occasionally in acinar lumen. Das and Biswal (1967) and Shivakumar (1997) observed the centroacinar cells in some of the acini in duck and Japanese quail respectively. Presence of centroacinar cells was also reported in fowl (Calhoun 1954), duck (Braun Blanquet 1969) and turkey (Malewitz and Calhoun 1957). The centro acinar cells were elongated and showed clear cytoplasm as reported by Braun Balnquet (1969) in duck.

The pancreatic acini emptied into small collecting ducts (intermediate tubules) through intercalated ducts. The intercalated ducts and intermediate tubules were lined by simple squamous epithelium. The slender intermediate tubules which were quite larger than intercalated ducts were seldom seen in sections as reported by Hodges (1974) in fowl. They showed secretory material in the lumen. Calhoun (1954) opined that exact pattern of duct system in pancreas of fowl was not understood. In the present study also due to absence of lobulation various orders of duct system like intralobular and interlobular ducts could not be distinguished. Striated ducts were not encountered as reported by Fitzerald (1969) in Japanese quail.

The larger intraglandular ducts were lined by simple cuboidal epithelium and showed secretory material in their lumen. The mean height of the epithelium was 2.9 ± 0.24 mm. The walls of the ducts initially composed of only a few fine collagen fibres gradually increased in thickness and complexity.

The extraglandular ducts possessed 3 layers i.e tunica mucosa; tunica muscularis and tunica adventitia (Fig. 4). The mucosa was lined by simple columnar epithelium. The thick tunica muscularis consisted of an outer circular and an inner-longitudinal layers of smooth muscle fibres (Fig. 4). The observations are in agreement with the findings of Hodges (1974) in fowl and McLelland (1979) in birds. Contrary to this Shivakumar (1997) described tunica muscularis as having an inner-circular and an outer-longitudinal layers in Japanese quail. He also stated that the goblet cells were observed in epithelium which, however, were not encountered in this study. The mucosa was strongly folded as villous like projections. Hence crypts giving glandular appearance were commonly observed. The mean height of the epithelium was 7.0 ± 3.8 mm. The lamina propria was formed of collagen, elastic and reticular fibres.

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Indian Journal of Animal Sciences **70** (2): 129-132, February 2000

Histological studies on the endocrine portion of pancreas of the domestic duck *(Anas boschas domesticus)**

G MADHAVI[1], T S CHANDRASEKHARA RAO[2], D PRAMOD KUMAR[3], Y NAGAMALLESWARI[4] and M SREENU[5]

Acharya N G Ranga Agricultural University, Tirupati, Andhra Pradesh 517 502

Received: 8 February 1999; Accepted: 13 August 1999

ABSTRACT

The islets of Langerhans in duck pancreas were more abundant in dorsal than in ventral lobe and were the main component of splenic lobe. Two types of islets, α (dark) and β (light) were observed. The islets were largest in splenic lobe, intermediate ones in ventral lobe and smallest in dorsal lobe. The a islets were larger than β islets. In addition to α, β and δ cells a fourth type of cell assumed to be endocrine in nature was observed only in a islets and exocrine tissue.

Key words: α islets, β islets, Duck, Endocrine pancreas

Information on histology of the endocrine portion of pancreas of the duck is limited. Hence the present study was undertaken.

MATERIALS AND METHODS

The adult local ducks (15) irrespective of the sex were utilized for the present study. Tissue pieces from dorsal, ventral and splenic lobes of the pancreas were collected after sacrificing birds and were processed for paraffin sections which were subjected to routine H&E and special histological staining methods like Van Gieson's method, Gomori's method, Weigert's method and Crossman's modification of Mallory's trichrome method (Singh and Sulochana 1997) and with different methods for pancreatic islet cells i.e Maldonado's method, Glenner Lillie method (Luna 1968), Gomori's chrome-alum haematoxylin method (Bancrofts and Stevens 1977), Aldehyde fuchsin method (Disbrey and Rack 1970) and Bielschowsky's silver impregnation method (Humason 1967). Micrometry was done wherever necessary and the data were subjected to statistical analysis (Snedecor and Cochran 1967).

*Part of the M.V.Sc., Thesis submitted by first author to ANGRAU in 1998.

Present address: [1] Veterinary Assistant Surgeon, Racherla, Dhone Mandal, Kurnool, Andhra Pradesh.

[2]Associate Professor and Head, Department of Anatomy; [5] Assistant Professor, Department of Surgery, College of Veterinary Science, Tirupati.

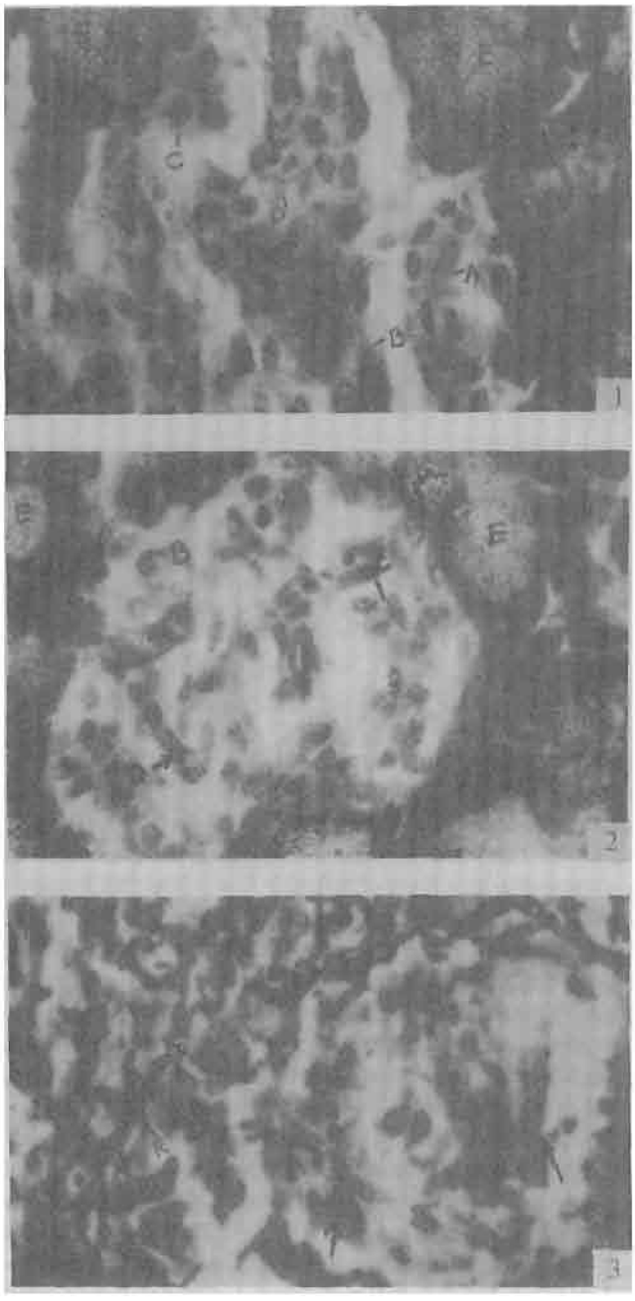
[3, 4]Assistant Professors, Department of Anatomy, College of Veterinary Science, Rajendranagar 500 030.

RESULTS AND DISCUSSION

The smaller endocrine portion of pancreas in duck was composed of islets of Langerhans surrounded by exocrine portion (Figs 1, 2). The islet cells were arranged in cords or strands separated by sinusoids (Figs 1, 2). Each islet was surrounded by a layer of reticular fibres (Fig. 3). Contrary to this Calhoun (1954) reported that islet tissue was not separated from the rest of pancreas by a connective tissue layer in fowl, whereas Das and Biswal (1967) reported that the islets were surrounded by collagen fibres in duck. The islets did not show any lobular appearance. Contrary to this Assenmacher (1980) reported that connective tissue divided the avian islets into lobules.

Based on type of cells these were divided into a and b islets consisting mostly of a and b cells, respectively, with few d cells in both the types of islets (Figs 1, 2). Similar observations were reported earlier in fowl (Calhoun 1954, Vijayaragavan and Seshadri 1976, Ladukar and Bhamburkar 1994), turkey (Malewitz and Calhoun 1957), duck (Hellerstrom 1963 and Braun Blanuqet 1969) and birds (Hazelwood 1973, Nickel *et al.* 1977, Guha and Ghosh 1978). Contrary to this mixed type of islets showing both a and b cells in addition to d cells were reported by Smith (1972), Mikami *et al.* (1985) and Shivakumar (1997) in Japanese quail and by Iwanaga *et al.* (1983) in chicken.

The diameter of islets ranged from 40 to 150 mm. The mean dia of the a and b islets (overall and lobe-wise) is shown in Table 1. The a islets were larger than b islets as reported by Hellerstrom (1963) in duck, Vijayaragavan and Seshadri (1976) and in fowl and Shivakumar (1997) in Japanese quail. There was a significant difference in mean diameter between



Figs 1-3. **1.** Photomicrograph of the pancreas showing a islet with a cell (A), elongated d cell (B), fourth type of cell (C) and sinusoid (D) and surrounding exocrine acini (E). H & E. ×1 000. **2.** Photomicrograph of the pancreas showing b islet with polyhedral b cell (B), elongated d cell (D) and sinusoid (C) and surrounding exocrine acini (E).H & E. ×1 000. **3.** Photomicrograph of the pancreas showing darkly stained a cells (arrows) and reticular fibres (R) surrounding islet.Bielschowsky's method. ×630.

α and β islets. Both the types of islets were largest in splenic lobe, intermediate ones in ventral lobe and smallest in dorsal lobe as reported by Guha and Ghosh (1978) in birds. There was a significant difference in mean diameter of a islets as well as b islets between splenic and dorsal lobes while the difference in mean diameter between splenic and ventral lobes as well as between ventral lobe and dorsal lobes was insignificant (Table 1).

The cellular proportion of a cells to d cells was 82 to 18% in a islets while the cellular proportion of b cells to d cells in b islets was 91 to 9%. The a islets appeared dark following application of silver stain (Bielschowksky's method) due to argyrophilia of a cells (Fig. 3) while b islets were light in appearance due to lack of affinity to silver by b cells. Hence the islets were termed dark and light islets respectively. These findings concur with earlier reports in fowl and duck (Hellman and Hellerstrom 1960) and birds (Guha and Ghosh 1978). The islets were more abundant in dorsal than ventral lobe and were the main component in splenic lobe as reported by Assenmacher (1980) in fowl. a islets were observed in all 3 lobes though they were predominantly seen in splenic lobe as reported by Hellerstrom (1963) and Braun Balnquet (1969) in duck, while in fowl the a islets were reported to be restricted to anterior portion of the gland (Lucas 1947, Vijayaragavan and Seshadri 1976) or region of fusion of dorsal and ventral lobes (Oakberg 1949) while they were reported to be limited to third lobe (a part of the ventral lobe) and splenic lobe in fowl by Mikami and Ono (1962) and in Japanese quail by Smith (1973) and Shivakumar (1997). The (islets varied in shape from round to elliptical to extensive and irregular groups of cells (Fig.1). The b islets were seen in all lobes of the pancreas as reported earlier in various species including duck (Oakberg 1949, Mikami and Ono 1962, Epple and Farner 1967, Braun Blanquet 1969., Smith 1973, Vijayaragavan and Seshadri 1976., Mikami *et al.* 1985, Watanabe *et al.* 1990, Takayangi *et al.* 1995 Shivakumar 1997). The b islets were somewhat round and well circumscribed (Fig.2).

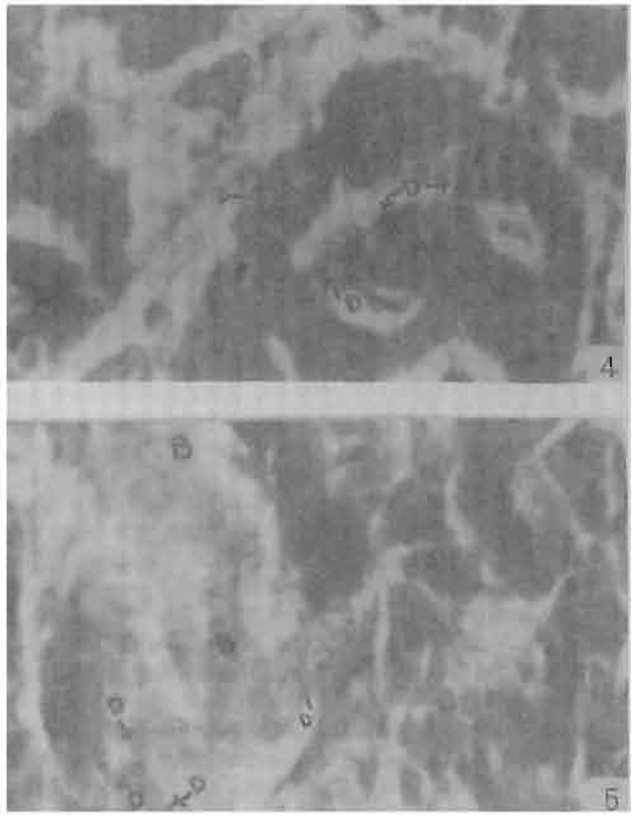
The α cells were columnar in shape (Fig. 1). The cells had an oval or round nucleus containing 1 or 2 nucleoli. The cytoplasmic granules stained red to pink following application of aldehyde fuchsin as reported by Assenmacher (1980) in birds. They also stained red to pink following application of Gomori's chrome-alum haematoxylin and Glenner Lillie methods (Fig.4) as reported by Bancrofts and Stevens (1977) and Luna (1968) respectively. The granules stained purple with Maldonado's method as suggested by Luna (1968). However, the granules were reported to be staining bright red with Mallory Heidenhain's Azan stain by Humason (1967) while in the present study the granules stained orange grey.

The β cells were polygonal and arranged in strands (Fig.2). The nucleus was round to ovoid with 1 or 2 nucleoli. These observations concur with the findings of Mikami and Ono (1962) in chicken. However, Assenmacher (1980) stated that the b cells were spherical to elliptical in birds. Free b cells were observed in exocrine tissue. These cells in Japanese quail were described as immature beta cells in the process of formation of the islets (Mikami *et al.* 1985, Shivakumar 1997). The cytoplasmic granules in b cells were described as staining

Table 1. Showing mean diameter of the islets (overall and lobe wise) and comparison of diameter of islets between three lobes

Overall mean diameter (μ m)			Mean diameter (μ m) Lobe wise		Comparison among splenic/dorsal/ventral lobes					
α islet	β islet	't' value		α islets	β islets	–	α islets	't' value	β islets	't' value
117.44±	58.11±	7.96*	Splenic Lobe	138± 4.2	67± 5.4	Splenic lobe vs Dorsal lobe	138± 4.2	100± 2.9	12.3	67± 5.4	44.6± 6.3	4.36*
			Dorsal Lobe	100± 2.9	44.6± 6.3	Splenic lobe vs Ventral lobe	138± 4.2	114.3±5.4	−5.68^{NS}	67± 5.4	62.66±1.85	−1.5^{NS}
			Ventral Lobe	114.33 ± 5.4	62.66± 1.85	Ventral lobe vs Dorsal lobe	114.33± 5.4	100± 2.9	−2.73^{NS}	62.66± 1.85	44.6± 6.3	−3.09^{NS}

*–Significant, NS– non significant.



Figs 4-5. 4. Photomicrograph of the pancreas showing a islet with a (A) and d (D) cells stained red to pink. Glenner Lilie method. ×630. 5. Photomicrograph of the pancreas showing b islands with b cells (B) stained purple and d cells (D) stained red to pink and surrounding exocrine acini (A). Glenner Lillie Method. × 630.

blue with chrome-alum haematoxylin (Bancrofts and Stevens 1977) and aldehyde fuchsin (Assenmacher 1980), violet blue with Maldonado's method (Luna, 1968) and purple (Fig.5) with Glenner Lillie method (Luna 1968). Similar observations were recorded in the present study. But the granules stained grey following application of Mallory Heidenhain's Azan method whereas Humason (1967) stated that the granules stained orange grey.

δ cells were less in number when compared to a and b cells as reported by Shivakumar (1997) in Japanese quail. They were elongated cells with darkly stained nuclei (Figs 1, 2), while Hellerstrom (1963) reported them as round or oval cells in duck. The cells were described to be elongated in fowl (Machino *et al.* 1966) while rod-shaped in Japanese quail (Smith 1974). They were freely dispersed in both a and b islets (Figs 1, 2). Contrary to this Smith (1974) stated that in Japanese quail these cells were present at the periphery of the b islets but were freely dispersed in a islets while Shivakumar (1997) reported that the cells were always found at periphery of any type of islets. The cytoplasmic granules of d cells, stained red to pink and violet blue following application of Gomori's chrome-alum haematoxylin and Maldonado's method, respectively, which concur with the findings of Bancrofts and Stevens (1977) and Luna (1968) respectively. The granules stained red to pink following application of aldehyde fuchsin while Assenmacher (1980) stated that they stained orange to grey in birds. The granules stained red to pink and grey following application of Glenner Lillie method (Fig.4) and Mallory Heidenhain's Azan method respectively.

Besides α, β and δ cells a fourth type of cell, assumed to be endocrine in nature was observed, in a islets along with a and d cells (Fig.1). They were also observed in exocrine tissue but in the form of spherical circumscribed areas. The cells were large and polygonal with eosinophilic cytoplasm and small cytoplasmic processes. The nucleus was spherical to oval. These findings concur with report of Shivakumar (1997) in Japanese quail. The cytoplasm of these cells did not show any affinity to special stains meant for islet cells unlike (, (and (cells. These cells might be homologous of pancreatic polypeptide immunoreactive cells described in fowl (Takayangi *et al.* 1995). These cells may be source of the third pancreatic hormone, avian pancreatic polypeptide, first identified by Kimmel *et al.* (1968) in chicken.

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Indian Journal of Animal Sciences **70** (2): 133-135, February 2000

Disposition kinetics of ampicillin in buffalo calves

M ALAM[1], N C BANERJEE[2] and K P YADAVA[3]

Birsa Agricultural University, Ranchi, Bihar 834 006

Received: 18 January 1999; Accepted: 13 August 1999

ABSTRACT

The pharmacokinetics of ampicillin after single oral dose (20 mg/kg b w) was studied in group of buffalo calves. The highest mean plasma concentration of drug (0.44 ± 0.08 µg/ml) was recorded at 4 hr which declined gradually to 0.18 ± 0.01 µg/ml at 24 hr of drug administration. The mean Cp ther ranged from 0.18 ± 0.01 to 0.44 ± 0.08 mg/ml between 30 min and 24 hr. The mean values of kinetic parameters for t½ ka, t½ β, Vd area, AUC, CLB, T/P ratio were 1.14 ± 0.12 hr, 15.71 ± 1.19 hr, 39.11 ± 1.64 L/kg, 11.53 ± 0.61 mg/ml hr, 29.31 ± 1.66 ml/kg/min and 0.70 ± 0.04 respectively. The mean values of K_{12}, K_{21}, Kel, K_{12} and K_{21} were 0.23 ± 0.03/hr, 0.37 ± 0.03/hr, 0.07 ± 0.007/hr and 0.61 ± 0.04 respectively. Dosage regimen based on the drug kinetics has been suggested for optimal clinical efficacy.

Key words: Ampicillin, Buffalo calves, Kinetics, Microbiological assay

Ampicillin, a broad-spectrum semisynthetic penicillin active against gram-(+) and gram-(-) bacteria has gained wide spread use in livestock. Limited data are available on the disposition kinetics of ampicillin especially in buffalo calves (Singh *et al.* 1984, Alam *et al.* 1997). The present investigation was envisaged to work out the various biokinetic parameters and also evolve appropriate dosage regimen of ampicillin trihydrate in buffalo calves.

MATERIALS AND METHODS

Animal and treatment

Investigations were conducted on a group of 7 clinically healthy male buffalo calves weighing 138-160 kg and approximately of 1½ years of age. The animals were maintained on standard ration and partial grazing. Drinking water was supplied to the animals *ad lib*. Ampicillin trihydrate was administered orally @ 20 mg/kg b w to each male buffalo calves.

Experimental and assay procedure

Blood samples were collected from jugular vein of each of the buffalo calves in sterilized test tubes containing sodium oxalate crystals as anticoagulant at stipulated intervals of 0.50, 1, 2, 4, 6, 8, 12, 24 and 48 hr following single dose oral administration. The blood samples collected were kept in refrigerator at 4°C and subjected to drug analysis within 24 hr of collection as per microbiological assay method (Ziv *et al.* 1973, and Cruickshank *et al.* 1975), using *Sarcina lutea* (β-55-1) as test organism obtained from Central Research Institute, Kasauli.

Table 1. Plasma concentration (µg/ml) of ampicillin trihydrate in buffalo calves (n=7) after single dose oral administration @ 20 mg/kg b w

Buff.	Time (hr)								
Calf No.	0.50	1	2	4	6	8	12	24	48
1	0.21	0.26	0.35	0.45	0.40	0.34	0.28	0.16	-
2	0.19	0.25	0.33	0.46	0.47	0.37	0.31	0.14	-
3	0.18	0.23	0.32	0.43	0.40	0.37	0.28	0.20	-
4	0.23	0.24	0.38	0.42	0.38	0.34	0.22	0.16	-
5	0.17	0.23	0.29	0.43	0.38	0.36	0.31	0.19	-
6	0.24	0.31	0.39	0.43	0.39	0.37	0.32	0.21	-
7	0.29	0.38	0.42	0.48	0.44	0.41	0.30	0.21	-
Mean	0.21	0.27	0.35	0.44	0.40	0.36	0.28	0.18	-
± S E	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	-

Pharmacokinetic procedure

Mean plasma ampicillin level-time profile for each animal was determined and kinetic values and dosage regimen was calculated (Ritschel 1976, Baggot 1977, Mercer 1978 and Notari 1980).

RESULTS AND DISCUSSION

Mean plasma drug concentration rose gradually to its peak

Present address: [1]University Professor, [2]Former University Professor and Head, Department of Pharmacology and Toxicology. [3]Dean, College of Veterinary Science and Animal Husbandry, Kanke, Ranchi 834 006.

Table 2. Kinetic parameters of ampicillin trihydrate in buffalo calves (n=7) after single dose oral administration @ 20 mg/kg b w

Parameters	Buffalo calf number								
	1	2	3	4	5	6	7	Mean	± S E
A mg/ml	0.43	0.69	0.42	0.47	0.37	0.38	0.32	0.44	0.04
B mg/ml	0.54	0.69	0.50	0.50	0.50	0.49	0.56	0.54	0.02
C°_{p} mg/ml	0.97	1.38	0.92	0.97	0.87	0.87	0.88	0.98	0.06
Ka/hr	0.56	0.55	0.56	0.92	0.40	0.93	0.61	0.65	0.07
β/hr	0.05	0.06	0.03	0.04	0.04	0.03	0.04	0.04	0.004
t½ Ka hr	1.23	1.24	1.23	0.74	1.72	0.74	1.13	1.14	0.12
t½ β hr	13.50	10.34	18.14	14.43	17.11	19.68	16.77	15.71	1.19
Vd(B) L/kg	36.47	28.98	40.00	40.00	40.00	40.81	35.71	37.42	1.59
Vd(C) L/kg	20.35	14.49	21.73	20.61	22.98	22.98	22.72	20.84	1.13
Vd area L/kg	34.01	32.92	42.41	42.05	43.22	42.05	37.14	39.11	1.64
AUC mg/ml hr	11.46	9.05	12.34	9.90	11.42	13.51	13.03	11.53	0.61
CLB ml/kg/min	29.08	36.76	27.00	33.64	28.50	24.67	25.56	29.31	1.66
T/P	0.67	0.78	0.74	0.87	0.63	0.73	0.51	0.70	0.04
fc	0.59	0.56	0.57	0.53	0.61	0.57	0.66	0.58	0.01
F	10.02	8.48	8.21	8.67	6.58	6.29	8.33	8.08	0.48
f	0.45	0.34	0.44	0.41	0.42	0.43	0.48	0.42	0.01

level at 4 hr thereafter, plasma drug level declined gradually at 24 hr post-drug administration (Table 1).However, no drug residual levels were detected in plasma samples collected at 48 hr of drug administration. The slower rate of absorption of the drug after enteral administration may be correlated with the fact that at buffalo rumen pH of 5.5, ampicillin with pKa of 2.7 will be ionized to the extent of more than 90%. Since, MIC for susceptible organisms ranges from 0.03 to 0.20 mg/ml (Bryant 1972, Hjerpe and Routen 1976), it would be obvious that the therapeutic level of the drug persisted in the plasma of buffalo calves till 12 hr suggesting comfortably a long interval for repetition of the drug in generalized and systemic infections caused by susceptible microorganisms, viz. *S. pyogenes, S. pneumoniae, S. aureus* and *H. influenzae*.

The mean values of t½ Ka, t½ b, Vdarea, ClB and F are given in Table 2. The kinetic parameters determined after oral administration showed larger Vd area. This indicated extensive drug dispersion in the tissue compartment and body water spaces. This would mean that the drug will be well dispersed into the extensive muscular and skeletal network of the body. The ClB value was on higher side suggesting its effective use in renal infections. Furthermore, the bioavailability (F) was also high which reflected that the drug penetrated well throughout the body tissues and organs.

Table 3. Microrate constants of ampicillin trihydrate in buffalo calves (n=7) after single dose oral administration @ 20 mg/kg b w

Parameters	Buffalo calf number								
	1	2	3	4	5	6	7	Mean	±S E
K_{12}/hr	0.19	0.19	0.21	0.38	0.13	0.36	0.18	0.23	0.03
K_{21}/hr	0.33	0.31	0.32	0.49	0.24	0.53	0.40	0.37	0.03
Kel/hr	0.08	0.11	0.06	0.08	0.06	0.06	0.06	0.07	0.007
t½ Kel hr	8.08	5.79	10.42	7.72	10.48	11.36	11.08	9.28	0.79
K_{12}/K_{21}	0.56	0.61	0.65	0.78	0.52	0.68	0.46	0.61	0.04

The values of micro-rate constant are presented in Table 3. The sum of K_{12} and K_{21} was higher in comparison to K_{2} which indicated that ampicillin trihydrate distributed rapidly in spite of concurrent elimination taking place. The T/P ratio was nearly equal to K_{12}/K_{21} ratio suggesting equal drug distribution between central and peripheral compartments.

The dosage regimen of ampicillin calculated on the basis of the kinetics of drug availability in buffalo calves for effective and safe therapy, taking into consideration of its MIC as 0.1 to 0.2 µg/ml was 20 mg/kg b w orally with a repeat at an interval of 12 hr.

ACKNOWLEDGEMENT

We thank the Dean, Facutly of Veterinary Science and Animal Husbandry, Birsa Agricultural University, Ranchi, for the facilities to conduct this work.

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Indian Journal of Animal Sciences **70** (2): 136-138, February 2000

Autogenous tensor fascia lata and dermal graft for gap repair of superficial digital flexor tendon in bovines: Clinical, radiological and histopathological study

O P GUPTA[1], NAVEEN KUMAR[2], A K SHARMA[3], G R SINGH[4] and N S PARIHAR[5]

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122

Received: 11 February 1999; Accepted: 13 August 1999

ABSTRACT

Male cow calves (8), 6 to 8 months old, were randomly divided into 2 equal groups 1 and 2. A 2.5 cm long defect was created in superficial digital flexor (SDF) tendon. Autogenous tensor fascia lata and dermal graft were used to fill the defect in groups 1 and 2 respectively. Clinical, radiological and histopathological observations revealed that both these autografts can be successfully employed in repair of SDF tendon.

Key Words: Autogenous dermal graft, Autogenous tensor fascia lata, Cow calves, Fasciagraphy, Superficial digital flexor (SDF), Tendon

Various biological and synthetic materials have been used as tendon transplant, but the transplantation of the relevant tissue obtained from the patient himself accords maximum success. Therefore, the present study was undertaken to evaluate the autogenous tensor fascia lata and dermal graft for gap repair in superficial digital flexor tendon in bovines.

MATERIALS AND METHODS

Male cow calves (8) between the age of 6 to 8 months old were randomly divided into 2 equal groups1 and 2. The animals were kept off feed for 12 hr prior to surgery and were tranquilized with triflupromazine hydrochloride @ 1 mg/kg body weight intravenously. A 2 cm. defect was created in SDF tendon under local infiltration analgesia and the defect was filled with autogenous tensor fascia lata in group 1 and autogenous dermal graft in group 2. The tensor fascia lata was obtained from the craniolateral aspect of femur and dermal graft from the lateral aspect of neck. Both the grafts were sutured with Bunnell-Mayer suturing technique using silk 1/0. Skin wound was closed in the routine manner. The limb was immobilized with plaster of Paris bandage. Oxytetracycline @ 5 mg/kg body weight was administered for 7 days intramuscularly. Plaster cast and skin sutures were removed post-operatively on 15th day.

Rectal temperature, heart and respiration rate were recorded for 7 days post grafting in all the animals. Gliding movements of the tendon were recorded on days 15, 30, 45 and 60 post grafting. Fasciagraphy was done on days 30 and 60 post grafting to evaluate the status of healing at the site of reconstruction. The tendon biopsy specimen for histopathological examination were taken on days 30 and 60 post grafting and were preserved in 10% formal saline solution. The tissues were processed by routine paraffin embedding technique and were stained with hematoxylin and eosin stain. The data were subjected to statistical analysis using two-way analysis of variance (Snedecor and Cochran 1984).

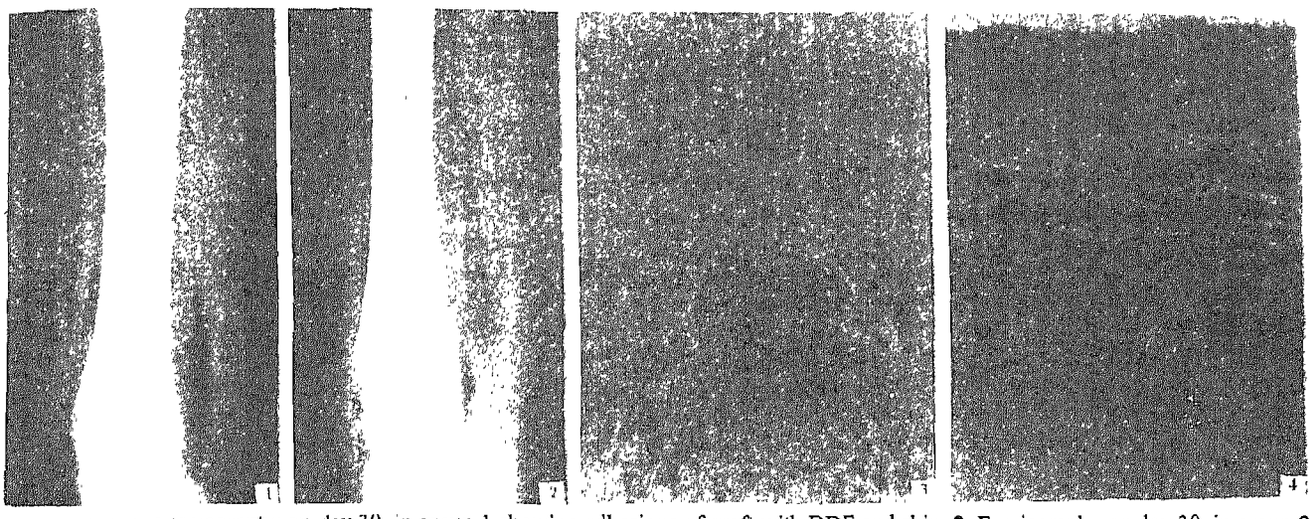
RESULTS AND DISCUSSION

Clinical

No significant difference in rectal temperature, heart and respiration rate was observed up to day 7. Animals of both the groups exhibited varying degree of lameness for the first 7 days. During this period, the animals showed minimal weight bearing and used to rest on toe. Slight limping for the first few days in buffalo calves has also been reported following autogenous tensor fascia lata grafting (Verma *et al.* 1983, Sharma 1988, Singh and Bhatia 1991). Lameness decreased gradually after 7 days and the animals showed normal gait and optimum weight bearing by day 15 post grafting. Early weight bearing following tendon autografting was also observed by Sharma (1988), Singh and Bhatia (1991), Verma *et al.* (1983 a, b) and Malik *et al.* (1998) in buffalo calves and Micheletto (1971) and Valdes *et al.* (1996) in equines.

Pain was not evident in any animal after removing plaster cast and skin sutures on day 15. However, hard non-painful swelling was observed at the site of grafting. The skin wounds

Present address: [1, 3]*Senior Scientist,* [2]*Scientist (Senior Scale),* [4]*Head, Division of Surgery;* [5]*Former Head, Division of Pathology.*



Figs 1-4. 1. Fasciagraphy on day 30, in group 1 showing adhesions of graft with DDF and skin. 2. Fasciagraphy on day 30, in group 2 showing dense homogenous swelling of the flexors and adhesions with the surrounding structures. 3. Microscopic examination on day 30, in group 1 showing less collagen formation and infiltration of neutrophils and mononuclear cells at the suturing site. H & E.×150.
4. Microscopic examination on day 60, in group 2 showing intermingling of dermal tissue with tendinous tissue and good amount of collagen fibres formation at the reconstructed site. H & E.×150.

were found completely healed. No gliding movements between flexor tendons were observed on days 15 and 30. However, on day 45 partial gliding and on day 60 full gliding movement were observed in both the groups. Improvement in gliding movements suggested decreased adhesions of tendon with the surrounding structures at the site of anastomosis, which confirms the findings by fasciagraphy on the other hand. Verma *et al.* (1983 a, b) reported full gliding movement of flexor tendon on day 42, following tendon autografting in buffalo calves.

Radiological

Fasciagraphy on day 30 in animals of both the groups revealed adhesions of transplanted graft with deep digital flexor (DDF) tendon and skin (Figs 1, 2). Both SDF and DDF tendon appeared as homogenous thickened mass. The site of reconstruction was more thickened and there was restoration of continuity of SDF tendon. On day 60, the swelling at the reconstructed site appeared less. However, mild to moderate adhesions were noted at the reconstructed site. Fasciagraphy provided useful information regarding the type and severity of adhesions formation and status of healing. Marked thickening and swelling at the reconstructed site indicates more fibroblastic activity (Peacock Jr 1964).

Histopathological

Histopathological examination on day 30 in group 1 revealed fibrovascular tissue reaction at the tendon graft junction. The connective tissue of the tendon sheath was more cellular with poor collagen formation. There was infiltration of neutrophills and mononuclear cells (Fig. 3). On day 60, complete union was observed between the fascial and tendon ends. The connective tissue was organized and collagen fibres were aligned in longitudinal direction. Valdes *et al.* (1996) reported more rapid gain in mechanical strength and histological maturity of reconstructed tendon after transplantation of an autogenic long digital extensor tendon in the repair of digital flexor tendon in equine. Fresh autogenic fascia lata grafts have been used successfully for gap repair of flexor tendon in equines, ovines and rabbits (Michelleto 1971) and buffalo calves (Verma *et al.* 1983b, Sharma 1988, Singh and Bhatia 1991). It has been claimed that fresh autogenic fascia activates the fibroblastic activity of surrounding tissues, thereby initiates early tenoblastic activity and finally the entire graft gets transformed into tendinous structure (Verma *et al.* 1983 b, Sharma 1988). In animals of group 2, intermingling of dermal tissue with tendinous tissue was seen on day 60 (Fig. 4). There was cellular reaction around the suture and comprised mainly by chronic inflammatory granulomatous reaction. Dermal tissue and tendinous tissue could not be identified with each other. There was gradual disappearance of the vascularity and the collagen fibres were aligned parallel to each other. Dermal tissue revealed mononuclear cell infiltration. Autologous dermal tissue as a suture material was satisfactory for the repair of Achilles tendon in dogs (De and Mukherjee 1988). Histological examination revealed early formation of fibro connective tissue in animals of group 2 as compared to group 1.

On the basis of clinical, radiological and histopathological observations, it is concluded that autogenic tensor fascia lata and dermal graft can be successfully employed in clinical situations were loss of a part of tendon occurs accidentally or maliciously.

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Indian Journal of Animal Sciences 70 (2): 139-141, February 2000

Yield and potency of follitropin extracted from pituitary glands of zebu cattle *(Bos indicus)* and water buffalo *(Bubalus bubalis)*

A MEHMOOD[1] and M ANWAR[2]

Animal Sciences Institute, NARC, P.O. NIH, Islamabad, Pakistan

Received: 4 December 1998; Accepted: 13 August 1999

ABSTRACT

Two methods were compared for follitropin extraction from the frozen-thawed pituitary glands of zebu cattle and water buffalo. The initial extraction was made by a gradual increase in the concentration of ammonium sulphate at different levels of pH, followed by precipitation of follitropin activity either by ethanol or by acetone. Follitropin yield (g/kg of tissue) differed nonsignificantly (cattle: 2.6 and 3.1; buffalo: 2.4 and 2.5) between species and between methods of extraction. Biological potency of the follitropin-extract also differed nonsignificantly and ranged at 88.2%-91.7% of standard FSH.P.

Key words: Follitropin extraction, Pituitaries, Water buffalo, Zebu cattle

Multiple ovulation and embryo transfer (MOET) technique is too expensive to be applied on a large-scale because of higher price of most commonly used gonadotropin, follicle stimulating hormone (FSH). Use of follitropin extracted from locally slaughtered animals may be helpful to overcome this impediment especially in developing countries. The present study was conducted to compare 2 methods of follitropin extraction (Ellis 1958, Cheng 1976) to assess follitropin yield and potency in water buffalo and zebu cattle. These methods use ammonium sulphate for initial extraction followed by precipitation of follitropin activity by ethanol or acetone.

MATERIALS AND METHODS

Pituitary glands of water buffalo and zebu cattle were obtained from a local abattoir, transported to the laboratory in ice-chilled sterilized saline and stored at –20°C until processed. After thawing, the anterior pituitaries were separated from the posterior parts, cleaned free from connective tissue, washed with 3 volumes of ice-cold 0.9% NaCl, and ground once in a meat grinder. Follitropin extraction was performed either by ammonium sulphate and ethanol (Ellis 1958) or ammonium sulphate and acetone (Cheng 1976). The extraction was accomplished at 4°-10°C using double distilled, deionized water. The low temperature was achieved by putting the extraction flask on an ice slab or by placing it in a refrigerator. The pH was adjusted with 1 N NaOH or 1 N HCl. Follitropin extraction was made without chromatographic separation to keep the method simple and less expensive.

AS-ethanol method

Ground tissue was suspended in 5 ml of 0.1 M ammonium sulphate/g of glands and pH of the suspension was adjusted at 7.4-7.5. After stirring for about 20 hr, the precipitate (ppt) was removed by centrifugation at 5 000 × g. The supernatant was adjusted to pH 5.5 and the ppt removed. The supernatant was readjusted to pH 7.4-7.5 and ammonium sulphate was added to a concentration of 2.2 M. After overnight flocculation the ppt was removed and concentration of the supernatant solution was increased to 3.0 M. The ppt obtained was resuspended in enough water to give 1.5 litre of solution per kg of tissue used. Ammonium sulphate was added to a concentration of 2.0 M, the pH was adjusted to 4 and the solution was allowed to stand overnight. The resulting ppt was dialyzed. Any ppt formed during dialysis was removed. The clear supernatant was adjusted to pH 5.5 and was lyophilized. It was termed fraction-I (F-I) of follitropin. A 1% solution of F-I at pH 5.5-6.5 was titrated with 0.1 M metaphosphoric acid to pH 3.5. The resulting ppt was removed and the clear supernatant solution was further titrated to pH 3. The small ppt was removed and the supernatant was adjusted to pH 4. Ethanol (95%) was added drop-wise to the supernatant to a concentration of 35%. The gummy ppt was dialyzed and lyophilized (follitropin, F-II).

Present address: [1, 2] Senior Scientific Officer, ASI, NARC, Islamabad, Pakistan.

Table 1. Recovery of follitropin (F-II) from frozen pituitaries of zebu cattle and water buffalo using ammonium sulphate and ethanol (AS-ethanol), or ammonium sulphate and acetone (AS-acetone)

Species	Extraction method	Follitropin yield (g/kg of tissue) mean ± SE
Cattle	AS-ethanol	2.6±0.60
	AS-acetone	3.1±0.7
Buffalo	AS-ethanol	2.4±0.2
	AS-acetone	2.5±0.3

Means do not differ (P>0.05).

AS-acetone method

Tissue was ground with ice cold distilled water (4ml/g) and the suspension was stirred at pH 6.0-6.5 for 3-4 hr. The supernatant (obtained after centrifugation at 12 000 × g for 20 min) was precipitated between 25% and 45% and between 45% and 70% saturation with ammonium sulphate. The ppt obtained at 70% saturation was redissolved, dialyzed against water and clear diffusate was freeze-dried (F-I). The freeze-dried powder (F-I) was dissolved in 2% (w/v) NaCl solution at a concentration of 10 mg/ml and pH of the solution was adjusted to 4.1. A reddish brown ppt was removed by centrifugation. The pH of the clear supernatant was immediately re-adjusted at 6.5-7.0. To the supernatant, 1 volume of cold acetone was added to precipitate a substantial amount of inactive proteins, which was removed by centrifugation. Another 2 volume of cold acetone was added to the remaining supernatant to make a final concentration of 75% acetone to precipitate the follitropin activity. After centrifugation, the ppt was redissolved, dialyzed against water and freeze-dried (follitropin, F-II).

Bioassay

The follitropin activity was determined by increase in ovarian weight of immature (3-4 weeks old) albino mice (Steelman and Pohley 1953). Follicle stimulating hormone from porcine pituitary (FSH-P) was used as standard. Preliminary data showed that when 10 to 20 IU of hCG/animal was given, 0.1-0.3 mg of FSH-P caused a significant increase in ovarian weight over that of the hCG control. Three different doses i.e. 0.1 mg, 0.2 mg and 0.3 mg of follitropin were tested each given on 3 days. Twenty IU of hCG/day was added to increase the action of follitropin in the ovary. Each dose group comprised 3 mice and was replicated 3 times. Both ingredients (follitropin and hCG) were mixed together in physiological saline and were given as a single subcutaneous injection (0.5 ml). Autopsy was performed 72 hr after the injection. The ovaries were removed, dissected free of surrounding tissue and weighed to the nearest 1/10 of a mg. Potency of unknown was calculated by determining the slopes of dose-response curves of each and obtaining a ratio from which the true potency could be ascertained. Calculations were made as per Steelman and Pohley (1953). Means (±SE) were calculated for 3 replicates. Data were analyzed with ANOVA and comparison made with LSD (Steel and Torrie 1980).

Table 2.Biological potency of follitropin extracted from frozen pituitary glands of zebu cattle and water buffalo using ammonium sulphate and ethanol (AS-ethanol), or ammonium sulphate afid acetone (AS-acetone)

Species	Extraction method	Potency (%)* mean ± SE
Fraction-I		
Cattle	AS-ethanol	86.0 ± 4.4a
	AS-acetone	56.0 ± 3.2b
Buffalo	AS-ethanol	83.1 ± 3.6a
	AS-acetone	57.1 ± 9.5b
Fraction-II		
Cattle	AS-ethanol	91.7±1.7
	AS-acetone	88.2±6.3
Buffalo	AS-Ethanol	91.4±1.8
	AS-acetone	90.3±13.8

*Compared to standard FSH-P containing 1 unit = 1 mg FSH activity.

a, b Means with different superscript within same fraction differ significantly (P<0.05).

RESULTS AND DISCUSSION

It took 2 days to prepare follitropin (F-II) by AS-acetone method and 5 days by AS-ethanol method. Data on yield (g/kg) of follitropin (F-II) are presented in Table 1. There was no effect of method of extraction or species on the follitropin yield (g/kg) indicating that the concentration of follitropin protein in the pituitaries of zebu cattle and water buffalo was not different from each other. A higher protein yield was obtained from cattle and buffalo pituitaries in this study as compared to the yield of bovine follitropin reported by Cheng (1976). It might be because of a different content of inactive proteins in the extract. However, the protein yield in the present study was comparable to that reported by Agarwal *et al.* (1993) from buffalo pituitaries.

The biological potency of fraction-I extracted by AS-ethanol method was significantly higher than that extracted by AS-acetone method (P<0.05) both in cattle and buffalo (Table 2). The potency of follitropin (fraction-II) did not differ (P>0 05) with respect to method of extraction or source of gland (Table 2). There was a 58% improvement in the follitropin potency from F-I to F-II both in zebu cattle and water buffalo by AS-acetone method in this study. However, the increase in potency from F-I to F-II was small in AS-ethanol method. This indicates a substantial amount of inactive protein in F-I extracted by AS-acetone method. Cheng (1976) was able to improve the potency of follitropin by 5-times from F-I to F-II using AS-acetone method. The AS-acetone method was faster than AS-ethanol method in terms of time spent on extraction of F-II. As the potency of F-II differed nonsignificantly between the 2 methods applied, AS-acetone method is recommended to save time.

Biological potency of the follitropin (F-II) extracted in this study was about 90% of FSH-P used as standard. Multiple ovulation was induced in water buffalo with FSH-P (Rahil *et al.* 1989), so there seems a scope to use follitropin extracted from zebu cattle and water buffalo pituitaries for superovulation in these species. The efficacy of this homologous follitropin to induce multiple ovulation in zebu cattle and water buffalo remains to be ascertained.

ACKNOWLEDGEMENTS

This work was supported by Grant No. ARF.11(ASD/89) of Pakistan Agricultural Research Council, Islamabad. Special thanks are extended to Dr R H Usmani for critical review of the article.

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Indian Journal of Animal Sciences 70 (2): 142-145, February 2000

Subacute exposure to inorganic mercuric chloride ($HgCl_2$) induce testicular toxicity in rats

N PRAKASH[1], V RAMKRISHNA[2] and ASHOK PAWAR[3]

University of Agricultural Sciences, Dharwad, Bidar, Karnataka 585 401

Received: 15 January 1999: Accepted: 4 August 1999

ABSTRACT

Investigation was carried out to determine the nature of testicular toxicity following subacute exposure to inorganic mercuric chloride ($HgCl_2$) in rats. Adult male Albino rats were divided into 3 groups each consisting of 8 animals. Group 1 served as untreated control, group 2 were exposed to inorganic $HgCl_2$ 5 PPM through drinking water for 13 weeks. Group 3 animals served as recovery group following treatment similar to group 2. Body weight and haematocrit values were monitored at regular intervals. On sacrification, testis were removed and weighed and subjected to histopathological examination along with liver and kidney tissue samples.

Testicular weight of rats exposed to inorganic $HgCl_2$ (groups 2 and 3) significantly ($P<0.05$) differed over control. The peritubular haemorrhage in the medullary region was evident in sections from kidney. Haematocrit values in treated rats were not different from untreated control group except for marginal decline in red blood cell count in group 2 and 3. The terminal testicular weights of $HgCl_2$—treated rats were significantly ($P<0.05$) different from control. The histopathological changes within the testis were mainly confined to Sertoli cells in group 2. The histotoxicological changes include discontinuation in basal lamina, vacuolation at multiple sites and disruption of spermatogenic cycle. The loss of architecture of testis was restored following a rest period. To conclude, subacute exposure to inorganic $HgCl_2$ (5 PPM) induce reversible testicular toxicity in rats.

Key words: Mercuric chloride, Subacute, Exposure, Toxicity.

Exposure to mercurial compounds, both organic and inorganic cause variety of tissue alterations and toxicity (Holt and Webb 1986, Urbach *et al.* 1992). Several metal cation including Hg^{2+} impair testicular homeostasis both *in-vivo* (Chowdhary and Arora, 1982) and *in-vitro* (Laskey and Phelps 1991). However, these studies are limited to either exposure to large dose or short-term acute exposure under culture systems, and histotoxicological alterations due to subacute exposure of $HgCl_2$ are not known. Hence present study was undertaken to determine the nature of testicular toxicity following subacute exposure of inorganic $HgCl_2$ and to know the recovery response in rats.

MATERIALS AND METHODS

Sexually matured Albino male rats, obtained from the veterinary college laboratory animal unit, were housed in laboratory and maintained at 25 ± 2°C with a light: dark cycle of 12hr each. All animals received normal rodent chow (NIN, Hyderabad) and water *ad lib.* The rats weighing 130 ± 5g were divided into 3 groups (n = 8 each) for control and experiments. Control animals (group 1) received distilled water. Groups 2 and 3 received inorganic mercuric chloride ($HgCl_2$, BDH) dissolved in distilled water at a concentration of 5 PPM and substituted for drinking water over a period of 13 weeks. Group 3 animals were further permitted a 30 days

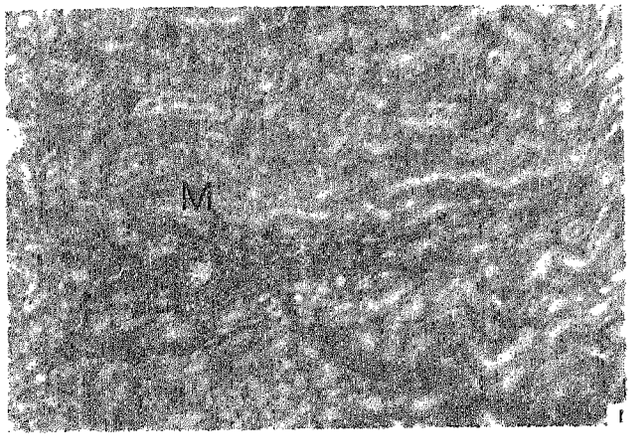
Table 1. Terminal body weight and testicular weight of control and experimental groups exposed to inorganic $HgCl_2$ (5 PPM) for 13 weeks

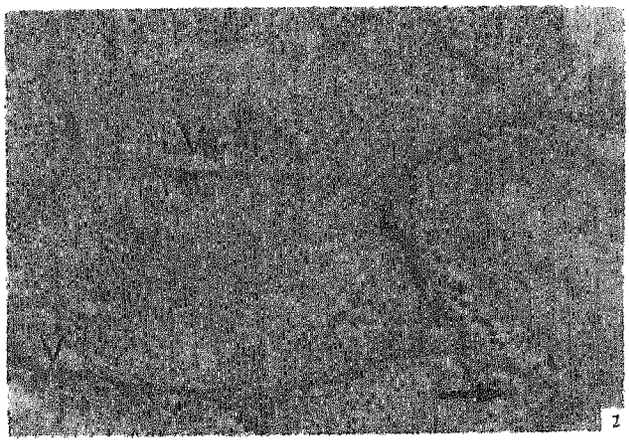
Group	Body weight (g)	Testicular weight (g)	Relative testicular weight (% body wt.)
Group 1 (control)	235±5.00	1.63±0.10	0.69
Group 2 ($HgCl_2$Tt.)	185±4.50*	1.02±0.20*	0.55*
Group 3 ($HgCl_2$Tt.+) 30 days rest)	190±4.50*	1.12±0.10*	0.58*

n=8 per group; data are expressed as mean±SD; *mean testicular weight of groups 2 and 3 were significantly different from untreated control ($P<0.05$).

Present address: [1]Assistance Professor and Head, Department of Pharmacology and Toxicology, Veterinary College, Bidar 585 401.

[2]Professor and Head. [3]Assistan Professor, Department of Anatomy and Histology, Veterinary College, Bidar.





Figs 1-3. 1. Photomicrograph of the kidney section from rat treated with inorganic $HgCl_2$ for 13 weeks showing peritubular haemorrhage in the medullary region (M). Masson's trichrome × 25. 2. Photomicrograph of the testis of rats treated with inorganic $HgCl_2$ for 13 weeks showing vacuolation, disruption of spermatogenic cycle and discontinuity of peritubular basal lamina (arrow). H & E × 25. V = Vacuolation, L = Leydig cell. 3. Photomicrograph of the rat testis following the recovery from inorganic $HgCl_2$ induced damage. Note down the normal radiating pattern of spermatogenic cycle. H & E × 100.

recovery period during which they received only distilled water and feed *ad lib.* The subacute dose used in this study was based on observation of Ramakrishna *et al.* (1996). However, body weight and blood samples were taken at regular intervals to monitor haematocrit values.

Animals of groups 1 and 2 were sacrificed at the end of 13th week (day 90) and group 3 animals after a rest period of 30 days (day 120). Testis were removed and weighed after separating from epididymis. Tissue samples were fixed in 10% neutral buffered formalin and paraffin section of 5-6 μm were stained with hematoxylin and eosin (H & E) or Mallory's trichrome. Tissue samples from kidney and liver were also subjected to histotoxicological study. Section were viewed and photographed.

RESULTS AND DISCUSSION

Neither mortality nor significant variation ($P>0.05$) in haematocrit values between control and $HgCl_2$ treated rats were observed. However, nonsignificantly marginal decline in red blood cell count was observed in groups 2 and 3 at the terminal phase of treatment (data not shown). The mean terminal body weight and testicular weights of different groups of rats are shown in Table 1. The mean body weight and testicular weight of groups 2 and 3 animals determined on day 90 and day 120, respectively, weighed significantly lesser ($P<0.05$) than that of control. Light microscopic evaluation of sections of kidney indicated congestion within glomerulus and peritubular haemorrhage in the medullary region of kidney sections from $HgCl_2$ treated animals (group 2; Fig. 1). No morphological changes in liver was found.

Testis from control group of animals showed all the stage of spermatogenesis and no abnormalities were detected. In contrast, the testis of group 2 rats showed evidence of disrupted course of spermatogenic cycle. Morphologically germinal epithelium is greatly reduced in size and degenerative changes in term cells are evident. Vacuolation in Sertoli cells at multiple sites are prominently observed in majority of the seminiferous tubules screened (Fig. 2). The peritubular basal lamina was discontinued at few sites. In majority of the tubules sloughing of sperjmatids and their flow towards the lumen was found. The degree of these changes compared to vehicle to vehicle treated control varied between tubules and between animals in group 2. The morphology of Leydig cells was normal and there was no perivascular or peritubular foci of lymphocytes in sections obtained from $HgCl_2$ treated rats (groups 2 and 3).

In the recovery group (group 3) testicular weight did not indicate any recovery of testicular function following 30 days of rest period. But return of disorganized peritubular basal lamina to normal and all stages of spermatogenic cycle in sequential radiating pattern was observed (Fig. 3). In few seminiferous tubules, Serotoli cells with vacuoles were still persistent but majority of sections revealed normal histology of testis.

Subacute exposure to inorganic $HgCl_2$ (5 PPM) neither induced changes in haematocrit values significantly ($P>0.05$)

nor affected integrity of nephrons in kidney sections. This rules out the possibility of secondary effects on testicular morphology as a result of vascular changes. The marginal decrease in red blood cell count at the terminal phase may be because of peritubular bleeding in the kidney (Fig. 3) and it indicates characteristic nephrotoxic behaviour of Hg^{+2} ions.

The significance of effect of Hg^{+2} on testicular endothelial cells/or blood testis barrier and role of these changes play in contributing to the degeneration of the seminiferous tubules can not be neglected. Such vascular changes leading to failure of testicular function occur following cadmium exposure (Mason *et al.* 1964). The reduced in Testicular weight may be due to the altered testicular steroidogenesis because of Hg^{+2} ion which inhibit 2b—hydroxy steroid dehydrogenate activity in rats (Chowdhary *et al.* 1985). Further, steroidogenesis was inhibited due to altered secretion of gonadotrophic hormone (LH) which is less likely as inorganic mercurials fair to enter blood—brain barrier. However, conclusion can not be drawn without the measurement of gonadotrophic hormone concentration in plasma and/or testicular tissue.

The possible mechanism of action of inorganic $HgCl_2$ on the testis is a direct effect on Sertoli cells. The Hg^{+2} interact with membrane bound-SH proteins. Sertoli cells play a major regulatory role in spermatogenesis and involved in synthesis of variety of proteins (Gangolli and Phillips 1993). Disruption of spermatogenesis, vacuolation and inhibition of protein secretion by Sertoli cells were observed following exposure to phthalate esters (Gray and Gangolli 1986). Similarly, chronic administration of cobalt in rats impair Sertoli cell structure and integrity and cause vacuolation (Anderson *et al.* 1992). Such direct toxic effect on Sertoli cells was also reported following exposure to sub-neurotoxic doses of 2, 5 hexandione (Boekelheide, 1988) or phthalate ester in pre-pubertal rats (Creasy *et al.* 1983). Metals are actively involved in enzyme activities and an enzyme activated by a metal is inactivated by other. Hence relative concentration of metal and its affinity for ligand is important. Mercurials (Hg^{2+}) affect organ distribution and elimination of zinc following repeated administration (Grosicki and Kossakowski 1992). Zinc is required for normal spermatogenesis. DNA and RNA polymerases are zinc metalloenzymes and thus require zinc for activation (Slater *et al.* 1971). Subacute exposure to $HgCl_2$ might have displaced testicular zinc similar to other metal cations, viz: cadmium and cobalt (Gunn *et al.* 1963, Anderson *et al.* 1989). Sequalae to depletion of testicular zinc, mercuric chloride (Hg^{+2}) might have exerted direct toxic effects on testis.

In the present study Leydig cells were intact. This might be due to the sub-acute dose used in the study since, severe liquification and degenerative changes appeared following a larger dose of $HgCl_2$ in rats (Ramakrishna *et al.* 1996). Similarly, no damage to Leydig cells were observed in rats following chronic exposure to 400 ppm of cobalt (Anderson *et al.* 1992). However, Hg^{+2} ions might have extended its influence on its steroidogenic ability as in the *in-vivo* exposure (Chowdhary *et al.* 1985) although it is unfair to draw conclusion without quantifying plasma/testicular testosterone concentration.

Recovery from testicular toxicity (group 3) was evident by restoration/repopulation of seminiferous tubules during rest period following $HgCl_2$ exposure (Fig. 3). The spermatogenic cycle was complete and normal radiating pattern of distribution of spermatids was evident. Otherwise, further extension of adopted dose of $HgCl_2$ would have rendered sterility in rats.

To conclude, subacute exposure to inorganic $HgCl_2$ impair spermatogenic cycle in rats. The histotoxicological changes were confined to Sertoli cells. These morphological changes were reversible after a rest period following subacute per oral exposure to inorganic $HgCl_2$ in rats.

ACKNOWLEDGEMENT

The authors thank Director of Instruction (Veterinary) for providing necessary facilities and encouragement.

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Indian Journal of Animal Sciences 70 (2): 146-148, February 2000

The influence of genotype and ewe body condition on reproductive performance

R C MALIK[1], N M AL-KHOZAM[2], M A RAZZAQUE[3] and T A AL-MUTAWA[4]

Kuwait Institute for Scientific Research, Aridland Agriculture Department, Foor Resources Division, P.O. Box 24885, Safat 13109, Kuwait

Received: 20 August 1998; Accepted: 17 August 1999

ABSTRACT

Ewes from 5 genotypes—Naeemi, Chios, Texel, Border Leicester Merino (BLM) and Naeemi × Border Leicester Merino—were scored for body condition before breeding and their reproductive performance was compared. Large differences were detected between genotypes with respect to fertility, prolificacy, birth weight and litter weight. Birth weight of lambs born to Naeemi ewes was lesser than the lambs born to BLM, Texel and Naeemi × BLM ewes ($P<0.05$). Birth weight of Naeemi and Chios lambs was similar. Litter weight was higher for the BLM ewes, and this genotype also had the highest prolificacy. Naeemi ewes had the lowest fertility.

Fatness of ewes described as body condition score (BCS) varied at breeding from 2–5 and had significant effects on birth weight. There was tendency for the birth weight to decrease with increasing BCS. Birth weights of lambs were significantly higher ($P<0.05$) for ewes with BSC up to 3 and lower for ewes with scores over 3. The coefficient of regression on birth weight on BCS was -0.41 ± 0.08 ($P<0.01$). Fertility, prolificacy and litter weights were not affected by BCS although ewes with higher scores tended to produce lower litter weights. The results show that ewes should be maintained in a moderate condition (BCS = 3) for breeding to produce lambs with heavier birth weights. Fat ewes (BCS>3) have the tendency to increase their own body weight rather than lamb birth weight. Body condition scoring system could be used to optimize dietary requirements of breeding ewes and birth weight of lambs.

Key words: Ewe body condition, Genotype, Reproduction, Sheep

Large differences in reproductive efficiency have been reported in Kuwait (Malik *et al.* 1996), in Saudi Arabia (Etawil and Narendran 1990) and in other countries (Gootwine *et al.* 1994a, Graili *et al.* 1994). Body condition score has been used to measure fatness of breeding ewes. This parameter can be used irrespective of the breed, body weight or age.

The condition of the breeding ewes before joining with rams is important. Therefore this study was undertaken with the aims-i) to evaluate the reproductive performance of ewes of 5 diverse genotype, and ii) to examine if ewe body condition at breeding had any effect on reproductive performance.

MATERIALS AND METHODS

Different genotypes—Naeemi, Chios, Texel, Boirder Leicester Merino (BLM) and Naeemi × BLM (F_1)—were used in the trial. Records (179) of 3-6-year-old ewes scored for body condition before joining were available for this study. The following body condition scoring system on a 5-point scale (Edey 1983) was used.

Very lean: Backbone prominent and sharp, with easily palpable individual spinous processes. Fingers can be easily passed under the transverse processes of the lumbar vertebrae

Lean: Backbone prominent but smooth. The lumbar transverse processes have smooth and round ends. The fingers can be passed under the ends of the traverse processes with a little pressure.

Medium: Backbone can be felt as a small elevation but is smooth and rounded at the top. The individual spinal processes are well covered and firm pressure is needed to feel them.

Fat: The spinous processes of the vertebrae are just

Table 1. Least-squares means with standard errors of body condition score (BCS) and weight of ewe at lambing by genotype

Genotype	(n)	Ewe BCS	Ewe weight
Naeemi	21	$3.51^{ab}\pm0.19$	$67.1^{a}\pm2.34$
Border Leicester Merino (BLM)	38	$3.50^{ab}\pm0.14$	$76.9^{c}\pm1.99$
Chios	11	$3.35^{a}\pm0.26$	$80.0^{c}\pm3.13$
Texel	18	$3.52^{ab}\pm0.20$	$69.3^{ab}\pm2.67$
Naeemi×BLM	24	$3.91^{b}\pm0.17$	$74.9^{bc}\pm2.37$

The means superscripted with any one same letter are not significantly different from each other ($P<0.05$).

Present address: [1,3]Research Scientist, [2,4]Research Associate.

detectable but the transverse processes cannot be felt.

Very fat: Neither the spinous processes nor the transverse processes can be felt. The skin over the spinous processes is concave.

All sheep were managed under an intensive system and were stall-fed throughout the year, with no provision for grazing. They were housed in partially enclosed sheds with fenced yards. Dry ewes were provided with 1kg/head/day of concentrate mixture and lucerne hay in the ratio of 4: 1 (12% crude protein and 12 MJ/kg digestible energy). This amount was increased to 1.25kg/head/day during the last 6 weeks of pregnancy. After parturition, ewes with single lambs were provided with 1.5kg/head/day during the suckling period. This ration was increased to 1.8kg/head/day for ewes with twins and 2kg/head/day for ewes with triplets.

The breeding programme involved the joining of Naeemi rams with Naeemi, BLM and Naeemi × BLM ewes. Chios and Texel ewes were joined with rams of their respective breeds. Ewe oestrus cycles were synchronized with progesterone-impregnated sponges, and ovulation was stimulated by intramuscular injection of 500 IU of pregnant mare serum gonadotrophin (PMSG). The rams were allowed to run with ewes for 35 days. The productive performance parameters recorded for the ewes were fertility (100 × ewes conceived/ewes joined), prolificacy (lambs born/ewe lambed), individual birth weight of lamb, and litter weight (kg birth weight/ewe lambed). Litter weight was based on single, twins or triplets/ewe. There was 1 ewe with quadruplets which was combined with ewes with triplets.

Data were analyzed by the least-squares analysis of variance (Harvey 1990). The model for the analyses of ewe weight and BCS included the effect of genotype only, for the analysis of fertility and fecundity (litter size) genotype and BCS were included as independent variables, and the statistical model for the analyses of birth and litter weight included the effects of BCS, genotype and sex. Birth type was used as a covariate for the analysis of birth and litter weights. Litter size was initially included as an independent variable for the analysis of BCS and ewe weight, found not significant, and deleted from the final analysis. Due to the above reason, BCS means for sheep producing singles, twins and triplets are not presented. Interactions were not included in the model. All the factors included in the model were regarded as fixed. Linear regression was used to relate birth weight with BCS. All data are presented as least-squares mesan (±SE).

RESULTS AND DISCUSSION

Mean pre-mating BCS and post-lambing body weights of ewes of 5 genotypes (Table 1) revealed no differences between the Naeemi, BLM, Chios and Texel ewes for BCS, but the Chios ewes had heavier body weights than that of Naeemi and Texel. The data indicate that BCS and body weight were relatively independent of each other and heavier body weight was not necessarily associated with higher BCS or vice-versa.

Genotype had a significant effect on fertility and prolificacy (Table 2). Only 38.7% Naeemi ewes conceived compared to 73.6-87.6% fertility rate for the other 4 genotypes. BLM ewes were more fecund (2.02 lambs/ewe) compared to 1.34, 1.35 and 1.54 lambs/ewe for Naeemi × BLM, Texel and Naeemi respectively. Gootwine *et al.* (1995b) reported an increase in mean prolificacy by 0.7 lamb in crossbread over the purebred Awassi ewes, a similar increase was noted for the BLM ewes over the Naeemi ewes in this study. Although no significant differences in prolificacy between BLM and Chios ewes were noted, higher litter weight of the BLM ewes compared to the Chios could be explained on the basis of higher fecundity of the former and heavier

Table 2. Least-squares means and standard errors of fertility, prolificacy, birth weight and litter weight by genotype and body condition score (BCS)

Category	Fertility (%)	Fecundity	Birth weight	Litter weight
Genotype				
Naeemi	38.7^{a}±6.6 (70)	1.54^{a}±0.17 (21)	3.52^{a}±.18 (30)	5.58^{a}±0.56 (21)
Border Leicester				
Merino (BLM)	81.0^{b}±7.0 (53)	2.02^{b}±0.14 (39)	4.26^{b}±0.14 (79)	7.91^{b}±0.47 (39)
Chios	83.0^{b}±12.1 (16)	1.63^{ab}±0.22 (12)	3.75^{ab}±0.23 (18)	6.01^{a}±0.73 (12)
Texel	87.6^{b}±9.8 (27)	1.35^{a}±0.19 (18)	3.98^{b}±0.22 (22)	5.62^{a}±0.64 (18)
Naeemi×BLM	73.6^{b}±8.5 (35)	1.34^{a}±0.17 (24)	4.04^{b}±0.19 (30)	5.68^{a}±0.57 (24)
BCS				
2	64.9^{a}±16.9 (7)	1.80^{a}±0.45(2)	4.46^{a}±0.46 (3)	7.86^{a}±1.48 (2)
3	72.0^{a}±4.5 (109)	1.41^{a}±0.08 (62)	4.11^{ab}±0.09 (94)	5.94^{a}±0.27 (62)
4	67.8^{a}±5.7 (64)	1.50^{a}±0.11 (35)	3.66^{c}±0.11 (55)	5.58^{a}±0.36 (35)
5	86.4^{a}±10.1 (21)	1.58^{a}±0.17 (15)	3.40^{cd}±0.17 (27)	5.26^{a}±0.57 (15)
Regression on BCS (Linear)			−0.41±0.08	

Mean comparisons have been made within each broad category.

Means superscripted with any one same letter are not significantly different from each other (P>0.05). The number of observations is presented within parentheses

birth weight of their lambs (Table 2). Litter weight and prolificacy were higher for BLM than Naeemi × BLM ewes (1/2 Naeemi + 1/2 BLM). Thus the level of BLM genes may account for the difference. Body condition score at breeding had no effect on fertility and fecundity of ewes, although ewes with BCS 2 and 3 (lean to medium ewes) tended to produce more kg of lamb than ewes with BCL higher than 3 (fat to very fat ewes).

Least-squares means for birth and litter weights by genotype and BCS (Table 2) revealed that significant differences existed between genotypes for birth and litter weights. Birth weight was higher for BLM, Texel and Naeemi × BLM ewes than Naeemi. Difference between Naeemi and Chios for birth weight was not significant. BLM ewes produced heaviest litter weight ($P<0.05$), whereas litter weight was similar for Naeemi, Chios, Texel and Naeemi × BLM. The linear regression of birth or litter weight on type of birth was not significant.

The effect of ewe BCS on lamb's birth weight was significant, but it was not significant for litter weight. Ewes with higher BCS had lambs with lower birth weight. Our results concur with those of Russell *et al.* (1981) and Al-Sabbagh *et al.* (1995) who suggested a negative relationship between BCS and birth weight. Russell *et al.* (1981) explained their results on the basis of a threshold of body condition beyond which it may have a negative effect on individual lamb weight. The linear regression of birth weight on BCS ($b= -0.41\pm0.08$, $P<.01$) suggested that any increase in BCS of the ewe above 3.5 is likely to result in a significant decrease in birth weight of the lamb.

Our results indicate that it would be advisable to keep breeding ewes in a moderate condition by maintaining an appropriate level of nutrition and avoiding overfeeding. Fat ewes are likely to increase their own body weight and reduce lamb's birth weight. Body condition scoring system can be used to optimize dietary needs of breeding ewes and birth weight of lambs. The aim in intensive management should be to have the BCS of breeding ewes at around 3 at joining and maintaining this condition up to lambing.

ACKNOWLEDGEMENT

The authors thank the Kuwait Livestock Transport and Trading Company for providing facilities to carry out this study.

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Indian Journal of Animal Sciences 70 (2): 149-150, February 2000

Seroprevalence of brucellosis in livestock and humans in Vidarbha region

D R KALOREY[1], V C INGLE[2] and N V KURKURE[3]

Nagpur Veterinary College, Nagpur, Maharashtra 440 006

Received: 22 January 1999; Accepted: 17 August 1999

Key words: Brucellosis, Seroprevalence

Brucellosis is a zoonotic disease affecting livestock and human beings. In cows it is commonly caused by *Brucella abortus*, whereas *Brucella meletensis* causes infection in goats which is characterized by stillbirth, abortion, decreased milk production and infertility. *Brucella meletensis* is more infectious to human being (Radostitis *et al.* 1994). Now a days goat farming is becoming popular as it provides self employment and additional source of income. In view of paucity of literature regarding prevalence of brucellosis and its zoonotic impact in Vidarbha region, present study was undertaken.

In this study herds with a history of abortions were selected. In herd A only cows were maintained, whereas in herd B goats and herd C having mixed livestock farming ie cows and goats cohabit. Additionally, zoonotic nature of brucellosis was studied by screening serum samples referred by Government Medical College, Nagpur.

Serum samples (72) from cows of herd A, goat serum samples (56) from herd B and cows serum samples (48) and 10 goat serum samples were collected from herd C where these animals cohabited and experienced an abortion storm in the recent past. Also serum samples from 21 in-contact animal attendants in herd A, 2 samples of persons having association with herd B animals, and 8 samples of the animal attendants handling herd C animals, were collected. This includes 4 serum samples referred by the Government Medical College, Nagpur.

The serum samples were tested by Rose Bengal plate agglutination test (RBPT) and by standard tube agglutination test (STAT) for assaying antibrucella agglutinin titers as per the standard procedure recommended by the Division of Biological Products, IVRI, Izatnagar. The standard antigens were procured from the IVRI, Izatnagar. The serum samples were subjected to dot-ELISA for assaying antibody against brucellae as per the procedure described by manufacturer.

Serum samples (72) from cows of herd A were screened and of these 12 (16.66%), 8 (11.11%) and 5(6.94%) were positive by RBPT, STAT and dot-ELISA respectively. Whereas in herd C prevalence of brucellosis was recorded to the tune of 25.0% and 18.76% by RBPT and STAT respectively. Higher seroprevalence and history of herd indicate endemic brucellae infection. Overall prevalence of brucellosis in cows was recorded to be 20.0% and 14.16% by RBPT and STAT respectively. The present finding is in close association with those of Isloor *et al.* (1994) whilst 7.49% and 3.55% prevalence was reported by Prahlad and Singh (1997).

In goats the seroprevalence of brucellosis of herd B was noted to be 3/56 (5.36%) and 2/56 (3.57%), respectively, by using RBPT and STAT. However, very high values 3/10 (30.0%) and 2/10 (20.0%), respectively, were observed in herd C. This might be due to poor management and hygienic practices and association with *Brucella* infected cows. Overall seroprevalence of brucellosis in goats was 6/66 (9.09%) and 4/66 (6.06%) by RBPT and STAT respectively. The result of present study are in close agreement with earlier reports of Ghosh and Verma (1995), Balbo *et al.* (1989) and Khire *et al.* (1998). However, comparatively lower prevalence was recorded by Prahlad and Singh (1997).

In the present investigation overall seroprevalence of brucellosis among in-contact animal attendants was only observed in herd C attendants 3/31 (9.67%) both by RBPT and STAT that indicates threat to human health due to zoonotic nature of disease specially in endemic area. The present finding is in accordance with that of Sharma and Purohit (1994). However, lower prevalence was observed by Desai *et al.* (1994). It is observed that under the field condition RBPT can be used as a quick reliable diagnostic test of brucellae infection. Result of present study also indicates highly contagious nature of the disease and warrants immediate isolation of infected animals from the herd. In view of zoonotic nature of disease early diagnosis and subsequent control measures may help in reducing frequency of human infection.

ACKNOWLEDGEMENT

Authors thank Dr H V S Batra, DRDO, Gwalior, for

Present address: [1]Associate Professor, [2]Assistant Professor, Department of Microbiology; [3]Assistant Professor, Department of Pathology.

providing ELISA diagnostic kit as a gratis.

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Indian Journal of Animal Sciences 70 (2): 151-153, February 2000

Clinical, virological and serological responses of sheep and goat inoculated with bluetongue virus type 2*

D SREENIVASULU[1] and M V SUBBA RAO[2]

Acharya N G Ranga Agricultural University, Tirupati, Andhra Pradesh 517 502

Received: 22 October 1998; Accepted: 13 August 1999

Key words: Bluetongue virus type 2, Serological responses, Virological responses

Bluetongue (BT) is an economically important insect transmitted viral infection of ruminants and the causative agent is bluetongue virus (BTV), a member of family Reoviridae, prototype virus of the genus Orbivirus. It is endemic in most of the tropical and subtropical regions of the globe and was reported in India in 1963 (Sapre 1964). Subsequently the disease is being reported annually from the Andhra Pradesh, Tamil Nadu, Karnataka and Maharashtra (Sreenivasulu *et al.* 1996). Eight serotypes and neutralizing antibodies to 18 serotypes were identified from different parts of the country (Prasad *et al.* 1992). Experimental inoculation studies were carried out to study clinical, immunological, virological and haemotological responses in cattle, sheep and goats using different serotypes and recorded variations in clinical, immunological and serological responses (Ghalib *et al.* 1985, Richards *et al.* 1988, and Flanagan *et al.* 1993). Chander *et al.* (1990) conducted similar studies in sheep using BTV-1 isolated at Hisar. However, information is lacking on the responses of viruses isolated from different parts of the country which are essential to understand the epidemiology and to develop the vaccine to control the disease. Hence the present investigation was taken up in sheep and goats to study clinical, virological and serological responses due to BTV-2 isolated from natural outbreak of sheep in Andhra Pradesh.

Sheep maintained in the Department of Animal Nutrition, and the goats available in the Department of Microbiology, College of Veterinary Science, Tirupati, were tested for the presence of precipitating antibodies to BTV. For experimental inoculation 4 sero-negative Nellore sheep and 3 sero-negative goats of 2 years age were selected after deworming. Bluetongue virus isolated from native sheep of Andhra Pradesh and identified as BTV-2 with plaque reduction test (Gard and Kirkland 1993) using type specific antiserum to the 24 internationally recognized BTV serotypes, further confirmed by the Veterinary Research Institute, Onderstepoort, Republic of South Africa, maintained in the Department of Microbiology, College of Veterinary Science, Tirupati, was used in the study. The titre of the virus was $10^{5.5}$ $TCID_{50}$ / ml at 10th, passage level *in-vitro* cell culture.

Three sero-negative Nellore sheep and 2 sero negative goats were inoculated with 10ml of the BTV-2 via intradermal and subcutaneous routes at multiple sites. One each of uninfected sero negative sheep and goats were kept as in-contact controls. The animals were daily examined for clinical signs besides recording rectal temperature. Blood samples (both heparinized and nonheparinized) were collected daily for 15 days after inoculation and were used for assessment of viraemia, leukocyte counts and serological responses.

Day-old chicken embryos (11) were used for assay of virus infectivity. Serial 10-fold dilution of virus were made in PBS. Each virus dilution (1/10 ml) was inoculated intravenously into each of 5 chicken embryos and incubated at 33.5°C. The embryos were examined daily for the mortality. The embryo infective dose 50 (EID_{50}) was calculated as per Reed and Muench(1938). Seroconversion was determined by agar gel immunodiffusion test (AGID) and serum neutralization test (SNT). The BTV antigen was placed in central well and 25 ml of test and reference serum samples were placed in peripheral wells alternating with each other. The plate was incubated at 25°C in a moist chamber and observed for 3 days for precipitation lines. SNT was carried out as per Ghalib *et al.* (1985) *in-vitro* cell culture. Leukocyte response was assessed by determining total leukocyte count as per Sastry (1976).

The temperature was 39.5°C on the day 6 and 40.5°C on the day 7 PI (Fig.1 A). On the day 8 and 9 PI temperatures were 40.4° and 39.8°C. Later it came down to 39°C on the 11 PI. BTV infected sheep had mild congestion of mucocutaneous borders of lips between day 10 and 11 PI. On day 12 PI, muscular stiffness was noticed. All infected sheep recovered and became normal by day 18 PI. Viraemia was detected on day 4 PI with BTV titre of 102.2 EID_{50} ml^{-1}

*Part of the Ph.D. Thesis of the first author, approved by Andhra Pradesh Agricultural University.

Present address: [1]Assistant Professor, Department of Microbiology, College of Veterinary Science.

[2]Principal, College of Veterinary Science, Gannavaram, Krishna, Andhra Pradesh 521 102.

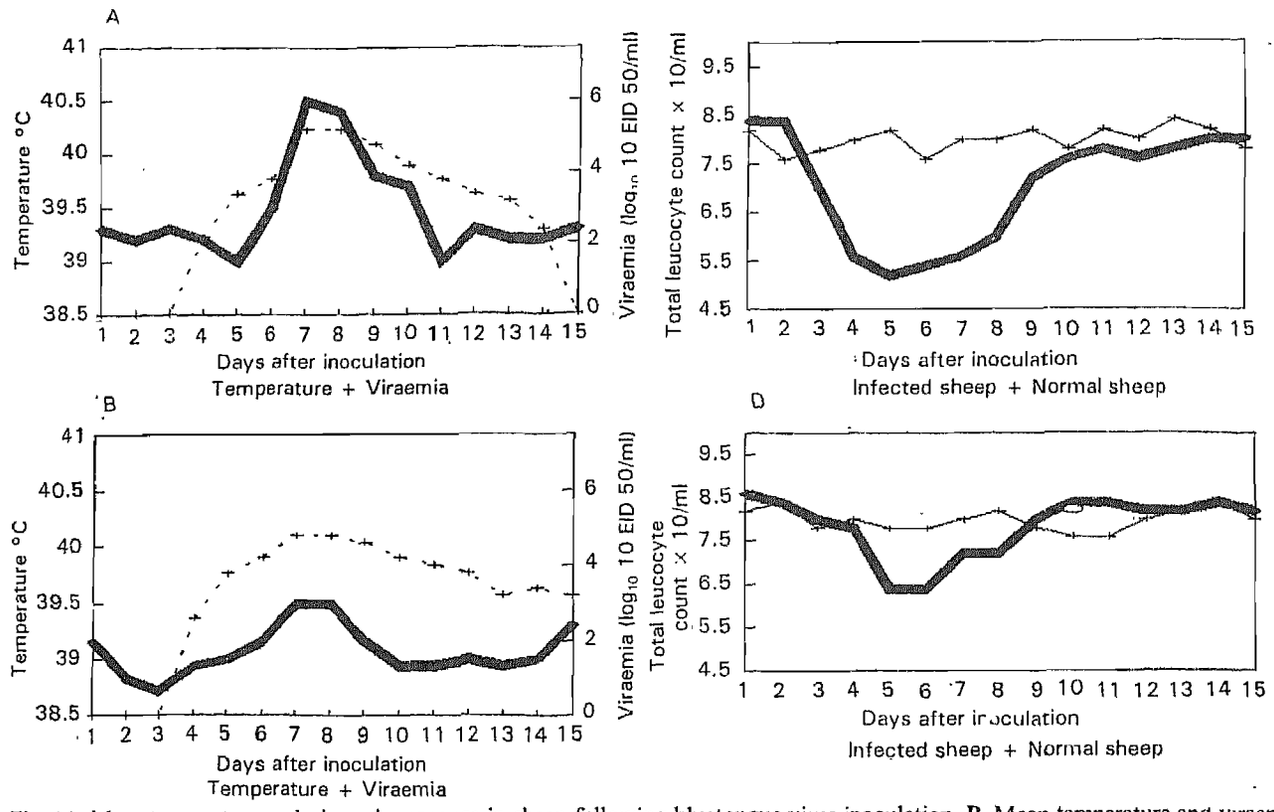

Fig. 1A. Mean temperature and viraemia response in sheep following bluetongue virus inoculation. B. Mean temperature and viraemia response in goat following bluetongue virus inoculation. C. Mean total leukocyte response in sheep following bluetongue virus inoculation. D.Mean total leukocyte response in goat following bluetongue virus inoculation.

of blood in infected sheep. A maximum of 105.2 EID_{50} ml^{-1} titres were noticed on days 7 and 8 PI (Fig.1 A). Leucopenia was observed between day 4 and 8 PI with total leukocyte counts ranging from 5.2 to 5.6× $10^6 ml^{-1}$ (Fig.1 C). Neutralizing antibodies and precipitating antibodies appeared on day 8 and 9 PI and maintained till the end of the experiment (15 days PI).

Experimentally inoculated goats had the maximum temperature of 39.5°C on days between 7 and 8 PI. Clinical symptoms were not observed in infected goats during study. However, viraemia was noticed on day 4 PI which persisted till the end of the experiment with the maximum titre of 104.8 EID_{50} ml^{-1}on day 8 PI (Fig.1 B). Leucopenia was observed between days 5 and 8 PI with total leukocyte counts ranging from 5.4 × 10^6 ml^{-1} to 7.2 ×$10^6 ml^{-1}$ (Fig.1 D). Neutralizing antibodies and precipitating antibodies appeared on day 9 and 10 PI. and maintained till the end of the experiment (15 days PI). Uninoculated in-contact sheep and goats were normal without clinical, virological and serological responses.

In this investigation experimentally inoculated sheep showed very mild clinical signs. Corriedale sheep inoculated with BTV serotypes 10, 11, 13, 17 also did not develop mouth lesions but exhibited pyrexia, excessive saliva and dysponea (Ghalib *et al.* 1985). Similar observations during the experimental infection was also reported previously by Chander *et al.*(1990). During the study infected sheep had viraemia that persisted for 10 days. Persistence of viraemia less than 3 weeks was observed in sheep infected with either Australian or US strains of BTV (Groocock and Campbell 1982, Ghalib *et al.*1985). However, Richards *et al.* (1988) reported the longer duration of viraemia (35-42 days) in BTV infected lambs.

Experimentally inoculated goats did not show any clinical signs. Pyrexia was also very mild with maximum temperature of 39.5 °C on day 7 and 8 PI. Viraemia was detected on day 4 PI and continued till the end of the experiment(15 days PI). However, the virus titres were less than that in sheep. Low virus titres along with prolonged viraemia was also reported in BTV inoculated goats by Luedke and Anakwenze (1972). Absence of clinical signs with prolonged viraemia in goats may have epidemiological significance. The cattle acts as a carrier with prolonged viraemia without exhibiting clinical signs (Gard *et al.* 1988, Ward 1994).

Leucopenia was observed between day 4 and 8 PI in sheep and goats. Haribabu (1985) also reported leucopenia in both experimentally and naturally infected sheep. The low leukocyte count may account for immuno-suppression reported in sheep infected with BTV(Ghalib *et al.* 1985).BTV inoculated sheep and goats developed humoral immune responses to BTV. Seroconversion was noticed on day 8 and

continued till the end of experiment. These results are in accordance with the reports of Chander *et al.* (1990), Johnson *et al.* (1992) and Flanagan *et al.* (1993). However, the immune responses did not result in virus clearance in goats. Infectious virus circulated together with the antibody in goats during the period under study. Similar observations were also noticed in both BTV-10 infected lambs and calves.

ACKNOWLEDGMENT

We thank the Acharya N G Ranga Agricultural University for providing facilities and financial support.

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Indian Journal of Animal Sciences 70 (2): 154-156, February 2000

Monitoring of *Brucella* infection associated with reproductive losses using multiple serological tests in organized goat and sheep flocks

S V SINGH[1], G S AGARWAL[2], H V BATRA[3], V K GUPTA[4] and N SINGH[5]

Central Institute for Research on Goats, Makhdoom, Uttar Pradesh 281 122

Key Words: Abortions, Brucellosis, Goat, Reproductive losses, Serological tests, Sheep

Information on abortions associated with brucellosis, both in the organized farms and farmers' flocks is limited, (Singh et al. 1994 and 1998). In this study goat and sheep flocks located at the Central Institute for Research on Goats (CIRG), Makhdoom, Mathura (UP) were monitored for reproductive losses (abortions and stillbirths) from 1985 to 1995, representing 21 parities. Flocks were screened against brucellosis using SAT and multiple serological tests (dot-ELISA, plate-ELISA, CFT and SAT). A new dot-ELISA kit was used as flock screening test in goats and sheep.

Serum samples

Serum samples from organized flocks of Jamunapari and Barbari goats and Muzaffarnagari sheep located at CIRG were collected and stored at -4°C till further use.

Serological tests

Female animals in which reproductive losses (abortions and stillbirths occurred and the general flocks, both were screened against brucellosis using SAT (from 1985 to 1992 and in 1995), and using multiple serological tests (dot-ELISA, plate-ELISA, CFT and SAT), at 6 monthly intervals (January 1993-first; September1993-second; and January 1994-third). During multiple tests screening flocks were first screened by dot-ELISA kit and the positive reactors were further tested using plate-ELISA, CFT and SAT tests.

(i) Dot-enzyme linked immunosorbent assay (dot-ELISA)

Dot-ELISA, a qualitative field test for bovines (Batra *et al.* 1989), was standardized for goats and sheep. Samples were processed as per the procedure given in the dot-ELISA kit obtained from DRDE, Gwalior, Madhya Pradesh.

(ii) Standard tube agglutination test (SAT)

Commercial *Brucella abortus* antigen for SAT was obtained from the Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh. The test was performed as per the procedure of Alton *et al.* (1975), Serum samples showing the titre of 1: 40 and above were considered as positive reactors for *Brucella* infection and were eliminated..

(iii) Complement-fixation test (CFT)

The CFT antigen was prepared from *Brucella abortus* S-99 and standardized according to Alton *et al.* (1975). Serum samples showing a titre of 1: 8 or above were interpreted as positive reactors.

(iv) Plate-ELISA test (p-ELISA)

Plate-ELISA was performed as per the method described by Batra *et al.* (1989). Sonic extract of *Brucella abortus* S-99 was used as antigen in the test. Animals positive in more than one test were considered as positive for *Brucella* infection in multiple tests screenings.

Brucellosis screening

Reproductive losses and brucellosis in organized goat and sheep flocks (1985-1995): Average annual reproductive losses (foetal losses) due to abortions and still births in 21 parturition seasons (1985-1995) were 17.8%, 11.8% and 8.7% in flocks of Barbari and Jamunapari goats and Muzaffarnagari sheep, respectively (Table 1). Of the cases of reproductive losses in goats (490) and sheep (192) using SAT, 3.0% does and 6.1% ewes were found positive for brucellosis (Table 2).

Screening of apparently healthy adult goat and sheep flocks using SAT:

(i) Before introduction of multiple serological tests screenings: Annual flock screening of Barbari (1989 and 1992), Jamunapari (1990 and 1992) and experimental goats (1990 and 1992), using SAT, revealed 2.6% and 0.7% nil and 1.2% and none animals as positive reactors respectively. Whereas, in Muzaffarnagari sheep flock there were 3.4% and 7.7% positive cases in 1989 and 1992 screenings, respectively, (Table 2).

(ii) *After the multiple serological tests screenings:* Screening of Barbari and sheep flock using SAT in 1995, showed 0.3% and 1.3% positive reactors for brucellosis respectively. Jamunapari flock was not screened in 1995. Results showed reduction in number of positive reactors, as

Present address: [1,4]Scientist (SS),[5]Principal Scientist and Head, Goat Health Division, CIRG, Makhdoom.

[2]Scientist,[3]Scientist E, Microbiology Division, DRDE, Gwalior, Madhya Pradesh.

Table 1. Per cent abortions, stillbirths and prevalence of brucellosis in organized flocks of goats and sheep from 1985 to 1995, based on SAT

Flocks	Females	Kids/lambs	Abortions	Still-births	*Brucella* reaction
Jamunapari+	1137	1452	104 (9.1)	30 (2.6)	01 (00.7)
Barbari+	2001	2496	335 (16.7)	21 (1.0)	14.(03.9)
Total	3138	3948	439 (14.0)	51 (1.6)	15 (03.0)
Sheep*	2206	1989	164 (7.4)	28 (1.3)	27 (14.0)
Grand total	5344	5937	603 (11.3)	79 (1.5)	42 (0 6.1)

*Muzaffarnagri sheep, +goat flocks, Figures in parentheses are percentages.

compared with earlier screenings using SAT. It has not been possible to screen all flocks annually by using SAT (Table 2).

Screening of flocks using multiple serological tests

In the first screening (Jan, 1993), only adult animals (874) were screened using dot-ELISA. Further testing of the positive reactors using CFT and SAT, finally revealed 9.8% positive animals for brucellosis. In the second and third screenings, 1 023 and 890 animals (all animals including young ones) were screened using dot-ELISA and further testing of reactors with SAT, CFT and plate-ELISA revealed 2.4% and 2.7% positive cases for brucellosis respectively. In Barbari, 6.9% 1.6% and 2.8%, in Jamunapari, 5.1%, 1.5% and 1.3% and in Muzaffarnagari 24.3%, 3.6% and 4.1%, were identified as brucellosis positive animals, during first, second and third screenings, respectively (Table 3). Screening of young animals (<6 months age) using dot-ELISA, many reactors were detected in second screening, which eventually became sero-negative in the third screening. The detection probability was about 3-8 times higher in the first multiple tests screening as compared to screening by SAT. Positive animals were eliminated from the flocks.

Abortion was the leading cause of reproductive losses and per cent abortions were highest in Barbari followed by Jamunapari and sheep flock. Losses due to abortions have been reported to be a major problem in organized flocks of small ruminants (Singh and Sengar 1990). Abortions and infected parturition in chronic carriers pose a threat to susceptible animals of the flock (Philpott and Anko 1972) and human beings (Piffaretti *et al.* 1987). Goats are usually kept as companion animal by poor people in Asian countries. Kolar (1987) reported more than 80% of human brucellosis cases contracted infection during parturition seasons.

In aborting goats 3.0% were sero-reactors for brucellosis using SAT. Barbari recorded the highest brucellosis incidence (3.9%) among aborting goats and Muzffarnagri sheep showed the highest incidence of brucellosis (14.0%) of all the flocks screened. As in aborting animals, in flock screening too sheep flock recorded highest positive reactors, followed by Barbari and Jamunapari. Between 2 flock screenings, Barbari recorded a downward trend but Jamunapari and sheep exhibited rise in the incidence. A decline in *Brucella* reactors (Barbari 0.3% and sheep 1.3%), was noticed in 1995 flock screening. Comparison between the two screenings (SAT and multiple tests) exhibited the limitations of SAT as single flock screening test. Study showed that use of SAT and removal of positive reactors only helped to keep the infection partially under control. SAT was cumbersome to perform on large flocks annually (Table 2).

For flock screenings, dot-ELISA has been useful as field based qualitative test. A very high per cent of positive reactors against brucellosis were detected both in goats (4.7%) and sheep (24.3%), in 1st multiple tests screening. Here too in goats, Barbari had highest per cent reactors than Jamunapari and experimental goat flock. The detection probability using multiple tests in goats was about 3 times higher as compared to SAT, as single test screening and was about 8 times (24.3%) in sheep flock. Elimination of large number of *Brucella* positive reactors from the flocks, led to a sharp decline in

Table 2. Brucellosis screening of adult goats and sheep flocks using SAT

Flocks	1989a		1990a		1992a		1995b	
	Tested	React.	Tested	React.	Tested	React.	Tested	React.
Jamunapari+	–	–	357	Nil	169	2(1.2)	–	–
Barbari+	3018	8 (2.6)	–	–	278	2(0.7)	310	1(0.3)
Experimental goats#	–	–	221	Nil	92	–	–	–
Sheep*	294	9 (3.0)	–	–	247	19 (7.7)	451	6 (1.3)
Total	595	17 (2.8)	578	Nil	786	23 (2.9)	761	7 (0.9)

+Goats, #experimental flock of western regional goats, *Muzaffarnagri sheep.

Figures in parentheses are percentages –screenings not performed.

[a]Before introduction of multiple serological tests screenings; [b]after introduction of multiple serological test screenings.

Table 3. Brucellosis screening of goats and sheep flocks using multiple serological tests

Screenings	First		Second		Third	
Flocks	Jan. 1993		Sept., 1993		Jan., 1994	
	Tot. Pop.	React.	Tot. Pop.	React.	Tot. Pop.	React.
Jamunapari+	195	10 (05.1)	271	4 (1.5)	297	4 (1.3)
Barbari+	245	17 (06.9)	315	5 (1.6)	323	8 (2.8)
Exp..Goats#	154	1 (00.6)	151	Nil	–	–
Total+	594	28 (04.7)	737	9 (1.2)	620	12 (1.9)
Muzaffarnagri*	280	68 (24.3)	386	14 (3.6)	360	15 (4.1)
Grand total	874	86(09.8)	1023	25 (2.4)	980	27 (2.7)

+Goats, #experimental flock of western regional goats, *Muzaffarnagri sheep.

Figures in parentheses are percentages. –Screening not performed **(dot-ELISA, plate-ELISA, CFT and SAT).

number of reactors in subsequent multiple tests screenings (second and third), 1.6% and 2.5%; 1.5% and 1.3%; and 3.6% and 4.2% in Barbari, Jamunapari and Muzaffarnagri sheep respectively. Utility of 2 or more than 2 serological tests, in flock screening of goats and sheep against brucellosis has been demonstrated by many workers.(Abd El-Ghani *et al.* 1983, Mahajan and Kulshreshtha 1991 and Jimeniez *et al.* 1992).

Multiple tests screenings at short intervals (at least 6 monthly) of goat and sheep flocks affected with endemic *Brucella* infection was essential to either eradicate or maintain infection to lower limits. Parturition in the same shed complex of the breeding stock and mixing of 2 species (goats and sheep), have been precipitating factors in perpetuation of infection. Presence of large number of positive reactors in all the 3 screenings in sheep flock suggested that probably the nidus of infection lies in this flock, though per cent abortions were lower than in goat flocks. Barbari flock being located in the vicinity of sheep flock had higher incidence of *Brucella* reactors. Addition of new animals in goat flocks resulted in abortion storms. Cross infection occurred during stray matings and mixing during grazing. Use of dot-ELISA helped in screening at short intervals. Identification of large number of reactors and their elimination have been instrumental in achieving a sharp reduction of *Brucella* reactors and also abortions even in sheep flock, where *Brucella* infection was as high as 24.3%.

ACKNOWLEDGMENTS

Authors are thankful to the Directors, CIRG, and DRDE, Gwalior for providing the necessary facilities; and Sri Jagdish of Goat Health Division for secretarial help.

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Indian Journal of Animal Sciences 70 (2): 157-158, February 2000

Spontaneous affections of pancreas in pigs

R SINGH[1] and N S PARIHAR[2]

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122

Received: 19 February1999; Accepted: 10 August 1999

Key words: Affections, Pancreas, Parasite, Pigs

Chronic parasitic pancreatitis in swine due to *Opisthorchis tenuicollis* encountered presently does not seem to have been documented earlier in the available Indian literature. Records, however, exist of the occurrence of the parasite in the intestines of 3 out of 142 pigs (Sarma and Gogoi 1986). This communication reports certain pathological conditions affecting pig pancreas.

Pigs (550 carcasses) were slaughtered for meat at Bareilly and Aligarh and the materials used for this study. The pancreata were grossly examined *in situ* and dissected out. Representative tissue pieces were preserved in 10% formalin saline, processed conventionally to obtain 4 μ thick paraffin sections and stained with haematoxylin and eosin and, if necessary, by special staining techniques.

Out of 550 pancreas, 54 had gross abnormalities consisting of red worm fluke infection (50) and necrosis of pancreatic fat (4). Soft fleshy globular parasites (2 mm × 5 mm) were identified as *Opisthorchis tenuicollis* on the basis of their morphology.

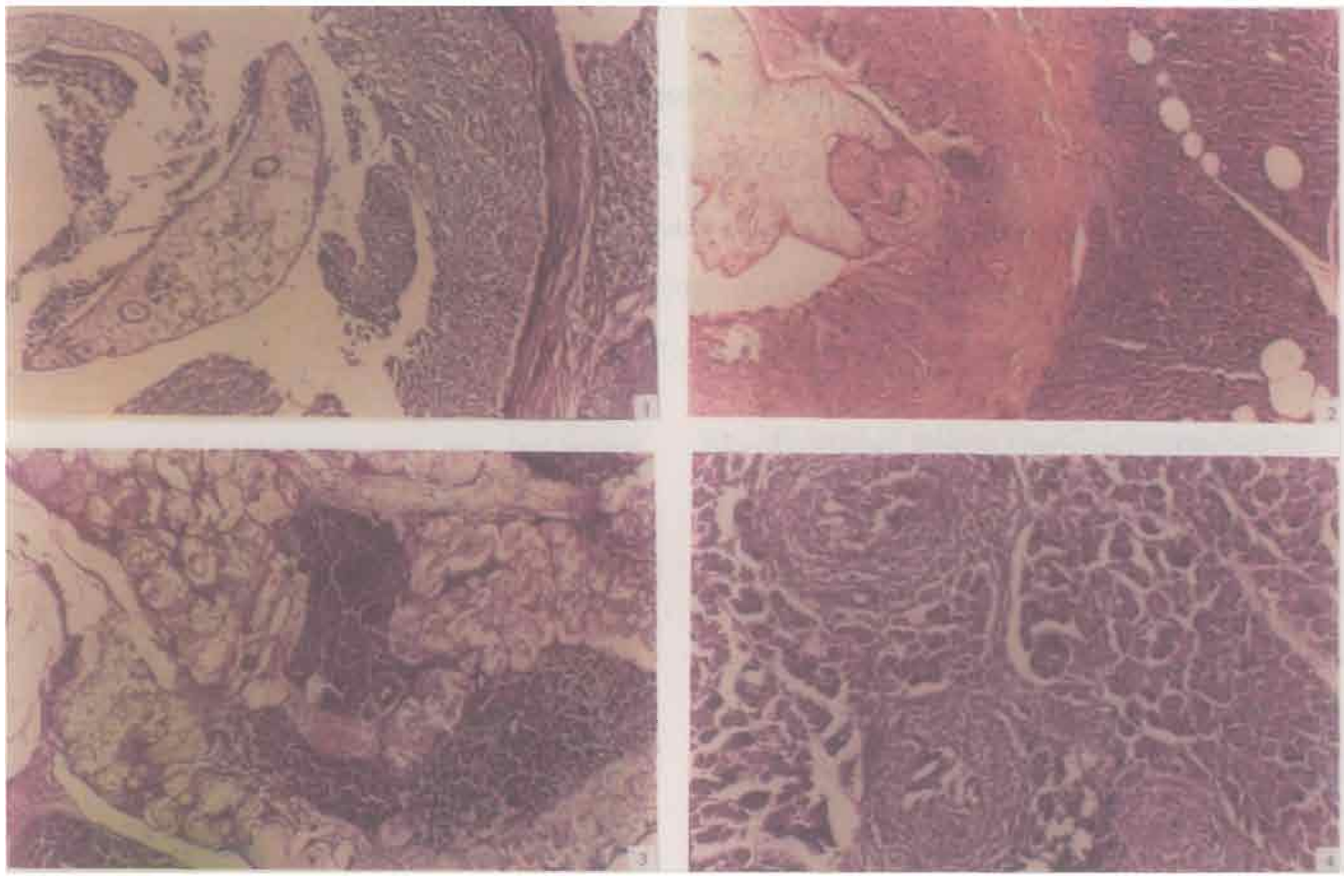
Infested pancreas were enlarged, jelly like, soggy with gelatinized fat over the surface and between the lobules. The ducts within the lobules were enlarged with walls thickened. The slimy mucoid contents within teemed with pink-coloured globular fleshy translucent flukes identified as *Opisthorchis tenuicollis*. Microscopically the intralobular and collecting ducts were prominent with hyperplastic thickened epithelial lining and variable fibrosis around them. The mucosal lining, besides being thickened, had complex villi like folds alongwith numerous acinar structures. The ducts often revealed individuals or groups of actively secreting goblet cells. The lamina propria revealed infiltration with mononuclear cells in which plasma cells were often in plenty. Eosinophils were not always noticed. Occasionally the infiltrative changes extended in the adjacent area with formation of lymphoid follicles (Fig. 1). The lumina of the ducts were filled with debris in which degenerated and exfoliated cells, mucinous secretions and parasitic worms without body cavity were apparent (Fig. 2). The connective tissue between the lobules was prominent around the ducts with mild leukocytic infiltration. Some of the pancreas in the affected cases also revealed lesions of lipomatosis, infrequently with fatty necrosis.

In 31 pancreas (5.63%) classified as having lipomatosis lesions, the fatty tissue was observed prominently in the interlobular and intralobular connective tissue as small foci or as large-confluent accumulations. The fat containing cells were of large sized occurring in the connective tissue septa or within the lobules between the acini. Lipomatosis has already been reported to occur in this species also as a part of general obesity apparently without any clinical significance (Jubb *et al.* 1993). The fatty tissue in 4 out of 31 cases had undergone focal necrosis. The necrosed tissue was dull whitish gray and flaky instead of yellow and shining. Microscopically the necrotic fatty cells revealed eosinophilic or amphophilic material arranged irregularly. Usually the tissue adjacent to such necrotic foci did not reveal any significant reaction except occasional mild mononuclear cell infiltration (Fig. 3).

In 3 (0.55%) cases it was observed that a few lobules revealed occurrence of small nodules which were evident microscopically. These nodules consisted of masses of macrophages alongwith a few lymphocytes. Giant cells were rare. In the centres of many of these nodules, structures resembling Schistosoma eggs were encountered (Fig. 4). In one of the cases the intact parasites were also found in the pancreatic vein. This is apparently the first report of natural parasitic infestation by schistosomes in pigs. Experimentally pseudotubercles in pancreas by *Schistosma incognitum* (Ahluwalia 1972) and by *Schistosoma japonicum* (Yason and Novilla 1984-85) have been reported.

Occurrence of mononuclear cell infiltrations in the interacinar (intralobular) and interlobular connective tissue in foci in 6 cases was observed but without evidence of any etiological agent. In 1 case certain cells in the acini in various lobules revealed vacuolation of their cytoplasm. The interstitial mononuclear cell reaction and fatty changes apparently could have been parts, respectively, of inflammatory and degenerative changes in these cases.

Present address: [1]Former Principal Scientist, A-124 Rajendra Nagar, Bareilly.



Figs 1-4. 1. Mononuclear cell and lymphoid follicular cell reaction adjacent to fluke-infested pancreatic ducts. H & E.×120. 2. Thickened and fibrosed intralobular pancreatic duct containing *O. tennuicollis* flukes. H & E.×48. 3. Lipomatosis in the pancreas. Fatty tissue has undergone necrosis.H & E.×48. 4. Granulomatous nodules in pancreatic lobules with remnants of eggs resembling those of *Schistosoma* sp. H & E.×150.

ACKNOWLEDGMENTS

The authors thank the Director and Joint Director (CADRAD), IVRI, Izatnagar, for the facilities provided.

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Indian Journal of Animal Sciences 70 (2): 159-160, February 2000

Comparative histochemical studies of *M.latissimus dorsi anticus et posticus* and *M.supracoracoideus* in ducks and quails: Muscle fibre type*

A N RAMESHA[1], R V PRASAD[2], K N CHANDRAMOULY[3] and KRISHNA KAKADE[4]

University of Agricultural Sciences, Hebbal, Bangalore, Karnataka 560 024

Received: 2 January 1999; Accepted: 13 August 1999

Key words: Ducks, Histochemical studies, Muscle fibre types, Quails

The information of muscle fibre types in ducks and quails was meager, therefore an attempt to classify the fibre types and to compare them in *M. latissimus dorsi anticus et posticus* and *M. supracoracoideus* is undertaken in ducks and quails. The *M. latissimus dorsi anticus et posticus* and *M. supracoracoideus* were collected and processed for histological and histochemical procedures. The paraffin sections were stained by Harris haematoxylin and eosin, phosphotungstic acid haematoxylin and periodic acid Schiff's methods (Luna 1968). The frozen sections of 20 mm were incubated for succinic dehydrogenase, phosphorylase, lipase, adenosine triphosphatase activities and for the demonstration of lipids and myoglobin concentrations (Pearse 1972). The diameter of fibres were measured using an ocular micrometer (Erma).

Histochemical and histoenzymic distribution of carbohydrate, lipid, myoglobin, succinic dehydrogenase, adenosine triphosphatase, phosphorylase and lipase in fibre types of *M. latissimus dorsi anticus et posticus* and *M. supracoracoideus* is summarized in Table 1. Based on the above parameters the fibre types were classified as type I, intermediate and type II fibres. The phosphorylase reaction showed strong reaction in type II fibres, followed by moderate reaction in intermediate and weak reaction in type I fibres of *M.latissimus dorsi anticus* (Fig. 1).

In all the 3 muscles studied in both ducks and quails the diameters of type I fibres were narrow while they were broad in type II fibres and in intermediate fibres the diameters were lying between the types I and II fibres. Similar findings have been reported earlier (George and Berger 1966). In the present study oxidative metabolism was involved with lipid as the metabolite for energy production in type I fibres as appreciated by higher concentration of the lipid, myoglobin, ATPase, lipase and SDH activities (Table 1). In type II fibres glycogen was anaerobically metabolized to yield energy as appreciated by a higher concentration of glycogen and phosphorylase

Table 1. The staining intensities of different histochemical parameters of type I, intermediate and type II fibres in the wing muscles of duck and quail

Parameters	*M. latissimus dorsi anticus*						*M. latissimus dorsi posticus*						*M. supracoracoideus*					
	Type I		Intermediate		Type II		Type I		Intermediate		Type II		Type I		Intermediate		Type II	
	D	Q	D	Q	D	Q	D	Q	D	Q	D	Q	D	Q	D	Q	D	Q
Lipids	3	3	2	2	1	1	4	4	3	3	2	2	3	3	2	2	1	1
Glycogen	0	0	1	1	2	2	0	0	1	1	2	2	0	0	1	1	2	2
Myoglobin	3	4	2	3	1	2	3	4	2	3	1	2	4	3	3	2	2	1
Succinic dehydrogenase	3	3	2	2	1	1	3	4	2	3	1	2	3	3	2	2	1	1
Adenosine triphosphatase	3	3	2	2	1	1	3	3	2	2	1	1	3	3	2	2	1	1
Phosphorylase	1	1	2	2	3	3	1	1	2	2	3	3	1	1	2	2	3	3
Lipase	4	3	3	2	2	1	3	4	2	3	1	2	3	4	2	3	1	2

D-duck, Q-quail.

The intensities of staining reactions were arbitrarily recorded as follows.

0 negative reaction, 1 weak reaction, 2 moderate reaction, 3 strong reaction, 4 very strong reaction.

*Part of M.V.Sc. [1]Thesis submitted by the senior author to University of Agricultural Sciences, Bangalore.

Present address: [2]Associate Professor, [3]Professor and Head, [4]Professor, Department of Anatomy and Histology, Veterinary College.

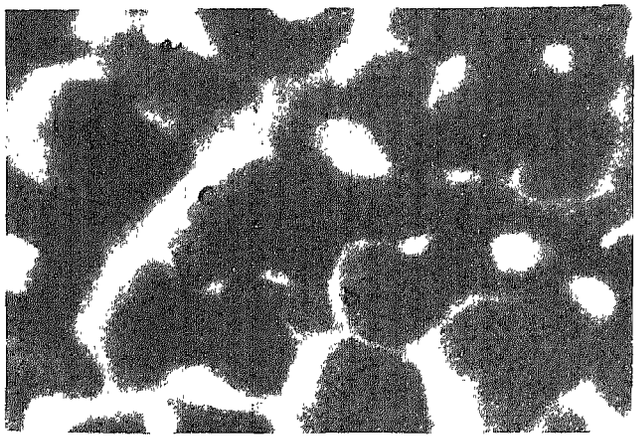

Fig. 1. Photomicrograph of *M. latissimus dorsi anticus* of duck showing strong reaction for phosphorylase in Type II fibres, moderate reaction in intermediate fibres and weak reaction in Type I fibres. Iodine method for phosphorylase. × 500.a, type I fibre, b, intermediate fibres, c, Type II fibres.

activities (Table 1). These features find agreement with George and Berger (1966), Morita *et al.* (1969) and Nishiyama (1966).

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Indian Journal of Animal Sciences **70** (2): 161-162, February 2000

Comparative histochemical studies of *M.latissimus dorsi anticus et posticus* and *M.supracoracoideus* in ducks and quails:— Percentage of fibre types*

A N RAMESHA[1], R V PRASAD[2], K N CHANDRAMOULY[3] and KRISHNA KAKADE[4]

University of Agricultural Sciences; Hebbal, Bangalore, Karnataka 560 024

Received: 2 January 1999; Accepted: 13 August 1999

Key words: Ducks, Fibre types, Muscle fibres, Quails

The percentage distribution of fibre types in the muscles of ducks and quails were recorded and an attempt has been made to correlate the percentage of fibre types and the type of physiological activity. The muscle fibres were classified into type I, intermediate and type II fibres based on succinic dehydrogenase and phosphorylase activities (George and Berger 1966).

M. latissimus dorsi anticus et posticus and *M. supracoracoideus* were collected from 10 each of adult ducks and quails. The frozen unfixed sections of 20μm were incubated for succinic dehydrogenase and phosphorylase activities (Pearse 1972).

The paraffin sections cut at 5-6μm were stained by haematoxylin and eosin (Luna 1968). In each of the muscle, the different fibre types were classified as type I (narrow), intermediate and type II (broad) based on histological and histochemical parameters and the diameter of fibres. A total of 1 000 fibres in each muscle were randomly selected to calculate the percentage distribution of each fibre type based on histoenzymic reaction and their mean and standard error were statistically analyzed (Snedecor and Cochran 1967).

In the present study the percentage of fibre types in *M. latissimus dorsi anticus* was 51.4 type I, 13.5 intermediate and 35 type II in quails and 51.6 type I, 12.17 intermediate and 35.17 type II in ducks as reported by Rosser *et al.* (1987) in the *M. latissimus dorsi pars cranialis* of Japanese quail.

In *M. supracoracoideus* 53.13%, 8.13% and 38.75% in quail and 50.5%, 10.5% and 39% in duck were recorded as percentage distribution of type I, intermediate and type II fibres respectively. This is in accordance with the findings of Salt (1963) who reported predominance of type I fibres in the *M. supracoracoideus* of the duck.

M. supracoracoideus and *M. latissimus dorsi anticus* of both ducks and quails contained predominantly type I fibers and may serve to hold wings in position during gliding to maintain body posture (Telesara and Goldspink 1978).

M. latissimus dorsi posticus type I, intermediate and type II fibres, respectively, recorded 35.5%, 15.6% and 48.9% in quails whereas in ducks the percentage of these fibres were 38.0%, 11.6% and 50.45% respectively. Thus there was predominance of type II fibres in *M. latissimus dorsi posticus* of ducks and quails similar to the study of Nishida *et al.* (1997).

The fast glycolytic fibres are adapted for relatively short contraction and the fibres are capable of developing maximal forces for saltatory motion. Therefore these fibres do not support sustained flight due to their dependence on the insufficient anaerobic pathway for energy production. (Sickles and Pinkstaff 1981). *M. latissimus dorsi posticus* in the duck and quail observed in the present study may help these birds in short bursts of flight.

*Part of M.V.Sc. [1]Thesis submitted by the senior author to University of Agricultural Sciences, Bangalore.

Present address: [2]Associate Professor, [3]Professor and Head, [4]Professor, Department of Anatomy and Histology, Veterinary College.

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Indian Journal of Animal Sciences 70 (2): 163-165, February 2000

Kinetics of intravenously administered ceftriaxone in lactating goat

K D YADAV[1], C JAYACHANDRAN[2], M K SINGH[3], H N JHA[4] and S P SINHA[5]

Rajendra Agricultural University, Patna, Bihar 800 014

Received: 5 May 1998; Accepted: 10 August 1999

Key words: Ceftriaxone, Goat, Kinetics, IV

Pharmacokinetic studies of ceftriaxone were reported in rabbit (Joly *et al.* 1988), sheep (Guerrini *et al.* 1985), calf (Soback and Ziv 1988), and mares (Gardner and Aucoin 1994), but available literature is almost devoid of any such studies in goat. The present study describes disposition, kinetics and distribution of ceftriaxone in various biological fluids in lactating goat.

Clinically healthy lactating goats (6) on non-descript breed between 1.5 and 2 years of age, and 25 to 30 kg weight were used. Sterile ceftriaxone sodium powder USP equivalent to ceftriaxone anhydrous 1 g was dissolved in 10 ml of sterile distilled water and was injected @ 20 mg/kg body weight in each goat by intravenous (IV) route. For collection of urine, a Foley's balloon cathter (No. 12) was inserted into the bladder through urethra and kept in position by inflating the balloon by injecting 20-30 ml of water. Milk was collected manually. Samples of plasma, milk and urine were collected at 2.5, 5, 7.5, 10, 15, 20, 30 and 45 min and 1, 1.5, 2, 2.5, 3, 4, 6 and 8 hr. The urine was collected beyond 8 hr at 10, 12 and 24 hr post IV administration of ceftriaxone. Assay of ceftriaxone in these body fluids were carried out by cylinder plate diffusion method using *Sarcina lutea* (ATCC 9341) as the test micro-organism (British Pharmacopoeia 1980).

Since the profile of log plasma drug concentration versus time showed a biphasic curve, kinetic parameters were calculated from formulae derived for a 2-compartment open model (Gibaldi and Perrier 1975, Baggot 1977, Notari 1980). Rational dosage regimen can be calculated for a drug having biological half life ($t_{1/2}\beta$) between 4 and 16 hr (Baggot 1977). Since $t_{1/2}\beta$ of ceftriaxone is well below 4 hr in this study, the dosage regimen has been suggested on the basis of maintenance of therapeutic concentration taking into account of the lag phase of bacteria.

At 2.5 min, plasma concentration of ceftriaxone was 33.08 ± 2.22 μg/ml and the drug was detectable up to 3 hr in plasma (Table 1). The drug appeared only at 20 min, reached its peak concentration at 45 min and was detectable up to 2 hr in milk. In urine, the drug appeared at 2.5 min, attained its peak concentration at 45 min and was detectable even beyond 24 hr.

The minimum bactericidal concentration (MBC) for *E. coli* was 0.06 μg/ml for ceftriaxone (Pangon *et al.* 1987). Regamey (1985) reported minimum inhibitory concentration 90% (MIC 90%) for ceftriaxone susceptible organism to be below 2 μg/ml. Hence, in this study 1 μg/ml was considered as therapeutic concentration. The therapeutic concentration (1 μg/ml) of the drug was maintained from 2.5 min to around 1.5 hr and 2.5 min to > 12 hr in plasma and urine, respectively. However, the drug failed to attain its therapeutic concentration in the milk samples.

Table 1.Concentrations (μg/ml) of ceftriaxone in various biological fluids in lactating goat following single intravenous dose of 20 mg/kg

Time	Value in mean ± SEM (n = 6)		
	Plasma	Milk	Urine
2.5 min	33.08 ± 2.22	ND	14.33 ± 7.03
5 min	26.75 ± 2.04	ND	69.33 ± 15.91
7.5 min	20.66 ± 1.22	ND	151.50 ± 30.12
10 min	17.50 ± 1.38	ND	239.50 ± 35.57
15 min	12.87 ± 1.34	ND	413.33 ± 19.73
20 min	9.52 ± 0.85	0.10 ± 0.04	764.17 ± 30.73
30 min	5.90 ± 0.39	0.24 ± 0.03	1109.17 ± 128.58
45 min	3.35 ± 0.27	0.42 ± 0.04	2295.83 ± 240.52
1 hr	1.36 ± 0.27	0.39 ± 0.04	1986.67 ± 138.21
1.5 hr	0.96 ± 0.17	0.23 ± 0.02	1314.17 ± 110.61
2 hr	0.62 ± 0.10	0.11 ± 0.02	880.00 ± 37.04
2.5 hr	0.36 ± 0.06	ND	706.67 ± 19.94
3 hr	0.11 ± 0.08	ND	497.50 ± 36.46
4 hr	ND	ND	283.33 ± 8.51
6 hr	ND	ND	154.17 ± 17.81
8 hr	ND	ND	70.67 ± 8.19
10 hr	ND	ND	32.17 ± 4.38
12 hr	ND	ND	8.13 ± 1.91
24 hr	ND	ND	0.65 ± 0.33

ND, Not detected.

Present address: [1]Touring Veterinary Officer, Gauharpur, Patna 800 009, [2,4,5]Associated Professor, [3]University Professor, Department of Pharmacology.

Distribution rate constant (α) and distribution half life (t½ α) were noted in the present investigation in lactating goat (Table 2). Similar t½ α was reported in mare (Gardner and Aucoin 1994). Guerrini *et al.* (1985) noted a higher value in sheep which denote a quicker distribution of ceftriaxone in sheep as compared to goat and mare.

The shorter t½ β of ceftriaxone in goat is further supported by higher rate constant of drug elimination from central compartment (Kel) and total body clearance (Table 2). The t½ β in sheep (Guerrini *et al.* 1985) and in mare (Gardner and Aucoin 1994) after IV administration of ceftriaxone are more or less similar to that obtained in goat in this study. However, higher t½ β in calf (Soback and Ziv 1988) and rabbit (Joly *et al.* 1988) were reported.

The values of rate of transfer to drug from central of peripheral (K_{12}) and peripheral to central (K_{21}) compartment were calculated. However, Guerrini *et al.* (1985) noted higher values for K_{12} and K_{21}, in sheep. The values of fraction of drug available for elimination from central compartment (Fc), tissue to plasma concentration ratio (T » P), area under plasma concentration curve (AUC) and volume distribution (Vd area) are shown in Table 2.

Vdarea obtained for ceftriaxone in lactating goat is comparatively higher than the values obtained in sheep (Guerrini *et al.* 1985) and calf (Soback and Ziv 1988). A large volume of distribution (> 1 /L/kg) of a drug indicates its wide distribution throughout the body or extensive tissue binding or rapid excretion or combination of all the above. Ceftriaxone is highly lipophilic in nature (Martindale 1982) and is excreted well through the urine. The high values of volume distribution obtained in goat for ceftraixone may be because of its high excretion in urine (Table 1) and its possible storage in fat depots. The total body clearance (CLB) obtained in the present study in goat was quite higher as compared to the values in sheep (Guerrini *et al.* 1985), calf (Soback and Ziv 1988) and mare (Gardner and Aucoin 1994).

The therapeutic concentration of 1 μg/ml was maintained in plasma from 2.5 min to around 1.5 hr after IV dose of 20 mg/kg in lactating goat. By taking into account the lag phase of bacteria, ceftriaxone may be administered every 4 hr @ 20 mg/kg for treating septicaemia and other systemic infections in goat. But, frequent administration of the drug may not be found popular with clinicians. Since the drug did not reach the therapeutic concentration in milk, ceftriaxone may not be of much use in treating mastitis. The drug maintained its therapeutic concentration from 2.5 min to even beyond 12 hr but lesser than 24 hr in lactating goat. By considering the lag phase of bacteria, ceftrioxine may be administered @ 20 mg/kg every 24 hr for treating urinary tract infections caused by ceftriaxone susceptible bacteria.

Table 2. Kinetic parameters of ceftriaxone in lactating goat following single intravenous dose of 20 mg/kg

Kinetic parameters	Value (Mean ± SEM) n=6
Zero time concentration (μg/ml)	
Distribution (A)	25.11 ± 3.58
Elimination (B)	3.78 ± 1.24
Theoretical zero time concentration (C^{o}_{p}=A+B)	28.89 ± 4.62
Rate constant (hr^{-1})	
Distribution (α)	3.625 ± 0.543
Elimination (β)	0.856 ± 0.103
Half life (hr)	
Distribution (t½ α)	0.21 ± 0.02
Elimination (t½ β)	0.86 ± 0.08
Micro-rate constant for drug transfer (hr^{-1})	
Central to peripheral compartment (K_{12})	0.664 ± 0.193
Peripheral to central compartment (K_{21})	1.212 ± 0.219
Elimination from central compartment (Kel)	2.604 ± 0.287
Fraction of drug available for elimination from central compartment	
Fc	0.33 ± 0.02
Tissue to plasma concentration ratio	
T ≈ P	2.11 ± 0.28
Area under plasma concentration curve	
AUC (mg/L.hr)	10.98 ± 0.75
Volume distribution	
Vd_{area} (L/kg)	2.35 ± 0.31
Total body clearance	
Cl_B ($ml.kg^{-1}.min^{-1}$)	31.03 ± 2.08

ACKNOWLEDGEMENTS

We thank Dr B B Verma, Principal, Bihar Veterinary College, Patna 800014, for providing necessary facilities to carry out the present study. The authors duly acknowledge the supply of free samples of standard pure ceftriaxone powder for carrying out the assay by Aristo Pharmaceuticals India.

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Indian Journal of Animal Sciences **70** (2): 166-168, February 2000

Evaluation of polytetrafluoroethylene (PTFE) and frozen tracheal mucosa for reconstruction of artificial tendon sheath in cow calves: Clinical, radiological and histopathological study

NAVEEN KUMAR[1], O P GUPTA[2], G R SINGH[3], A K SHARMA[4] and N S PARIHAR[5]

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122

Received: 6 January 1999; Accepted: 13 August 1999

Key words: Air-tendogram, Artificial tendon sheath, Calves, Frozen tracheal mucosa, PTFE, Tendon

The role of PTFE and deep frozen tracheal mucosal grafts in the prevention of peritendinous adhesions was studied. Male cow calves (8), aged 5 to 6 months, were randomly divided into 2 equal groups A and B. The animals were kept off feed for 12 hr prior to surgery. and tranquilized with triflupromazine hydrochloride @ 1mg/kg body weight intravenously. The animals were secured in lateral recumbency and posterio-medial aspect of lower hind-limb was prepared for aseptic surgery. A crescent-shaped curved skin incision was made on the medial aspect between the fetlock and hock, under local infiltration analgesia and superficial digital flexor (SDF) tendon was exposed. Tendon sheath of 2 cm was removed and the tendon was severed transversely. The severed end of SDF tendon was anastomosed by Bunnell-Mayer technique using silk No. 1. Construction of new tendon sheath was done with PTFE graft in animals of group A and with frozen tracheal mucosa (– 4°C) in animals of group B. PTFE graft was autoclaved before reconstruction. Bovine trachea was collected from freshly sacrificed animals and washed thoroughly in sterile normal saline containing 2.5g of streptomycin in 40 ml of solution. Tracheal mucosa was carefully separated and used as a cuff for reconstruction of tendon sheath. The PTFE and tracheal mucosa were placed around the anastomotic site after anastomosing the tendon and edges of the graft were sutured with silk No. 2/0 to encase the anastomosed area. Skin wound is closed in the routine manner. The limb was immobilized with plaster of paris bandage incorporating hock and fetlock joints. Oxytetracycline dihydrate was administered @ 5mg/kg body weight for 7 days intramuscularly. Plaster of paris cast and skin sutures were removed 15-day postoperatively.

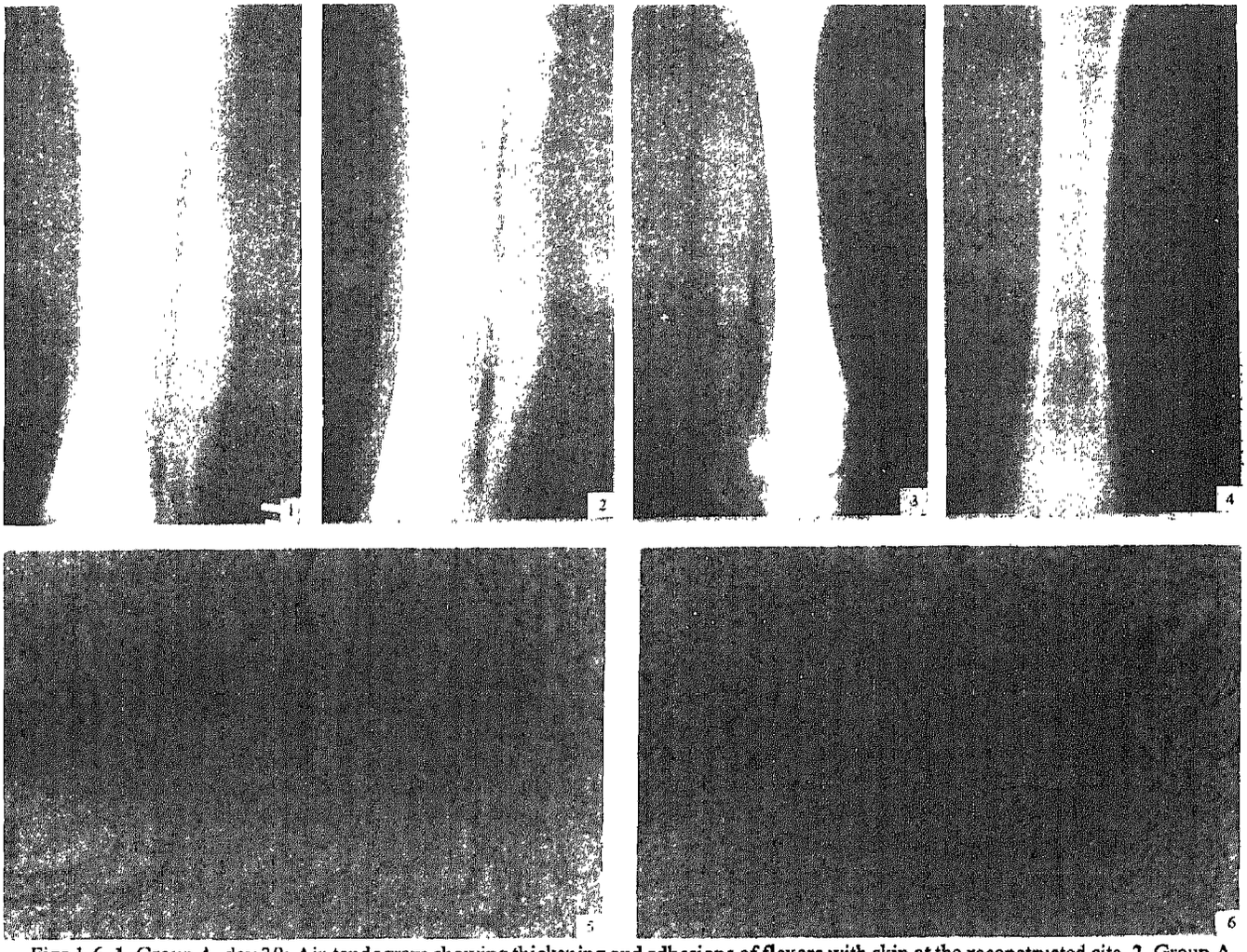
Rectal temperature, heart rate and respiration rate were recorded daily up to 7 days, after reconstruction in all the animals. Tendon gliding movements were observed at the operated site in animals of both the groups before surgery and on days 15, 30, 45 and 60 post-operatively. Air-tendograms (fasciagraphy) was performed as per method described by Saikia and Nigam (1980) to evaluate the status of healing and the presence of adhesions at the site of tendon reconstruction on day 30 and 60 post-operatively. The tendon biopsy specimens for histopathological examination were collected on days 30 and 60 post-operatively preserved in 10% formal saline solution and were stained with haematoxylin and eosin. The data of clinical parameters subjected to 2-way analysis of variance (Snedecor and Cochran 1984). A probability of $P<0.05$ was considered statistically significant.

No significant difference ($P>0.05$) in rectal temperature, heart and respiration rate was recorded up to day 7. The animals exhibited varying degree of lameness for the first 5 days. During this period the animals showed minimal weight bearing and used to rest on toe. Lameness and reduced weight bearing on the operated limb was due to pain and injury at the site. Verma *et al.* (1983a) observed limping gait for a week in buffalo calves following tendon autografting. Lameness decreased gradually thereafter and the animals showed normal gait and optimum weight bearing by day 15 post-operatively.

All the animals showed hard non-painful swelling at the site on day 15. Partial gliding movement between flexor tendons was observed on day 45 in animals of group A. Similar observations were also reported following reconstruction of tendon with autografts (Verma *et al.* 1983 a and b) and allografts (Dass *et al.* 1987) in buffalo calves on day 14. However, full gliding movement of flexor tendon was observed on day 42, following autografting in buffalo calves (Verma *et al.* 1983a). Full gliding movement was observed in group A on day 60, whereas gliding movements were partial in group B.

Air-tendogram in animals of group A on day 30 post-operatively (Fig. 1) revealed that skin, superficial and deep

Present address: [1]Scientist (Senior Scale), [2, 4]Senior Scientist, [3]Head, Division of Surgery, [5] Former Head, Division of Pathology.



Figs 1-6. **1.** Group A, day 30: Air-tendogram showing thickening and adhesions of flexors with skin at the reconstructed site. **2.** Group A, day 60: Few filmy adhesions of skin with tendon sheath constructed with PTFE. **3.** Group B, day 30: Marked thickening of flexors and severe adhesions of skin with S D F and D D F tendon. **4.** Group B, day 60: Relatively less adhesions and thickening of the reconstructed tendon compared with that on day 30 in the same group. **5.** Group A, day 60: Well developed fibrous connective tissue showing wavy appearance and parallel arrangement of collagen fibres at the site of reconstruction. H & E. × 50. **6.** Group B, day 60: Regenerating tendinous tissue intermingled with normal tendinous tissue with evidence of vascular reaction. H&E. × 50.

digital flexor resembled as homogenous thickened mass. The reconstructed site appeared thickened and adhesion with deep digital flexor and suspensory ligament was not observed. On day 60 (Fig. 2) the swelling at the reconstructed site was less as compared with that on day 30. Air-tendograms of group B on day 30 post-operatively (Fig. 3) revealed marked thickening of superficial and deep digital flexor severe adhesions of skin with SDF and DDF tendon. Adhesions with and suspensory ligament was not evident. On day 60, the swelling at the reconstructive site was relatively less but over all findings were similar to those of day 30 (Fig. 4).

Air-tendograms in animals of group A revealed that PTFE behaved as an inert material and adhesions between the reconstructed site and surrounding structure were relatively less as compared to group B. Selway (1975) also reported the use of PTFE as an artificial tendon sheath which resulted in the formation of long, gliding adhesions rather than short restrictive adhesions. Adhesions and thickening of both flexors in group B indicated severe inflammation at the site where freezed tracheal mucosa was used for the reconstruction of tendon sheath. Peacock Jr. (1964) also reported increased thickening and swelling at the site indicating more fibroblastic activity.

Microscopically, on day 30 in group A, the remnants of suture material were present within the different size masses predominantly of mononuclear cells. Regenerating tendinous tissues were intermingled with normal tendinous tissues with evidence of vascular reaction (Fig. 5). Collagen fibres were arranged in haphazard manner at the site of anastomosis. On day 60, the collagen fibres were arranged parallel to each other and less cellular reaction was observed at the site. Few adhesions were seen between PTFE and newly formed fibrous

connective tissues. Selway (1975) also used PTFE as an pseudo-sheath around tendon and reported formation long gliding adhesions leading to normal gliding movement of the tendon.

On day 30 in group B, the area between the ends of the anastomosis was acquired by fibrovascular connective tissues. There were adhesions between tendinous tissues and frozen tracheal mucosa. Remanants of the suture material were also observed. On day 60, the evidence of tracheal tissue around the anastomosis had practically disappeared except for the presence of little rarefied fibrovascular tissue. The area between the ends of the anastomosis was acquired by well developed fibrovascular connective tissue arranged in the tendinous structure (Fig. 6). The focus of suture was indicated by chronic inflammatory granulomatous reaction. The remnants of suture material were present within the different size masses predominantly of mononuclear cells. Various biological materials viz. formalin fixed arterial encasement (Nigam *et al.* 1975), paratenon (Stark *et al.* 1977) and human amniotic membrane (Singh 1994, Saini *et al.* 1998) have been successfully used to minimize the adhesions between tendon and skin. Nigam *et al.* (1975) reported formation of numerous young fibroblast at the anastomotic site running parallel to the newly formed capillaries with less cellular reaction.

On the basis of findings of the experiment, it can be concluded that PTFE can be used for reconstruction of artificial tendon sheath, whereas, freeze dried tracheal mucosa causes more thickening and inflammation at the site. On the other hand, PTFE behaved as an inert material.

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Indian Journal of Animal Sciences 70 (2): 169-170, February 2000

Use of carbon fibres for the reconstruction of superficial digital flexor tendon in crossbred calves: scanning electron microscopic

NAVEEN KUMAR[1], A K SHARMA[2] and SATISH KUMAR[3]

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122

Received: 1 January 1999; Accepted: 13 August 1999

Key words: Carbon (SDF) fibres, Fibroblasts, Scanning electron microscope, Superficial digital flexor tendon (SDFT)

A study was conducted to evaluate carbon fibres for the reconstruction of superficial digital flexor tendon (SDF) in bovines. Male crossbred cow calves —(12) aged 6 to 18 months and weighing between 80 and 250kg—were used. A 2.5cm long defect was created in SDF tendon at midmetarasal region under lumbosacral subarachnoid (spinal) anesthesia using xylazine (@ 0.05mg/kg body wt) and ketamine (@ 2.5mg/kg body wt, maximum dose 100mg). The defect was repaired with 2 strands of carbon fibres (each consisting of 6 000 filaments of 7.5 μ diameter) using modified locking loop suture (Brown and Pool 1983). Skin incision was closed with nylon and limb was immobilised up to hock with plaster of paris bandage. The tissue biopsies were collected on 7, 14, 30 and 90 post-operative days for SEM studies.

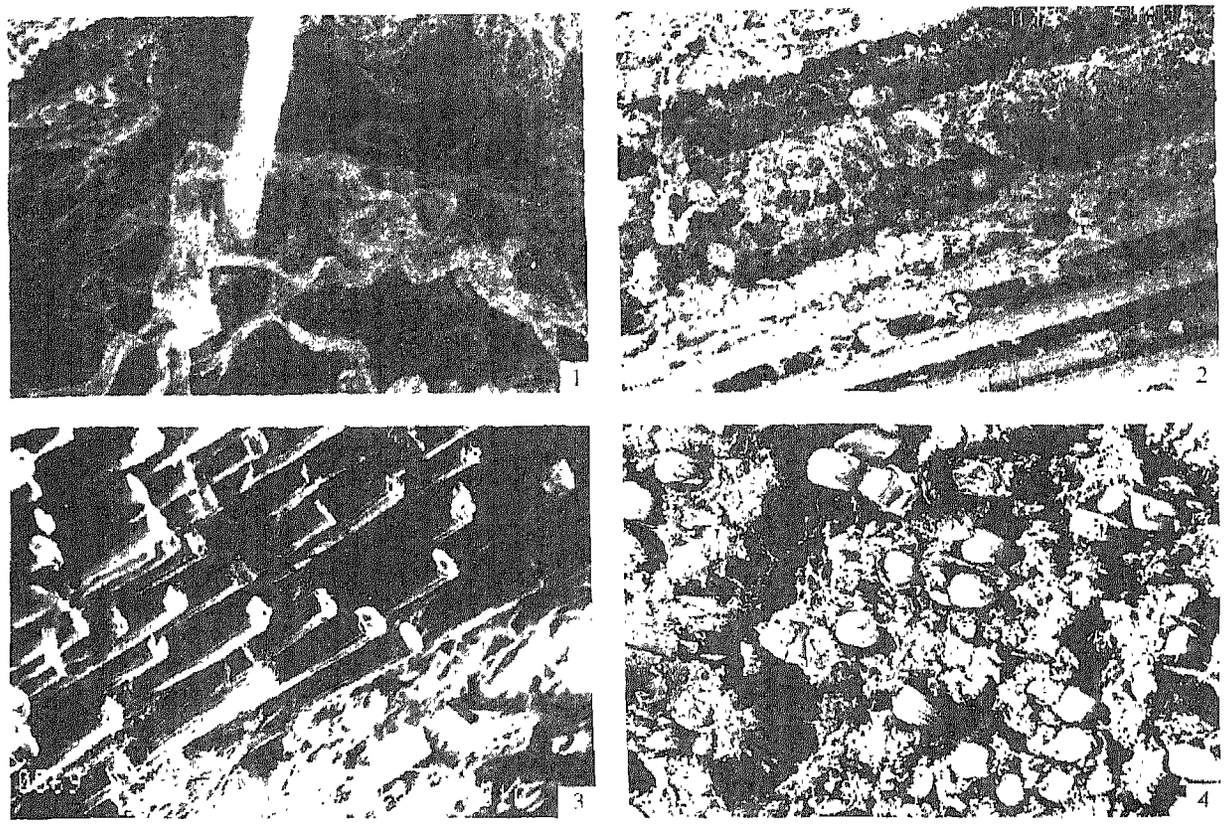
The biopsy samples were cut into small pieces with desired information, checked under magnifying glass and fixed into Karnovsky fixative at 4°C. After fixation for 3–4 days tissues were washed 3 times with phosphate buffer of pH 7.2. Specimens were dehydrated using serial gradient of alcohol in water. Finally tissues were washed 3 times in absolute alcohol. Hexamethyldisilazane (HMDS) technique was used for drying the specimens (Nation 1983). Specimens were mounted on aluminium stubs using adhesive carbon tape and sputtered with gold ions. The specimens were observed at 5 kv under scanning electron microscope. Magnification range of the unit was calibrated using colloidal latex particles of 1 μ size.

On day 7, the gap between the resected tendon ends was filled with highly vascularized granulation tissue having lossely filled tissue matrix and newly formed collagen fibrils. These fibrils were thin and slender in appearance (Fig. 1). Amorphous neoformed highly cellular tissue were present in abundance in between carbon fibre strands. Red blood cells (RBCs), macrophages and fibroblasts were seen in abundance at this stage. RBC was oval and biconcave in shape. Relatively small round smooth cells having beaded appearance were macrophages (Mendes *et al.* 1985a). The stellate or fusiform cells which have spread out their processes along the carbon fibres were immature fibroblasts (histiofibroblasts Mendes *et al.* 1984 a).

On day 14, the strands of carbon fibres were completely encircled by denser and well organised neoformed fibrous connective tissue. This new tissue was denser and more organised than on day 7. Fragmentation or breaking of the carbon fibres was not observed. At the exterior of the carbon fibre strands the neoformed fibrous connective tissue had grown in between the carbon fibre filaments which resulted in thickening of carbon fibre implant. Whereas, in the interior of carbon fibre strands very little tissue was formed in between the carbon fibre filaments and hence they were tightly packed compared to the exterior of the carbon fibre implant (Fig. 2). The ingrowth of fibrous tissue and collagen fibres in between the carbon fibre strands caused in increase in the diameter of the implant. Similar arrangement of collagen fibres was also observed by Jenkins *et al.* (1977) and Howard *et al.* (1984). Further, carbon fibre implant provides scaffold for migration of fibroblasts and early induction of neotendon similar in morphology to mature tendon (Jenkins *et al.* 1977, Forster *et al.* 1978).

On day 30, the collagenic network, encircled the carbon fibres and very little tissue was formed in the interior of carbon fibre strands. On day 90, the carbon fibre strands were completely surrounded by neoformed connective tissue. The tissue surrounding the carbon fibres was densely packed, well organised neoformed connective tissue resembling normal mature tendon (Fig. 3). Whereas, the new tissue which had grown in between the carbon fibre strands was relatively less organized and showed some occasional empty spaces/cavities (Fig. 4). These cavities/empty spaces ranged from 5 to 15 μ in diameter. Similar findings were also reported by Mendes *et al.* (1985b). Absence of tissue in midsection of carbon fibre implant with only occasional clumps of material following replacement of anterior cruciate ligament was also observed in rabbits (Amis *et al.* 1988). Intact carbon fibres were still

Present address: [1]Scientist (Senior. Scale), [2]Senior Scientist, [3]Senior Scientist, National Biotechnology Centre.



Figs 1-4. **1.** Scanning electron micrograph on day 7 showing carbon fibre filament surrounded by new formed collagen fibrils. × 1 000. **2.** Scanning electron micrography on day 14 showing very little tissue in the interior of carbon fibre strand (small arrow), whereas exterior of strand surrounded by tightly packed neoformed fibrous connective tissue (large arrow). × 1, 000. **3.** Scanning electron micrograph on day 90 showing densely packed well organised mature tendon tissue around the carbon fibre strand (arrow). × 500. **4.** Scanning electron micrograph on day 90 depecting cavities measuring from 5 to15 μ in diameter in the neoformed tendon. × 1, 000.

visible at this stage. Numerous uneven ridges on the surface of carbon fibres running along the filament were clearly visible on higher magnification (× 2, 000). The longitudinal grooving on the carbon fibre filament is a normal surface texture (Goodship *et al.* 1980, Amis *et al.* 1984). SEM in this study was useful in understanding the tendon healing process following the repair with carbon fibres.

ACKNOWLEDGEMENTS

The authors thanks to Dr O P Bahl and Dr R B Mathur, Carbon Technology Unit, National Physical Laboratory, New Delhi, for their collaboration and providing carbon fibres for the study.

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Indian Journal of Animal Sciences 70 (2): 171-173, February 2000

Endocrine response in fracture healing: Radiological study

S B SURYAWANSHI[1], S K MAITI[2], G R SINGH[3] and H C SETIA[4]

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122

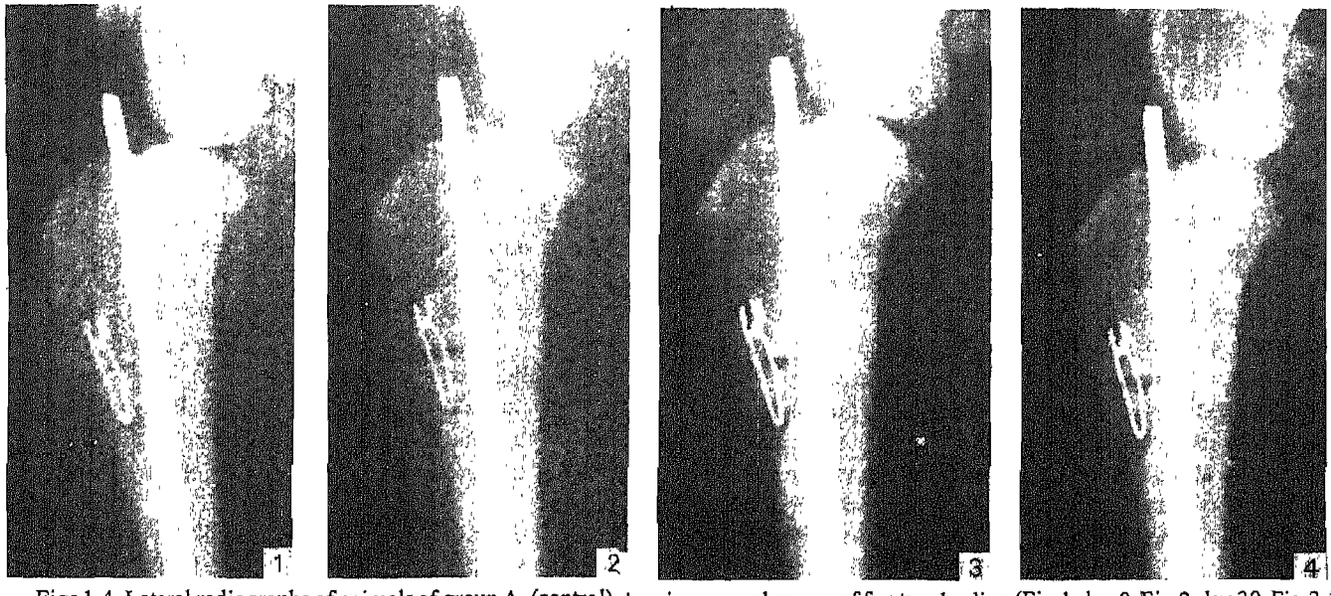
Received: 6 January1999; Accepted: 10 August 1999

Key words: Dog, Fracture healing, Insulin, Thyroxine, Tibia, X-ray

Healing of fracture is a consequence of cellular behaviour in a living organism and as such can be modified by almost all endogenous or exogenous factors that affect the metabolic function of cells (Cruess and Dumont 1975). These factors include transforming growth factor-β (Maiti and Singh 1996), bone morphogenetic proteins (Maiti and Singh 1998), hormones, vitamins and prostaglandins (Norrdin and Shih 1988). The anabolic hormones like thyroxine and insulin accelerated bone formation in experimental animals (Udupa and Singh 1964, Pandey 1985). Hence, the study was proposed to evaluate the effects of thyroxine and insulin therapy in the management of tibial metaphyseal fracture in dogs.

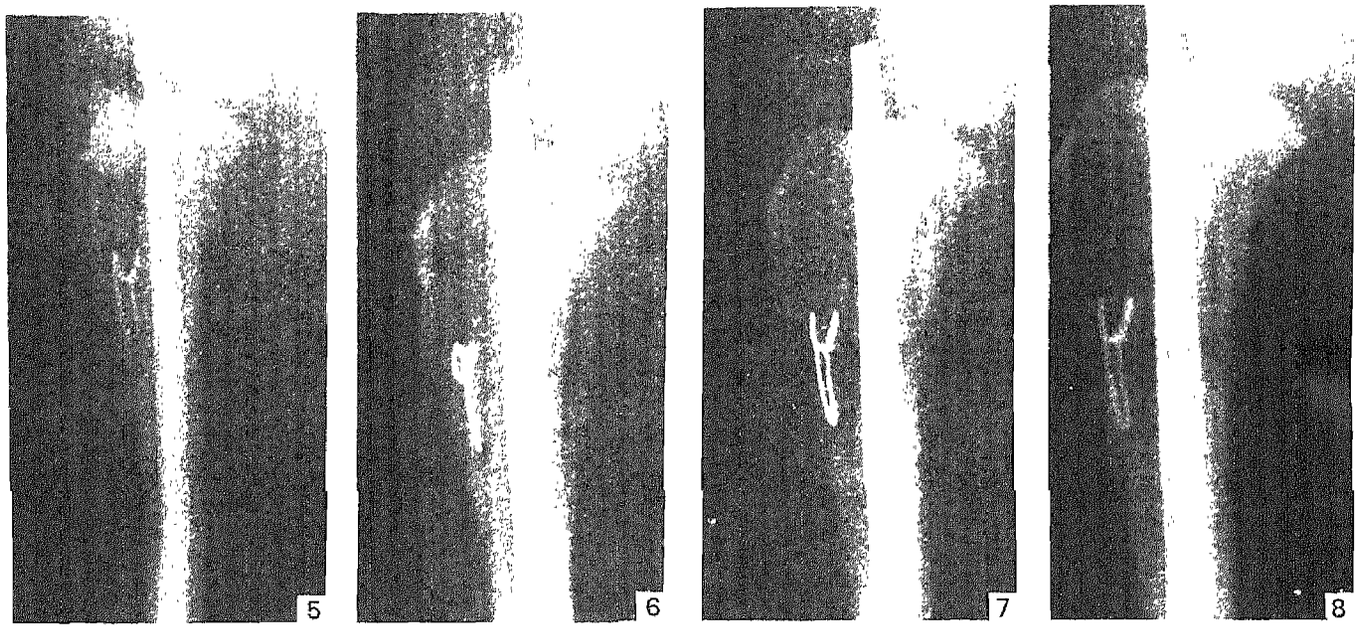
Clinically healthy, adult mongrel dogs (15) of either sex were randomly divided into 3 groups (A_1, A_2 and A_3) of 5 animals each. In all the animals, unilateral metaphyseal fracture was created in the proximal tibia and then immobilized with cross intramedullary pinning with interfragmentary wiring under thiopental (5%) anaesthesia. 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 lorexane cream for 5 days post-operatively. In group A_2, thyroxine sodium tablets (0.013 mg/kg body wt, orally) and in A_3, insulin injection (2 IU/kg body wt, s/c) were given on day 7 and then on every alternate day till day 45 post-operatively, at one specific time. Group A_1, remained as control, in this group no hormonal therapy was given.

Craniocaudal and mediolateral radiographs of the fracture site were taken immediately after surgery and subsequent on days 15, 30, 45 and 60 post-operatively. The radiographs were observed for status of fracture reduction and fixation immediately after surgery and subsequently for the maintenance of reduction, progress of fracture healing and



Figs 1-4. Lateral radiographs of animals of group A_1 (control) showing normal course of fracture healing (Fig.1-day 0, Fig.2-day 30, Fig.3-day 45 and Fig. 4-day 60).

Present address: [1]M.V.Sc. Scholar, [2]Scientist (Senior Scale), [3]Head, Division of Surgery, [4]Technician (T-6, Radiology).



Figs 5-8. Lateral radiographs of animals of group A_2 (Thyroxin treated) showing accelerated course of fracture healing (Fig.5-day 0, Fig.6-day 30, Fig.7-day 45, and Fig. 8-day 60).

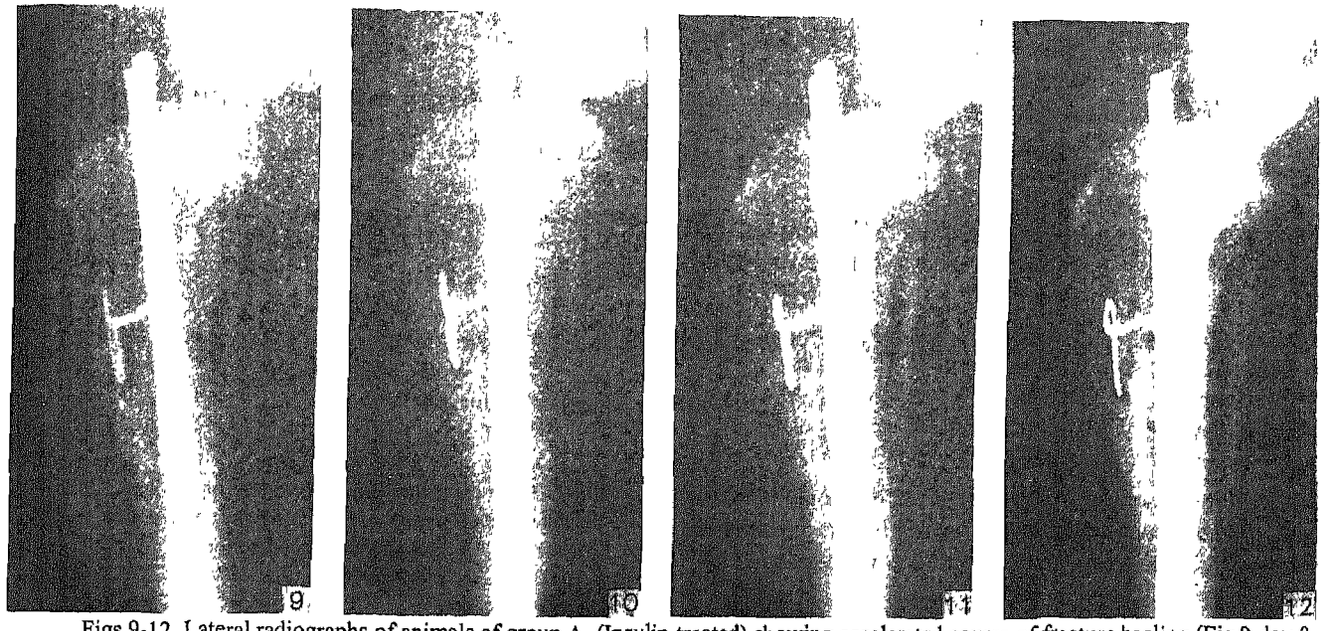
complication, if any, at different intervals.

Radiographs taken immediately after surgery showed satisfactory reduction and alignment of fracture fragments in all animals. However, little fracture gap was invariably present in all the animals (Figs 1, 5, 9) and the gap was relatively more at the cranial edge of fracture site compared to caudal edge. This could be attributed to the greater tensile forces generated by the extensors of the stifle like quadriceps femoris muscle acting on the cranial edge. The inter-fragmentary wire applied at this edge though provided good apposition, could not neutralize the tensile forces completely. At the caudal edge the gap was very negligible due to compressive forces (Bloomberg 1993). Radiographs taken at subsequent intervals showed maintenance of implant and fixation in all the animals indicating proper reduction and fixation of fracture.

In the group A_1 (control) radiographs taken at different intervals did not show any change in the position of either pins, wires or fracture fragments except in 1 animal where 1 pin was seen to have crossed the distal cortex of the tibia and in another animal, splintered fracture occurred at day 15. However, fixation was maintained and no other complication was seen till the end of the observation period. Periosteal proliferation at the site of bone defect was not visible in the radiographs on day 15, though the periphery of the defect became smooth and uniform. In the day 30-radiograph, there were indication of new bone proliferation from the periphery. In almost all the animals periosteal and intercortical callus was seen adjacent to the area of bone defect which obliterated the defect site (Fig.2). On day 45, fracture gap further reduced by bridging callus and it became more organized (Fig.3). Though fracture healing was perfect at the caudal border of the defect, little gap was still present at the cranial border and remodelling process continued in all animals by day 60 (Fig.4).

Radiographs taken at different intervals in thyroxine-treated group (A_2) revealed excellent bone healing in almost all the animals of this group without any complication, except in 1 animal, wherein splintered fracture occurred, as seen in 1 animal of group A_1. At day 15, the periphery of the fracture line became uniform and smooth. In all the animals there was presence of new bone formation, as evident by periosteal and intercortical new bone formation adjacent to the area of bone defect. Fracture line appeared irregular and hazy. On day 30, periosteal bridging callus became organized and obliterated the fracture gap (Fig.6). Further organization of bridging callus and obliteration of fracture gap was seen in all animals on day 45. Bone defect at caudal border was completely obliterated by new bone (Fig.7). By day 60, there was complete obliteration of fracture gap even at the cranial border by organized new bone and earlier remodeling of periosteal callus started in all animals (Fig.8). There were no radiographic signs of either delayed/non/malunion or bone infection in any animal, at any interval. In general, the healing was best in this group of animals when compared among 3 groups.

The fixation was maintained in all the animals of A_3 group up to the end of the observation period and all the animals showed normal course of fracture healing. without any complication. On day 15, though the periosteal callus was not markedly pronounced as in group A_2, the radiographs revealed an increased density at the fracture site. By day 30, periosteal callus was distinguishable. The fracture gap reduced



Figs 9-12. Lateral radiographs of animals of group A_3 (Insulin-treated) showing accelerated course of fracture healing (Fig.9-day 0, Fig.10-day 30, Fig.11-day 45 and Fig. 12-day 60).

substantially in all animals (Fig.10). The day 45 radiographs revealed further reduction in the fracture gap and the bridging callus became more organized (Fig.11). On day 60, excellent healing at the fracture site was observed with the presence of periosteal and intercortical callus and the cortical continuity appeared to be normal (Fig.12). In general, the healing was better in A_3 than that in group A_1 but the amount of callus was lesser than that of group A_2.

The animals of hormone-treated groups (A_2 and A_3) showed early signs of fracture healing compared to control group (A_1). This indicates that exogenous administration of thyroxine and insulin has helped to enhance osteogenesis and fracture healing. The thyroid hormone accelerates bone healing by influencing different phases of endochrondral ossification (Pitt-Rivers and Tata 1959). Thyroxine produces reduction in the immature tissue components forming more of cartilaginous tissue in the callus initially followed by quicker transformation into bone leading to early remodeling (Koskinen 1967, Udupa and Gupta 1965) which was also found in this study. Whereas, insulin is associated with increased collagen synthesis, stimulation of osteoblastic activity and proper utilization of glucose and hexophosphates (Singh and Udupa 1966). These hexophosphates are important source of phosphorus for calcification of bone matrix. Pandey (1985) reported early replacement of the hypertrophied cartilage tissue by mature collagen fibres and new bone formation in the insulin-treated fracture cases. Similar observation was also observed in this study. These findings suggest that thyroxine and insulin administration helped to enhance the process of fracture healing, however, thyroxin had little edge over the insulin.

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Indian Journal of Animal Sciences 70 (2): 174-175, February 2000

Fertility status in crossbred heifers in relation to their steroid hormonal profiles before and after insemination*

M BALAKRISHNAN[1], M G GOVINDAIAH[2], M SREENATH[3] and A V RAI[4]

University of Agricultural Sciences, Bangalore, Karnataka 560 024

Received: 28 December 1998; Accepted: 17 August 1999

Key words: Crossbred heifers, Fertility, Hormonal profile, Insemination

The information regarding the steroid hormonal profiles in fertile and repeat breeding crossbred heifers in India is scanty. The present study was aimed to provide information in this regard.

Crossbred (HF × zebu; 3: 1) prepubertal heifers (n: 27), aged 18 to 22 months, with body weight ranging from 190–220 kg were selected from the dairy herd of the National Dairy Research Institute, Bangalore. The estrus was observed thrice/day (0700, 1400 and 1800 hr). Jugular blood samples (20 ml) were collected in heparinated tubes between 14.30 and 15.30 hr as per the given schedule: (i) from the beginning of the experiment, at 15-day-interval up to first observed estrus; (ii) every day for 3 consecutive days (first AI) and thereafter, (iii) at 3-day-interval until the onset of next estrus or 60 days of pregnancy. Only first 3 service cycles were followed. Separated, frozen and thawed (before assay) blood plasma samples were assayed for progesterone (P_4) and estradiol 17ß (E_2) concentrations, by radioimmunoassay, following the procedures of Balakrishnan *et al.* (1986, 1992). The breeding data were classified in to 2 groups: (i) fertile (FG– conceived within 3 cycles), and (ii) repeat breeders (RBG– not conceived within cycles).

The data were subjected to ANOVA and wherever significant ($P \leq 0.05$) effects were found, the means were compared, using Duncan's multiple range test (Steel and Torrie 1960).

The overall mean (±) age and body weight of heifers at maturity (first detectable estrus), were 698.5±15.8 days and 239.1±5.5 kg respectively. In the first estrus none of the heifers conceived.Out of the 27 animals, 13 were conceived within 3 services (AI), remaining 14 were RB(s) having significantly lower body weight (183.79± 6.44 kg) around the age at maturity. In FG, there was a progressive and significant ($P \geq 0.05$), increase in the P_4 levels after 6 to 21 days of AI (3 032, 3 750 and 4 105 pg/ml respectively) compared to RBG (Fig.1). It continued to rise up to 6 067 pg/ml on 60 th day of pregnancy, whereas, in RBG it dropped gradually before the onset of next cycle. The peak E_2 concentration (21 pg/ml) on the day of estrus in FG dropped to none pg/ml on the following day (Fig.1). From day 18 onwards it started increasing slowly in consistent with the increase in P_4 levels up to 60 days of early pregnancy (ranging between 12 and 28 pg/ml). In case of RBG, the peak E_2 concentration on the day of estrus (13 pg/ml) dropped rather slowly up to day 3, thereafter ranged between 7 and 9 pg/ml up to 21 days of post AI.

During follicular phase (FP), the E_2 level in FG (16 pg/ml) was higher ($P \leq 0.05$) than the RBG (13 pg/ml) (Table 1). However, RBG showed higher ($P \leq 0.05$) E_2 concentration (10 pg/ml) during luteal phase (LP) compared to FG (6 pg/ml). The overall P_4 concentration in the FG heifers (2 294 pg/ml) was significantly ($P \leq 0.05$) higher than the RBG (893 pg/ml). However, the E_2 level in the FG was significantly lower ($P \leq 0.05$) than the RBG (9 vs 10 pg/ml).

Table 1. Mean concentrations of progesterone and estradiol 17ß during different phases of estrous cycle in fertile and repeat breeding crossbred heifers

Fertility groups	Progesterone *Pg/ml*	Estradiol 17ß *Pg/ml*
Follicular phase (-2.0 and 2 days)		
Fertile	232.00	16.33[a]
Repeat breeders	184.00	12.59[b]
Early luteal phase (3, 6 and 9 days)		
Fertile	1245.00[a]	7.00
Repeat breeders	460.00	7.87
Luteal phase (12, 15 and 18 days)		
Fertile	4235.00[b]	5.73[a]
Repeat breeders	1685.00	10.07[b]

*Part of Ph.D. Thesis of the first author, awarded by the University of Agricultural Sciences, GKVK, Bangalore, Karnataka 560 064.

Present address: [1]Senior Scientist, [3]Technical Officer, SRS of NDRI, Bangalore 560 030,

[2]Professor and Head, Department of Animal Genetics, Biostatistics and Breeding, [4] Former Director of Instructions (Veterinary), Veterinary College, UAS, Hebbal, Bangalore 560 024.

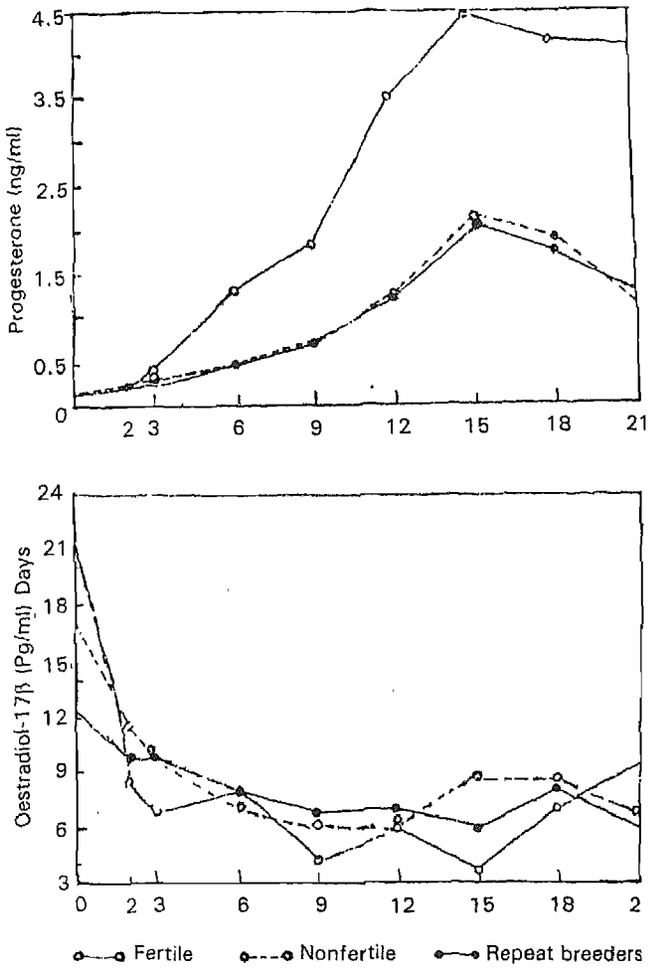

Fig. 1. Blood plasma progesterone (top) and oestradiol 17ß (bottom) concentrations in fertile and repeat breeding crossbred heifers from the day of estrus (0 day) to 21 days of post-insemination.

There is a pertinent concordance between the results obtained in the present study and earlier reports of Shemesh *et al.* (1983) in *B. taurus* and Balakrishnan *et al.* (1986) in crossbred heifers. During the period following AI, the increase in P_4 levels were significantly higher in FG heifers than other groups. The estrous cycle length observed in this study, both prior to and after AI were normal and are in agreement with the earlier reports (Balakrishnan *et al.* 1992) in crossbred heifers. The reason for the longer mean estrous cycle length following AI might be due to environment and management effects causing early embryonic death might have occurred in some of the heifers. Linares *et al.* (1982) reported that maternal production of P_4 may be normal even during abnormal embryo development in cattle. In the light of conformed reports that pregnancy failure might not be simply due to inability of the CL to secrete sufficient P_4. Further investigation in this regard is warranted.

The hormonal profiles, as recorded in the present study, were observed to be following the typical pattern in the fertile and repeat breeding heifers at different phases of the cycle which may be useful to identify reproductive problem animals at an early age and by adapting appropriate cost effective management tools.

ACKNOWLEDGEMENTS

We thank Dr R Nagarcenkar, (Former Director, NDRI, Karnal), Dr S R Sampath, (Former Head, SRS, Bangalore) for the facilities provided and to Mr K S Gurumurthy and A Louis for their technical assistance.

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Indian Journal of Animal Sciences 70 (2): 176-177, February 2000

Laparoscopic detection of ovulation rates and pregnancy in different sheep breeds

B A BUCHOO[1], N A WANI[2], M Z KHAN[3] and G M WANI[4]

S K University of Agricultural Sciences and Technology, Post Box No. 953, G.P.O.Srinagar, Jammu and Kashmir 190 001

Received: 21 May 1999; Accepted: 13 August 1999

Key words: Pregnancy diagnosis, Laparoscopy, Sheep ovulation rate

Detection of ovulation and pregnancy, aspiration of oocytes, embryo transfer and in utero semen deposition were performed by laparoscopy (Synder and Dukelow 1974, Blockey *et al.* 1979, Wani and Sahni 1988, Wani and Buchoo 1990). Pregnancy diagnosis in sheep has a limitation in comparison to large animals. Therefore the owner remains unaware about the fate of its pregnancy for a pretty long time and at time becomes economical burden when the animal turns to be dry. In the present study an attempt was made to detect the corpora lutea, an indirect method of pregnancy diagnosis and compare the results with actual lambing. Also an attempt was made to compare the ovulation rates of different breeds of sheep under our agroclimatic conditions.

Animals, 5 each, from Corriedale, Hampshire, Southdown and Polldorset were grouped together and maintained under identical conditions at the university sheep breeding farm. The animals showing estrous were tupped and laparoscopied 10 to 14 days after mating. The number of functional corpora lutea, unovulated follicles were counted in right and left ovaries and recorded. The birth of live young ones was also recorded. The observations were analyzed as per Snedecor and Cochran (1967).

Ovulation rates were higher (Table 1) in Southdown sheep than other 3 breeds but the differences were statistically nonsignificant ($P<0.05$). All the ewes who exhibited estrous among Polldorset breed had ovulated. While as 20% ewes from other 3 breeds showed unovulatory estrous. The right ovary was more active in all breeds except in Hampshire where left ovary was seen more active, however, statistically differences were nonsignificant may be because of small number of experimental animals in each group. Wani and Sahni (1988) have also seen right ovary more active in sheep than the left ovary. The ovulation rates differ in different breeds of sheep and is in agreement with the findings of Cumming, (1979). However, breed differences, nutrition and agroclimatic conditions play a role in this neuroendocrine phenomenon (Davis and Cumming 1976).

Table 1. Ovulation rates in different breeds of sheep

Breed (n=5 in each breed)	Ovulation rate	Unovulated follicles (No.)	Anovulatory estrous (%)	Ovulations Right: Left ovary
Corriedale	0.8 ± 0.2^a	5.20 ± 1.9^a	20.00^a	3.0: 1.0
Southdown	1.2 ± 0.37^a	5.60 ± 0.87^a	20.00^a	5.0: 1.0
Polldorset	1.0 ± 0.00^a	7.00 ± 0.95^a	0.00^a	4.0: 1.0
Hampshire	0.8 ± 0.2^a	6.80 ± 1.62^a	25.00^a	1.0: 2.0

Values with same superscript in a column does not differ significantly.

All the animals detected pregnant on the basis of presence of functional corpus luteum delivered a live young lamb, showing 100% accuracy of the method for early pregnancy detection (Table 2). In Southdown sheep out of 5 animals 2

Table 2. Laparoscopic detection of corpus lutcum as an indicator of early pregnancy in sheep

Breed	Tag No.	No.of corpora lutea	Birth of lamb
Corriedale	90C126	0.00	–
	FC100	1.0	single
	87C176	1.0	single
	91C191	1.0	single
	88C192	1.0	single
Southdown	91S6	1.0	single
	89S6	2.0	single
	89S4	1.0	single
	88S4	0.0	–
	87S2	2.0	single
Hampshire	87H3	1.0	single
	91H8	1.0	single
	87H2	1.0	single
	88H3	0.0	–
	89H3	0.0	–
Polldorset	91P18	1.0	single
	91P11	1.0	single
	91P15	1.0	single
	90P7	1.0	single
	89P17	1.0	single

Present address: [1] Associate Professor, Veterinary Surgery, [2]Assistant Professor, [3] Associate Professor and Head, Animal Reproduction and Gynaecology, [4] Director, Extension Education.

were having twin corpora lutea but delivered a single viable lamb, may be because of early embryonic deaths or fertilization of only 1 oocyte.

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Indian Journal of Animal Sciences 70 (2): 178-179, February 2000

Skin blood flow during pre-and post-treadmill exercise in buffaloes

PARVEEN KUMAR[1], S V SINGH[2], R C UPADHYAY[3] and ANJULI AGGARWAL[4]

National Dairy Research Institute, Karnal, Haryana 132 001

Received: 10 December 1997; Accepted: 13 August 1999

Key words: Buffalo, Skin blood flow, Treadmill

The information in buffaloes regarding normal values of skin blood flow (SBF) in pre-and post-exercising conditions is not available. Therefore study was conducted on buffaloes to measure the SBF at various anatomical sites using non-invasive laser Doppler flow meter.

Six male murrah buffaloes (average age 1.5 to 2 years, weighing about 100 to 150 kg) were maintained under standard feeding and managemental conditions as followed at the institute.

They were trained to walk on treadmill @ speed of 5 km/hr. Laser Doppler Skin Blood Flow Meter was used to measure SBF at rump (middle), hump (base), fore and hind legs (lateral gluteal muscle)and fore head(middle). The observations were taken at these sites during pre-and post-30 min treadmill exercise in all the animals during summer and hot humid seasons.The average maximum and minimum temperature (°C) and relative humidity (%) were 34.7 ± 1.5, 20.2 ±1.8, 39 during summer and 32.4±0.7, 25.0 ±0.4 and 70 during hot humid season.

The average pre-exercise value of SBF at rump was 6.27 perfusion units (PU's) (range 3.53±0.57 to 11.34 ±0.48 PU's) during summer, whereas the respective value during hot humid season was 6.07 PU's (range 2.15±0.18 to 10.68 ±0.44 P.U's). However, the variations in SBF values were observed within individuals. Variations in SBF during exercise have also been reported by Oberg *et al.* (1979) and Tenland (1982). After animals were subjected to treadmill exercise, SBF increased to 13.99 PU's (6.88±0.40 to 17.30±1.01 PU's). The increase in skin blood flow occurred by 2-4 times over pre-exercise values with an average increase of 2.23 times. The skin blood flow increased may be due to increase in body temperature which resulted from metabolic heat production. Sweat glands are activated, skin blood flow is augmented as a function of core temperature and skin temperature (Wenger *et al.* 1975).No significant difference in SBF values was observed between the 2 seasons when the data was subjected to statistical analysis.

The SBF values at hump were 12.16 and 12.26 P.U's during hot and hot humid seasons respectively. The individual variations were observed as values ranged from 4.88±0.36 to 15.29 ±0.31 PU's during summer and respective values during hot humid season were 4.26 ±0.39 and 21.26 ±0.71 PU's. For SBF the individual variations may be due to differences in age, body weight and physiological status. Nilsson *et al.* (1980) also reported that acute physiological stimuli may cause well known vascular response which leads to variable blood flow to skin. Similar increasing trend was also observed after treadmill exercise at hump region. The SBF increased by 2-4 times in different animals over their initial pre-exercise values during summer and hot humid seasons. However, in

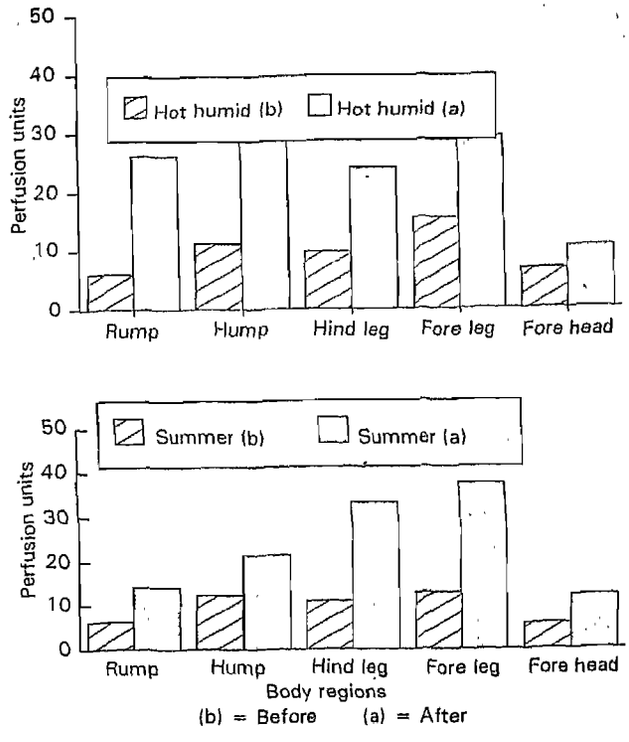

Fig. 1. SBF in buffaloes during hot humid and summer season.

Present address: [1,2]Scientist (SS), [4]Scientist, Division of Dairy Cattle Physiology.

[3]*Principle Scientists, CIRG, Makhdoom, Uttar Pradesh.*

some of the animals there was negligible increase during hot humid season (Fig.1).

The SBF values were 10.84 and 12.78 PU's during summer and 9.72 and 15.54 PU's during hot humid season at hind leg and fore leg respectively. The increase over pre-exercise values in SBF were 326 and 292% during summer and 247 and 188% during hot humid season at hind leg and fore leg region respectively. Tenland *et al.* (1983) reported normal SBF values in dorsal fore arm skin of human ranging from 14-28 PU's (0.143±0.021 to 0.276 ±0.068 volts). Manohar (1990) also reported that blood flow increased due to exercise over their initial values in ponies. Since the reference values in buffaloes are not available, therefore, the results could not be compared.

The average resting SBF values at fore head region ranged between 6 and 7 PU's which increased by 1.5 to 2 times over their initial values. The increase blood flow to this region helped to maintain the blood temperature and energy supply to the working muscles. Rowell (1974) also reported that with the increase in relative intensity of exercise, blood flow to visceral organs is reduced and these processes helps to maintain muscle perfusion and blood pressure. Probably reduced blood flow to splanchnic area helps to direct the flow of blood to skin which helps in heat dissipation and thermoregulation. The study revealed that treadmill exercise induced 2-3 times increased skin blood flow at different sites to maintain the internal milieu.

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Indian Journal of Animal Sciences 70 (2): 180, February 2000

Effect of extra vitamin E supplementation on hydropericardium syndrome of chicken

SANJEET KUMAR[1], M U KHAROLE[2] and JITENDER KUMAR[3]

CCS Haryana Agricultural University, Hisar, Haryana 125 004

Received: 7 January 1999; Accepted: 17 August 1999

Key words: Chicken, Hydropericardium syndrome (HPS), Vitamin E

The information on hydropericardium syndrome (HPS) in broilers was meager. This disease, well known among farmers as "Litchi disease", has got introduced in Jammu and Kashmir, Punjab, Haryana, and the occurrence of this disease is being reported from almost all parts of the country. The course of the disease is usually 10-14 days during which a mortality of 30-60% has been observed (Jaffery 1988).It is known that vitamin E and selenium play an important role in prevention of transudative lesions seen in transudative diathesis of poultry by reducing the capillary permeability. Day-old broiler chicks (n=25) were procured from a commercial hatchery and were divided into 2 groups. The chicks of both the groups were kept in separate houses and given *ad lib.* water and feed. Vitamin E was dissolved in 95% ethyl alcohol and then mixed in the feed @ 200 mg/kg feed (McIlroy *et al.* 1993). HPS infected control group, having 15 chicks, received normal feed and infection with 0.1 ml of HPS inoculum (20% infected liver suspension) subcutaneously in neck at 21 days of age.

The 10 chicks in vit. E fed and HPS infected group were fed extra vit. E @ 200 mg/kg feed from day 1 till termination of the experiment ie 32 days. At 21 days of age, the chicks were inoculated with HPS infected liver suspension (20%) with a dose of 0.1 ml subcutaneously in the neck.

The chicks were observed daily for clinical signs, mortality, gross and microscopic lesions up to 252 hr post-infection (hrPI) and average number of birds affected were calculated. Also, pericardial fluid was collected from sacrificed chicks and measured.

Mortality and gross lesion of hydropericardium was indicator of severity of disease and vascular permeability were studied in the HPS inoculated vit. E fed broiler chicks and were compared with control HPS infected chicks fed on normal diet (without extra vit. E). The mortality due to HPS was low (70%) and intensity of hydropericardium was slightly lower (average 1.5 ml) in the infected broilers fed extra vit. E as compared to the control chicks (without extra vit. E, 73% and 2.0 ml). Mortality in vit. E fed group was recorded earlier (48 hr PI) than the other group, but no mortality was seen after 66 hr PI in the vit. E fed group.

The gross and microscopic lesions observed in the control group (infected without vit. E) were slightly more severe than the vit. E fed group. Although, diffuse fatty degeneration and intranuclear basophilic inclusion bodies were seen in the hepatocytes of vit. E supplemented group. However, the differences observed between the treated (vit. E) and untreated (control) groups in respect of mortality, gross and microscopic lesions and amount of fluid in the pericardium were not significant. Vitamin E is known to reduce the severity of cellular damage due to its antioxidative property and reduce transudate formation. Since no significant effect of vit. E was seen, the results of the present study indicated that vit. E was not able to affect the mortality, pathology or reduce the vascular permeability (hydropericardium). Thus, suggesting that in HPS vit. E alone has either no or very little role in the pathogenesis of hydropericardium or on reducing the severity of pathological changes.

Present address: [1]Veterinary Surgeon, Veterinary Hospital, Kathumar, Alwar, Rajasthan.

[2]Professor, Department of Veterinary Pathology; [3]Assistant Professor, Department of Veterinary Physiology, Veterinary College, Mathura 281 001.

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Indian Journal of Animal Sciences 70 (2): 181-183, February 2000

Productive quality of Awassi rams in Rajasthan

A K DANG[1], A JOSHI[2], S M K NAQVI[3] and J P MITTAL[4]

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

Received: 10 January 1999; Accepted: 13 August 1999

ABSTRACT

Superior productive performance of Awassi breed of sheep is likely to improve production of nondescript sheep in arid and semi-arid regions of India. An experiment was conducted to study reproductive adaptability of Awassi rams under semi-arid climate of Rajasthan. The sexual behaviour, libido and semen quality of 2 adult Awassi rams kept on semi-intensive management system were studied. The sexual behaviour studied in terms of sniffing/nosing of external genitalia, kicking with forelegs, moving in and out of the tongue, courting grunts was similar to rams of native breeds, and Awassi were able to donate good quality semen sufficient for one oestrous cycle and each of these ram was capable of ejaculating 3-4 times during the span of 15 min. The semen volume, sperm motility and sperm concentration, in first and in subsequent ejaculations were similar to native and Awassi rams. It was apparent from these results that Awassi rams are well adapted, in their sexual behaviour, libido and semen characteristics under semi-arid region of Rajasthan.

Key words: Awassi rams, Libido, Semen quality, Semi-arid environment, Sexual behaviour

Since the productive attributes of Awassi breed suits to traditional system of husbandry of arid and semi-arid regions of India, there seems to be a good scope for introducing this breed for enhancing mutton, milk and wool production. The potential of the Awassi breed has not been explored for improving reproductive adaptability under semi-arid environment of India. The knowledge of the sexual behaviour of this breed may help in its effective use for breeding purposes. Therefore an experiment was conducted to study the sexual behaviour and semen quality of Awassi rams maintained under semi-arid climate of Rajasthan.

MATERIALS AND METHODS

Mature Awassi rams (2), age between 5 and 6 years, were used in the present study. The study was conducted during July 1998 (MDT 34.0 ± 0.37EC, RH 80.2 ±1.48%). Like rams of other native breeds these rams were also maintained on the prevailing semi-intensive management system being followed at this institute. Testicular dimensions viz. scrotal circumference, testicular diameter and length were measured at the beginning of the study which was conducted into 2 phases. In the first phase, parameters pertaining to libido viz. the reaction time and number of mounts up to first ejaculate were studied for 18 days when rams were introduced individually to an anestrus ewe restrained in an iron crate. During this period the semen quality traits like ejaculate volume, sperm density and sperm concentration were also studied in first ejaculate collected with the aid of an artificial vagina early in the morning at 7.00 AM In the second phase, sexual behaviour viz. sniffing/nosing of external genitalia, kicking with forelegs, moving in and out of the tongue, courting grunts, flehmen response; libido and time taken for first ejaculate and time between various ejaculations during 15 min for 4 consecutive days. The semen parameters mentioned above were also studied for each ejaculate collected during this time. Scrotal circumference, testicular diameter and testicular length were measured with the help of a cloth tape and vernier calliper.

RESULTS AND DISCUSSION

The mean values of scrotal circumference, testicular diameter, testicular length were 32.20, 30.00, 6.64, 6.15 and 19.80, 20.00 cm in rams 1 and 2 respectively. Positive relationship was obtained between testicular dimensions and ejaculate volume. Similar relationship was obtained by Tiwari and Sahni (1982) for crossbred (Rambouillet × Malpura) rams under semi-arid conditions of India. The restraining of ewe in an iron crate was necessary, at the recording observations pertaining to sexual behaviour of rams for improving their testing efficiency for minimizing the distraction and reducing variability in the behaviour of stimulus ewe. The immobilization of female is considered to be the greatest

Present address: [1]Scientist, [3]Scientist (Senior Scientist), [4]Head and Principal Scientist, Division of Animal Physiology; [2]Scientist (Senior Scale), Division of Animal Biochemistry.

Table 1. Libido and semen characteristics of Awassi rams recorded for 18 days (mean±SE)

Items	Ram 1	Ram 2
Number of mounts	3.33±0.43	1.94±0.28
Reaction time (sec)	21.22±3.21	18.82±5.75
Ejaculate volume (ml)	1.10±0.05	0.54±0.05
Sperm density (0-3 scale)	3.00±0.00	3.00±0.00
Sperm motility (%)	82.22±1.05	82.35±1.03

single stimulus for copulatory behaviour of rams (Signoret 1975) as with bulls and boars. In a study by Zenchek *et al.* (1988) when it was given freedom to move about, the anestrus ewe became less attractive to Rambouillet crossbred rams. Average values of libido and semen quality traits recorded for 18 days daily are given in Table 1. Higher values were recorded for libido traits and ejaculate volume in ram no. 1 in comparison to the values recorded for ram no. 2. This may be attributed due to higher body weight and age of ram no. 1. Values of semen characteristics are in agreement as reported by Epstein (1985) for Awassi rams in their native tract and in Malpura and Chokla rams under semi-arid environment (Honmode and Tiwari 1974). These results indicate that rams of Awassi breed can donate good quality semen continuously for covering one oestrous cycle without affecting its quality under semi-arid conditions of Rajasthan.

Data pertaining to behavioural responses, libido and semen characteristics of Awassi rams has been presented in Table 2. The behavioural responses were minimum before the first ejaculate because as soon as the rams were released they tried to mount the ewe and ejaculate. Reaction time was almost similar in both the rams. The average reaction time of Awassi rams recorded in the present study was significantly higher than Malpura and Chokla rams obtained by Honmode and Tiwari (1974) and was lower than that recorded by Mittal (1982) for Magra and Marwari rams. Fraser (1985) reported that in sexually mature males mounting behaviour becomes specifically oriented so that the female is appropriately covered for intermission to be effected. Frequency of mounts generally decreased for each successive ejaculate as the rams were able to effectively orient themselves behind the ewe after their first experience. In previous studies conducted by Honmode and Tiwari (1974) on Malpura and Chokla rams number of mounts decreased whereas average reaction time increased showing a progressive fall in sexual desire. The time recorded in present study between various ejaculates was less then that reported by Wiggins *et al.* (1953) in Rambouillet, Columbia, Targhee and Corriedale rams and by Mittal (1982) for Magra and Marwari rams. After each ejaculate the rams stood still for 10-20 sec (refractory period) which is assumed to be a state of sexual exhaustion (Fraser 1975). The behavioural activities recorded as sniffing, kicking,

Table 2. Sexual behaviour, libido and semen characteristics of Awassi rams (mean±SE)

Items		Ejaculate			
		First	Second	Third	Fourth
Behaviour of rams					
Sniff/nosing of external genitalia	1	0.00	1.33±0.27	2.66±0.98	2.00±0.61
	2	0.00	3.50±0.71	–	–
Kicking with foreleg	1	2.00	12.50±1.31	9.25±2.30	20.25±1.51
	2	0.00	13.66±0.72	12.93±4.38	–
Tongue in and out	1	0.00	3.00±0.00	2.50±0.82	3.66±1.18
	2	0.00	5.00±0.00	2.00±0.00	–
Vocalization (courting grunts)	1	0.00	1.00±0.00	3.00±1.41	3.33±0.27
	2	1.50±0.35	2.00±0.00	2.00±0.00	–
Number of mounts	1	5.75±0.54	1.50±0.25	1.50±0.25	2.33±0.54
	2	2.50±0.55	1.33±0.27	–	–
Time taken (sec)	1	40±6.12	100±28.01	223.5±36.9	359±24.94
	2	41±13.5	253±56.17	–	–
Semen characteristics					
Ejaculate volume (ml)	1	0.97±0.19	0.50±0.03	0.47±0.06	0.36±0.02
	2	0.62±0.18	0.50±0.14	0.50±0.00	–
Sperm density (0-3 scale)	1	3.00±0.00	2.50±0.25	1.75±0.21	1.33±0.27
	2	3.00±0.00	2.00±0.47	2.00±0.00	–
Sperm motility (%)	1	88.75±1.08	83.75±2.07	83.75±2.07	78.33±1.36
	2	87.50±1.08	66.66±15.1	75.00±0.00	–
Sperm concentration (10^6/ml)	1	2450±198.0	2040±377.9	991.2±201.5	1060±99.77
	2	2612±142.6	1825±337.5	1640±0.00	–

1-Ram no. 1, 2-Ram no. 1

moving of tongue and vocalization were increased before the second ejaculate and afterwards. Most significant change was observed in kicking with forelegs. Price *et al.* (1992) obtained similar results in Fin × Targhee rams. Flehmen response was not observed in either of the rams as the ewe did not urinate during the observed for 15 min.

Results of semen characteristics in this study are similar to those reported by Juma and Dessouky (1969) for the Awassi rams in Iraq, Corriedale, Jaiselmeri, Magra, Marwari rams under arid (Mittal and Ghosh 1979, Mittal 1980) and Malpura and Chokla rams Honmode and Tiwari (1974) under semi-arid regions of India. According to Lynch *et al.* (1992) 4 to 5 ejaculations can be obtained from a ram before the latency or refractory period of 20 min. Four ejaculates could be collected from ram 1 and three ejaculates from ram 2 in the present study during 15 min, which is indicative of their high libido and mating performance. According to Hafez (1987) rams can ejaculate many times in a day for several weeks before severely depleting their epididymal reserves of sperm because of the small ejaculates and the large epididymal reserves. Fraser (1975) reported that rams which concentrate their breeding season into relatively short periods require high levels of libido for effective mating during that time.

The first ejaculate was having highest volume, pre cent motile sperms and sperm concentration but these semen attributes decreased progressively on subsequent ejaculates up to the 4th ejaculate in ram 1 and 3rd ejaculate in ram 2. However, the values of these traits in the last ejaculates can be considered satisfactory for breeding purposes. Such declining trends in semen quality of Rambouillet (Sahni and Tiwari 1975), Magra and Marwari (Mittal 1982) Fin × Cross (Amir *et al.* 1986) and Merino (Thwaites 1995) rams are already on record. In accordance with the results of this breed obtained by Epstein (1985) positive correlations were obtained between ejaculate volume and sperm density in both the rams.

It is apparent from these results that the sexual behaviour and semen quality of Awassi rams are satisfactory under semi-arid conditions of Rajasthan where sheep husbandry plays a dominant role in agro-economy of the region. Awassi and native Malpura breed of semi-arid region of India resemble each other in phenotypic appearance, the productivity of latter breed in terms of mutton, milk and wool yield can be improved by introducing blood of former breed. Efforts are being made for improvement of the productivity of Malpura sheep under field conditions through artificial insemination using semen of Awassi rams (Mathur *et al.* 1998, Mittal *et al.* 1998).

ACKNOWLEDGEMENT

The authors are highly thankful to the Director of this institute for providing necessary facilities for this study.

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Indian Journal of Animal Sciences 70 (2): 184-187, February 2000

Time trends in estimates of genetic parameters in a White Leghorn population subjected to long-term selection

R N CHATTERJEE[1], B S MISRA[2] and H N SINGH[3]

Chandra Shekhar Azad University of Agriculture and Technology, Mathura Campus, Mathura, Uttar Pradesh 281 001

Received: 27 January 1999; Accepted: 17 August 1999

ABSTRACT

Pullets (3 813) of 185 sires and 1 403 dams of 9 consecutive generations were examined in the present study. Females, on the basis of their family averages and males, on the basis of sire family averages were selected for first 100 days egg production every generation. The traits taken were age at sexual maturity (ASM), body weight at sexual maturity (WAM), first 100 days egg production (EP100), egg weight (EW) and average clutch size (CS). The phenotypic time trends for all these traits were nonsignificant except for EW which was negatively significant. The pooled heritability estimates were higher for ASM and EW, moderate for WAM and low for EP100 and CS. The time trends of heritability estimates (b ± SE) were all negative and not significant. This indicated that additive genetic variance of the traits in the population were not reduced significantly. The pooled estimates of genetic, environmental and phenotypic correlation of ASM with WAM were all positive, while ASM with EP100 and CS were mostly negative. The pooled estimates of genetic correlation between ASM and EW was positively significant (0.23 ± 0.01) and moderate. The estimates of genetic correlation between WAM and EW were all positive and moderate to high. EP100 was positively correlated with CS but negatively correlated with EW. There was negative association between EW and CS. The time trends in estimates of genetic, environmental and phenotypic correlation were mostly not significant.

Key words: Time trends, Genetic parameter, Released phenoxypie response, White Leghorn

The magnitude of heritability in a population provides a measure of additive genetic variability which determines the potential for changing population means by selection. Evidence of changes in the genetic constitution of population under selection is manifested by changes in heritability estimates over generations. Investigations pertaining to the effects of selection on genetic correlations had been very limited. However, Lerner (1958) presented theoretical models which allow for a change from a positive to a negative genetic correlation between two traits on which selection was applied.

The main objectives of the present investigation were to study changes over a period of time in the heritabilities and in the genetic, environmental and phenotypic correlations between certain traits in a population of White Leghorn subjected to long term selection for improving egg production.

MATERIALS AND METHODS

The data of 3 813 pullets of 185 sires and 1 403 dams pertaining to 9 consecutive generations of a White Leghorn population maintained at livestock research substation, Chak-Ganjaria, Lucknow were utilized. Nearly 150 females on the basis of their family averages (both fullsibs and halfsibs) and 20 cocks on the basis of their sire family averages for first 100 days egg production were selected every year. Fullsib and halfsib matings were avoided. The data were corrected for significant hatch effects in each generation separately using least square constants (Harvey 1966). The realized phenotypic response per generation was obtained from regression of generation means on generation number (Snedecor and Cochran 1989). Heritability estimates of the traits in every generation were calculated from paternal halfsib correlation using Harvey's (1987) mixed model least squares and maximum likelihood (LSMLMW) computer programme. Standard errors (SE) of heritability estimates were computed as per Dickerson (1960). The heritabilities obtained for each trait, were then pooled over generations by weighting the individual estimates inversely to their variances (Enfield *et al.* 1966). The SEs were also pooled to obtain a pooled estimate of SE for each trait. The estimates of correlations over the generations were computed using Harvey's (1987) mixed model least squares and maximum likelihood

Present address: [1]Senior Scientist (Poultry Science), Animal Science Section, CARI, Post Box No. 181, Port Blair 744101, Andaman & Nicobar Islands.

[2]Professor (Retd), [3]Professor, Department of Animal Genetics and Breeding, Veterinary College.

Table 1. Time trends in heritability estimates of the traits

Generation	ASM	WAM	EP100	EW	CS
	h^2_s±S E	h^2_s±S E	h^2_s±S E	h^2_s±S E	h^2_s±S E
S_0	0.45±0.29	0.36±0.14*	0.26±0.22	0.48±0.23*	0.39±0.27
S_1	0.49±0.35	0.34±0.31	0.31±0.30	0.46±0.28	0.33±0.30
S_2	0.47±0.21*	0.11±0.10	0.06±0.08	0.46±0.21*	0.11±0.10
S_3	0.60±0.24*	0.47±0.20*	0.32±0.16*	0.40±0.10**	0.31±0.15*
S_4	0.32±0.15*	0.17±0.11	0.34±0.15*	0.58±0.21*	0.19±0.11
S_5	0.50±0.25*	0.49±0.25*	0.02±0.10	0.25±0.18	0.15±0.14
S_6	0.23±0.17	0.14±0.15	0.16±0.15	0.42±0.21*	0.36±0.20
S_7	0.65±0.26*	0.27±0.15	0.19±0.13	0.41±0.25	0.02±0.07
S_8	0.43±0.22*	0.35±0.20	0.13±0.13	0.41±0.27	0.35±0.20
Pooled	0.42±0.005**	0.23±0.003**	0.14±0.002**	0.41±0.005**	0.14±0.002**
b±SE	−0.003±0.018	−0.003±0.019	−0.016±0.015	−0.011±0.011	−0.013±0.017

*, *Significant at P<0.05.* **, *significant at P<0.01.*

(LSMLMW) computer programme by paternal halfsib correlation. The correlations, estimated initially for each generation separately, have been pooled over generations (Enfield *et al.* 1966). The time trends of the estimates of heritability and genetic, environmental and phenotypic correlation were calculated from regression of the estimates on generation numbers (Snedecor and Cochran 1989).

RESULTS AND DISCUSSION

There was a nonsignificant decline of ASM and WAM as a correlated phenotypic response in these traits. These traits showed negative trend, which might be due to negative genetic correlation of the primary trait (EP100) with ASM and WAM (Tables 2 and 3). Similar findings were obtained by Singh *et al.* (1986) in White Leghorn. The realized phenotypic response of EP100 and CS was positive though not significant. The reasons, for small positive response in the primary trait (EP100), perhaps were small population size, individual measurement sampling, fluctuation of environmental trend, natural selection in opposite direction, high experimental error and / or ineffective selection criterion (Nordskog *et al.* 1967). In CS, the positive correlated phenotypic response was probably due to a high positive genetic correlation (0.87 ± 0.01) between EP100 and CS. Average egg weight showed a significant negative trend, which might be due to negative genetic correlation between EP100 and EW (Table 3).

The pooled heritability estimates of ASM, WAM, EP100, EW and CS were highly significant (P<0.01). Similar estimates for ASM were observed by Nayak and Mishra (1985). This value for WAM corresponds well with Bais (1996). The estimates of heritability (from sire component) for EP100, EW and CS are in conformity with the findings of Lal and Sandhu (1977), Singh *et al.* (1986), Krishna and Chaudhary (1987) and Renganathan *et al.* (1983).The time trends of heritability estimates for ASM, WAM, EP100, EW and CS were all nonsignificant (Table 1). However, the trends

Table 2. Time trends in the estimates of genetic, environment and phenotypic correlation of ASM with other traits and WAM with EP100 and EW

Gene-	ASM×WAM			ASM×EP100			ASM×EW			ASM×CS			WAM×EP100			WAM×EW		
ration	Gen	Env	Phe	Gen	Env	Phe	Gen	Env	Phe	Gen	Env	Phe	Gen	Env	Phe	Gen	Env	Phe
S_0	0.53±0.70	−0.05	0.05	−0.03±0.40	−0.40	−0.26	0.48±0.35	−0.16	0.07	−0.43±0.37	−0.27	−0.34	−0.02±0.76	0.14	0.11	0.68±0.61	0.35	0.35
S_1	0.38±0.40	0.10	0.21	<−1	0.36	−0.19	−0.19±0.47	0.28	0.10	−0.80±0.44	0.18	−0.22	0.28±0.49	−0.07	0.04	>1	−0.03	0.31
S_2	0.25±0.39	0.35	0.18	0.17±0.51	−0.11	−0.05	−0.38±0.27	0.30	−0.01	0.14±0.42	−0.41	−0.25	0.15±0.69	−0.04	−0.03	−0.73±0.28*	0.67	0.30
S_3	0.78±0.13**	0.20	0.51	−0.23±0.30	−0.19	−0.20	0.24±0.39	0.13	0.14	−0.41±0.30	−0.52	−0.45	0.04±0.31	−0.13	−0.06	0.24±0.40	0.18	0.17
S_4	0.016±0.34	0.42	0.36	−0.29±0.29	0.02	−0.08	0.20±0.27	0.08	0.13	0.06±0.34	−0.09	−0.06	−0.54±0.30	0.13	−0.04	0.04±0.32	0.34	0.21
S_5	0.81±0.16**	−0.24	0.28	0.82±1.67	−0.21	−0.07	0.91±0.21**	−0.33	0.12	0.01±0.43	−0.24	−0.15	>1	−0.17	−0.01	0.86±0.21**	−0.17	0.20
S_6	−0.83±0.44	0.33	0.12	−0.09±0.51	−0.34	−0.29	−0.53±0.35	0.11	−0.09	−0.29±0.41	−0.33	−0.31	0.19±0.57	−0.07	−0.03	0.20±0.41	0.31	0.27
S_7	0.09±0.32	0.08	0.08	−0.66±0.29*	−0.08	−0.27	0.27±0.27	0.02	0.17	−0.28±0.81	−0.29	−0.20	0.23±0.38	0.12	0.14	0.53±0.26*	0.05	0.18
S_8	0.62±0.26*	−0.22	0.11	−0.43±0.14**	−0.10	−0.17	0.14±0.30	−0.03	0.06	−0.11±0.34	−0.16	−0.14	0.20±0.43	0.14	0.15	0.36±0.29	−0.07	0.13
Pooled	0.57±0.01**	0.11	0.21	−0.29±0.02**	−0.12	−0.18	0.23±0.01**	0.044	0.08	−0.23±0.02**	−0.24	−0.24	−0.02±0.02	0.01	0.03	0.31±0.01**	0.18	0.24
TTr_G	−0.044±0.067			−0.064±0.07			0.007±0.061			0.04±0.04			0.01±0.04			0.06±0.08		
TTr_E	−0.02±0.03			−0.01±0.03			−0.002±0.03			0.008±0.03			−0.008±0.02			0.042±0.032		
TTrP	−0.008±0.02			−0.04±0.012			−0.001±0.01			0.02±0.015			0.01±0.011			−0.024±0.01		

TTrG, TTrE and TTrp are time trend of estimates of genetic, environmental and phenotypic correlation respectively.

*, Significant at P<0.05 **, significant at P<0.01.

Table 3. Time trends in the estimates of genetic, environment and phenotypic correlation of WAM with CS, EP 100 with other traits and EW with CS

Generation	*WAM×CS*			*EP100×EW*			*EP100×CS*			*EW×CS*		
	Gen	Env	Phe	Gen	Env	Phe	Gen	Env	Phe	Gen	Env	Phe
S_0	−0.22±0.67	0.24	0.15	0.48±0.42	0.07	−0.08	0.89±0.12**	0.69	0.75	−0.72±0.36*	−0.12	−0.16
S_1	−0.02±0.49	0.12	0.02	>1	−0.59	−0.07	>1	0.60	0.73	−0.72±0.54	−0.45	−0.11
S_2	>1	−0.26	−0.01	0.06±0.51	−0.06	−0.03	0.84±0.31*	0.64	0.65	−0.53±0.54	−0.45	−0.11
S_3	−0.47±0.28	0.03	−016	−0.86±0.25**	0.24	0.04	0.92±0.09	0.50	0.63	<−1	0.05	−0.14
S_4	−0.06±0.39	0.05	0.03	−0.10±0.27	−0.07	−0.08	0.71±0.17**	0.72	0.70	−0.22±0.31	−0.11	−0.28
S_5	0.12±0.42	−0.04	0.01	>1	−0.30	−0.17	0.54±0.81	0.77	0.73	−0.36±0.52	−0.27	−0.15
S_6	0.37±0.45	−0.17	−0.04	−0.19±0.41	0.01	−0.04	>1	0.63	0.72	−0.15±0.33	−0.15	−0.11
S_7	0.07±0.81	0.16	0.13	−0.34±0.13*	0.18	−0.01	0.94±0.64	0.78	0.76	−0.77±0.07**	−0.04	−0.10
S_8	0.25±0.33	0.11	0.16	−0.21±0.40	0.13	0.02	0.87±0.17**	0.69	0.71	−0.02±0.32	−0.18	−0.10
Pooled	−0.05±0.02*	0.03	0.04	−0.31±0.01**	−0.01	−0.04	0.87±0.01**	0.67	0.71	−0.68±0.01**	−0.16	−0.15
TTrG	0.07±0.03*			−0.08±0.08			−0.009±0.03			0.07±0.04		
TTrE	−0.005±0.02			0.04±0.03			0.013±0.011			0.008±0.02		
TTrP	0.005±0.01			0.004±0.009			0.003±0.006			0.006±0.007		

TTrG, TTrE and TTrP are time trend of estimates of genetic, environmental and phenotypic correlation respectively. *, Significant at $P<0.05$**, significant at $P<0.01$.

for all these traits were negative. This indicated the decline of additive genetic variance of the traits but not significant. The nonsignificant negative time trend of heritability estimates were probably because of ineffective selection criterion. The negative trend of heritability as a result of selection was also observed by Grewal *et al.* (1995). The estimates of heritability for ASM and EW were significant ($P<0.05$) in most of the generations, while for other traits the estimate was not significant in most of the generations. The reasons for nonsignificant estimates in respective generations probably were small population size and / or non-normal distribution of data (Ibe and Hill 1988).The estimate of heritability for ASM, WAM, EP100, EW and CS was highest in S7, S5, S4, S4 and S0 generation, respectively, while the estimate for the traits was lowest in S6, S2, S5, and S7 generation respectively (Fig. 1).

The pooled estimates of genetic correlation of ASM with WAM and EW were highly significant ($P<0.01$). The environmental and phenotypic correlations between these traits were also positive. However, the correlations (genetic, environmental and phenotypic) of ASM with production traits (EP100 and CS) were negative. These values are in close agreement with Shrivastava *et al.* (1993). There was nonsignificant correlations between WAM and EP100, while, the genetic, environmental and phenotypic correlations of WAM with EW were 0.31 ± 0.01 ($P<0.01$), 0.18 and 0.24 respectively. The estimates of correlation between WAM and CS were very low in magnitude. There was negative genetic correlation between EP100 and EW, which was highly significant ($P<0.01$). These findings are in close agreement with the findings reported by Das *et al.* (1983). Some nonsignificant estimates of correlation, in different generations, were probably because of small population size or the data were not normally distributed (Ibe and Hill 1988). The negative genetic correlations between traits indicated that, selection for either of these trait would reduce the other, while, the positive genetic correlations indicated that selection for either of these traits would improve the others as a correlated response. The time trends in estimates of genetic correlation of ASM with WAM and EP100 were negative, while, with EW and CS were positive. The time trends of WAM with other traits were all positive. The trends of genetic correlations of EP100 with EW and CS were negative, while, EW with CS was positive. All these time trends were not significant, but the time trend of estimate of genetic correlation between WAM and CS was positively significant ($P< 0.05$), though the magnitude was small. Thus, the nonsignificant (mostly) time trends in estimates of genetic correlations did not provide a strong argument against the general tendency for negative time trends in the genetic correlations observed by Frairs *et al.*(1962).The time trends in estimates of environmental correlation between traits were mostly negative and not significant, while, the trends of phenotypic correlations

Fig 1.Time trend of heritability estimates of different traits

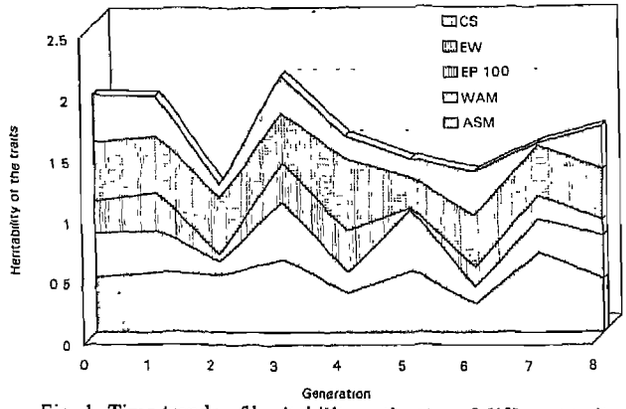

Fig. 1. Time trends of heritability estimates of different traits.

between traits were mostly positive and not significant. The behaviour of genetic correlation, in different generations (direction and magnitude) might have been due to genetic variance of any one of the either trait approaching zero (King 1961).

The realized phenotypic response of the traits were not significant except for EW which was negatively significant and not desirable. The additive genetic variance of the traits in the population was slightly reduced as indicated by nonsignificant negative time trends of heritability estimates. This indicated that the selection criterion was not that much effective. The pooled estimates of correlation between traits were in good agreement with other workers as discussed. Mostly, there was no time trend of correlation estimates. The realized phenotypic response, estimates and time trends of heritability and correlations have given indications for the traits to be included in selection index or else, the selection criterion is to be changed for the studied population.

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Indian Journal of Animal Sciences 70 (2): 188-190, February 2000

Effect of expander-extruder processing on nutritive value of maize cobs based complete diet of buffaloes

G V N REDDY[1] and K JOJI REDDY[2]

Acharya N G Ranga Agricultural University, Rajendranagar, Hyderabad, Andhra Pradesh 500 030

Received: 1 September 1998; Accepted: 6 August 1999

ABSTRACT

Expander-extruder processed maize cobs (40%) based complete diet, corresponding mash diet and a conventional diet (containing concentrate mixture and ground-maize cobs in 60: 40 ratio as in complete diets i e as such basis) were fed to 3 permanently fistulated male buffaloes using 3×3 latin square design to assess the effect of these diets on rumen fermentation pattern and nutrient utilization. The DMI and water intake/kg DMI were comparable on all the diets. Total N, TCA-precipitable N and residual N were increased ($P<0.01$) from conventional diet to mash and expander-extruder processed diet where the trend in NH_3-N concentration was reverse. All the N fractions except NH_3-N and TVFA concentrations peaked at 4- hr post feeding. The digestibilities of DM, OM and CP were significantly ($P<0.05$) higher on expander-extruder processed diet compared to other 2 diets. All the animals were on positive N, Ca and P balances under 3 treatments. Expander-extruder processing increased the DCP and TDN contents of diet and the intakes on all the diets were much higher than the recommended level for maintenance of adult buffaloes. The results indicated that the rumen fermentation pattern in buffaloes was normal on maize-cobs based diets and that expander-extruder processing stimulated to some extent the rumen fermentation pattern and enhanced the nutrient utilization of complete diet.

Key words: Buffaloes, Complete diet, Expander-extruder, Maize cobs, Nutritive value, Rumen fermentation

The information on incorporation of poor quality roughages in complete diets improved their palatability and nutrient utilization was meager. Therefore present attempt was made to study the effect of expander-extruder processing of complete diet containing maize-cobs on rumen fermentation pattern and nutrient utilization in buffaloes.

MATERIALS AND METHODS

A complete diet was formulated using maize-cobs 40, maize grain 10, groundnut-cake 10, deoiled rice-bran 28.5, molasses 10, mineral mixture 1 and common salt 0.5% and processed into mash form. Vitamin A was added @ 10g/100 kg. Half of the complete diet was subjected to expander-extruder processing to form pellets.

Pellets were compared with corresponding mash and a conventional diet containing concentrate mixture (maize grain 16.7, groundnut-cake 16.7, deoiled rice bran 47.4, molasses 16.7, mineral mixture 1.7 and common salt 0.8%). Vitamin A supplement was added @ 20g/100kg and ground-maize-cobs in 60: 40 ratio as in complete feeds. These diets were fed *ad lib.* to 3 permanently fistulated adult male buffaloes in a 3×3 latin-square design. After 30 days feeding, feed residues, faeces and urine were collected for 7 days, followed by a 3-day rumen sample collection. A 10-day switch over period was observed between periods. On the day of rumen liquor collection animals were offered water 1 hr before the '0' hr fluid collection and after last collection. The rumen liquor samples were collected at 0, 2, 4 and 6 hr post feeding. pH and ammonia N of the rumen fluid were determined immediately after sample collection. Rumen liquor samples were preserved by adding 1 ml saturated mercuric chloride/ 100 ml rumen fluid and stored in a refrigerator for further analysis.

Rumen liquor samples were analyzed for total N (micro kjeldahl), trichloroacetic acid (TCA) supernatant N, TCA-precipitable N (Cline *et al.* 1958), residual N, food and protozoal N (Singh *et al.* 1968), ammonia N (Schwartz and Schoeman 1964) and TVFA (Barnett and Reid 1956). The feed, faeces and urine samples were analyzed for proximate principles (AOAC 1985), calcium (Talapatra *et al.* 1940), phosphorus (AOAC 1985) and fibre fractions (Goering and Van Soest 1970). DE and ME values were calculated using

Present address: [1]Professor and Head, Department of Veterinary Biochemistry, College of Veterinary Science, Rajendra Nagar, Hyderabad 500 030

[2]Professor and Head, Department of Veterinary Biochemistry, College of Veterinary Science, Gannavaram, Distt. Krishna.

Table 1. Per cent chemical composition of experimental feeds (on DM basis)

Item	Maize cobs	Concentrate mixture	Complete diet Mash	Extruded pellets
Organic matter	92.1	85.7	90.2	90.2
Crude protein	4.2	15.6	12.1	12.5
Crude fibre	32.9	13.1	22.4	22.1
Ether extract	0.5	1.5	1.4	1.4
Nitrogen-free extract	54.5	55.5	54.3	54.2
Total ash	7.9	14.3	9.8	9.8
Neutral detergent fibre	77.3	30.1	50.5	51.0
Acid detergent fibre	38.7	18.0	27.3	27.0
Hemicellulose	38.6	12.1	23.2	24.0
Cellulose	29.6	12.1	20.1	20.3
Lignin	5.7	3.8	4.7	4.2
Silica	3.4	2.1	2.5	2.5
Calcium	0.4	0.2	1.2	1.2
Phosphorus	0.1	1.0	0.8	0.8

the factors suggested by NRC (1978). The data were analyzed as per the procedures of Snedecor and Cochran (1968).

RESULTS AND DISCUSSION

The chemical composition of the experimental diets is presented in Table 1. The dry matter intake (DMI) and water intake/kg DMI were comparable on all the diets (Table 3). However, the DMI was 3.6 and 12.3% higher on complete mash and pelleted diet, respectively, compared to conventional diet. Although, the conventional diet was offered in a concentrate: roughage ratio of 60: 40 as that of complete diet, but the actual intake ratio obtained was 67: 33 due to the lower intake of maize-cobs and complete intake of concentrate mixture.

All the N fractions except ammonia N (Table 2) peaked at 4 hr post-feeding. This may be due to active degradation of protein and hydrolysis of NPN substances, for microbial protein synthesis. Subsequent decline in the concentration of different N fractions may be due to change in rumen volume through inflow of saliva (Murali *et al.* 1989) and followed similar pattern in 3 treatments.

Total N, TCA-perceipitable N and TVFA concentrations were higher ($P<0.05$) in expander-extruder processed complete diet than mash and conventional diets which indicated that expander extruder processed diet can provide better rumen fermentation medium compared to other 2 diets. pH values were inversely related to TVFA concentrations on different diets at different times after feeding (Table 2) indicating that pH of rumen liquor was dictated by quantity and type of VFA present in the reticulo-rumen (Reddy and Reddy 1986 and Gowd *et al.* 1987).

The digestibility of DM, OM and CP (Table 3) were significantly ($P<0.05$) higher on expander-extruder processed diet compared to other 2 diets. The digestibility of these nutrients were comparable on conventional and mash diets,

Table 2. Mean[a] values of nitrogen fractions (mg/100ml,) pH and TVFA (meg/L) in the rumen fluid of buffaloe bulls as affected by experimental diets

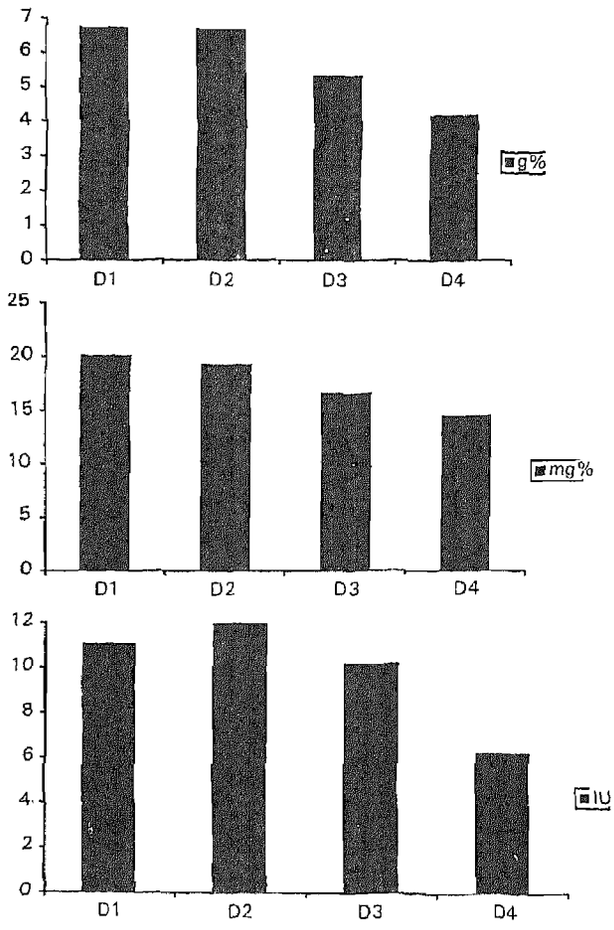
Diet	Hours after feeding 0	2	4	6	Mean
		Total nitrogen			
Conventional	60.4	98.3	110.2	91.5	90.1[b]
Mash	63.1	100.5	113.5	95.1	93.1[c]
Pellets	65.9	105.6	116.8	97.3	96.4[b]
Mean	63.1[b]	101.5[d]	113.5[e]	94.6[c]	
		TCA precipitable nitrogen			
Conventional	23.4	37.2	42.0	39.4	35.5[b]
Mash	27.5	40.1	44.7	41.2	38.4[c]
Pellets	29.9	42.1	50.4	44.2	41.7[d]
Mean	27.0[b]	39.8[c]	45.7[e]	41.6[d]	
		Ammonia nitrogen			
Conventional	5.1	13.3	10.7	6.0	8.8[c]
Mash	4.2	10.0	8.7	5.2	7.0[b]
Pellets	4.4	10.7	7.4	5.2	7.0[b]
Mean	4.6[b]	11.2[c]	9.0[d]	5.5[c]	
		pH			
Conventional	6.7	6.5	6.5	6.6	6.6
Mash	6.8	6.6	6.5	6.6	6.6
Pellets	6.9	6.5	6.5	6.6	6.6
Mean	6.8[e]	6.5[c]	6.5[b]	6.6[d]	
		TVFA			
Conventional	70.4	90.1	101.2	85.0	86.7[b]
Mash	73.2	93.6	107.5	89.4	90.9[c]
Pellets	76.5	97.1	113.7	92.3	94.9[d]
Mean	73.4[b]	93.6[d]	107.5[e]	88.9[c]	

[a]Mean of 9 values.

[bcde]values bearing different superscripts within the row as well as within the column differ significantly ($P<0.01$).

indicating that the blending of concentrate and roughage in mash diet was advantageous to the extent that the concentrate proportion was more in the conventional diet (67%). The higher N balance on expander-extruded diet may be due to processing effect which might have supplied more energy as a result of higher OM digestibility resulting in better utilization of absorbed nitrogen. The animals were on positive N, Ca and P balances indicating that these diets could supply these nutrients in required proportion.

The DCP content was significantly ($P<0.05$) higher on expander-extruder processed diet compared to other 2 diets (Table 3). The DCP content and intake was higher than the recommended level (3.08% and 154g, respectively) on all the diets. The TDN content of expander-extruder processed diet was significantly ($P<0.05$) higher compared to conventional diet and mash diet was in between the other 2 diets. The TDN content and intake was higher than the recommended level (48.6% and 2.43 kg, respectively) on all the diets (Table 3). The narrow protein: energy ratios on all



Figs 1-3. Effect of neem kernel-meal on: 1. Blood haemoglobin (g%); 2. Plasma calcium; 3. Plasma ALT (IU) activity, in laying hens.

crude protein, 48 ether extract, 157 crude fibre and 146 total ash. Inclusion of NKM at 150 and 200 g/kg levels significantly ($P<0.01$) reduced the feed consumption (Table 1), indicating poor palatability of experimental diets. Lowered feed intake resulted in an absolute reduction in the intake of protein, energy and calcium and thus leading to a significant ($P<0.01$) decline in egg production. Sadagopan *et al.* (1981) also recorded a decrease in egg production in layers fed diets containing higher levels of NKM. Poor palatability and reduced feed efficiency were earlier reported in broiler chicks fed neem- seed- meal (Salawu *et al.* 1994).

Biochemical parameters

Feeding NKM at 150 and 200 g/kg levels significantly ($P<0.01$) reduced the haemoglobin content (Fig. 1), indicating a possible impact of limonoid derivatives on erythropoesis. Significantly($P<0.05$) lower level of plasma calcium (Fig. 2) may be attributed to lowered availability of calcium due to @ 20% reduction in feed intake in layers fed NKM at higher levels. The ALP activity was significantly ($P<0.05$) lowered

Table 1. Feed intake and egg production in layers fed different levels of NKM

Attribute	Level of NKM (g / kg)				
	0	100	150	200	SEM
Feed intake(g/ bird / day)	95.9[a]	92.4[a]	76.9[b]	74.1[b]	1.46 **
Egg production (%)	72.9[a]	71.7[a]	53.3[b]	44.2[c]	2.02 **

Means bearing different superscripts in a row differ significantly; ** $P<0.01$.

in hens fed NKM at 200 g / kg level (Fig. 3) indicating disturbed alanine synthesis. Tissue specific inhibition of detoxifying enzymes in liver, lung and kidney has been reported in rats due to the administration of azadirachtin (Mahboob *et al.* 1995). Similarly hepatic microsomal enzyme inhibition was also reported by Vijjan and Tandon (1985) due to feeding water extract of neem -seed-cake in rats.

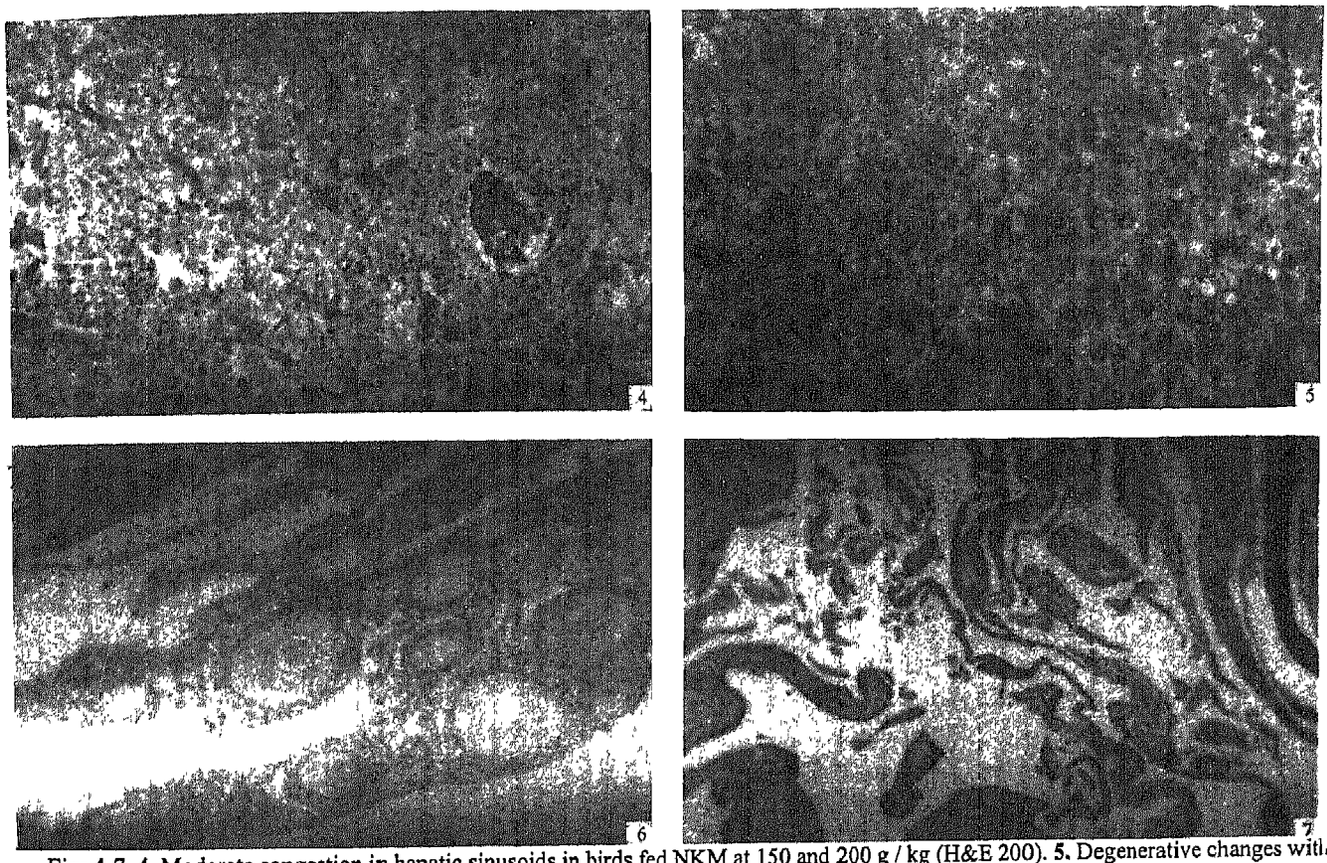
Tissue changes in visceral organs

Microscopic examination of different visceral organs revealed moderate congestion in intestine, liver, kidneys and spleen of birds fed test diet containing 150 and 200 g/kg NKM (Fig. 4). The mucosa of intestine was thickened with haemorrhagic patches in birds fed 200 g / kg NKM. Varying degree of degenerative changes in hepatic parenchyma with hydropic degeneration, cloudy swelling of hepatocytes and mild to severe fatty infiltration occupying almost entire liver parenchyma was observed in birds fed 200 g / kg NKM (Fig. 5). In kidneys lesions were mild characterized by moderate inflammatory changes in tubular epithelium with vacuolation of cytoplasm in layers fed diets containing 150 and 200 g / kg NKM. Toxicity and degenerative changes in liver and kidney were also reported in birds given water extract of neem fruits (Singh *et al.* 1985).

Ovarian changes

In ovary and oviduct of laying hens fed 150 and 200 g / kg NKM, reduction in height of glandular epithelial linings and loss of cilia with atrophy of ovarian follicles were noticed. However, in control and at 100 g / kg NKM feeding, large number of mature and immature ova of different shapes and sizes were observed in the ovarian follicles (Fig. 6), whereas at 200 g / kg level of NKM feeding the ovarian follicles were nearly devoid of ova, indicating a sub-functional state of ovaries (Fig. 7). Oral feeding of neem extract to rats for longer duration did cause thickening of follicular wall in ovary and scattering of stromal and glandular cells (Sangeeta *et al.* 1989). Sinha *et al.* (1984) reported spermicidal action of neem oil in rheseus monkeys. Vijjan and Parihar (1983) reported pronounced testicular degeneration in rats fed neem-seed-cake. The anti-fertility effect was also observed in cockerels fed NKM (Tyagi *et al.* 1996).

The findings of this study indicate and confirm the adverse effects of feeding NKM at higher levels on laying



Figs 4-7. **4.** Moderate congestion in hepatic sinusoids in birds fed NKM at 150 and 200 g / kg (H&E 200). **5.** Degenerative changes with fatty infiltration in liver in birds fed NKM at 200 g / kg (H&E 400). **6.** Normal ovary with follicular activity in hens fed control and 100 g / kg NKM diets. (H & E 100). **7.** Atrophied and sub-functional ovary in hens fed 200 g/kg NKM (H& E 100).

performance, biochemical parameters and internal organs. However, inclusion of NKM up to 10% (10 g / kg) of the dietary level in laying hens appears safe.

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Indian Journal of Animal Sciences 70 (2): 194-196, February 2000

The effects of bentonite on egg performance of laying hens

F INAL[1], N GULSEN[2], B COSKUN[3] and C ARSLAN[4]

University of Selçuk, 42031, Konya, Turkiye

Received: 29 December 1998; Accepted: 30 July 1999

ABSTRACT

The experiment was conducted to evaluate the effects of bentonite on egg production, egg weight, egg specific gravity, feed consumption and feed efficiency of laying hens. Babcock Brown laying hens at 32 weeks of age were fed diets containing 0 (control), 1.5, 2.5, and 3.5% bentonite for 3 months.

Egg yield was not affected by bentonite. Damaged egg rates (broken plus abnormal size) decreased by feeding 1.5 and 2.5% bentonite, whereas 3.5% did not have any effect. Egg weight 61.42, 61.15, 61.21 and 60.41g of experimental groups respectively. Both egg weight and egg specific gravity were not influenced by bentonite. Feed efficiency (kg feed/kg egg) was 2.47, 2.45, 2.43 and 2.59 in the groups fed 0, 1.5, 2.5, and 3.5% bentonite respectively.

Key words: Bentonite, Egg performance

The clay minerals from smectite group are called bentonite. Bentonite is a soft and colloidal aluminohydrosilicate which includes 75–80% montmorillonite (Çelik 1993, Demirel *et al.* 1995). Bentonites are generally white, yellow, pink or greenish. A high quality bentonite is hydrophylic, and holds water approximately 6–7 folds (Çelik 1993).

Turkey is the second rich country as bentonite resources which provides nearly 20% of world bentonite beds. The 70–80% of bentonite produced in Turkey is used for domestic market as civil engineering and agricultural purposes and the remaining is exported (Demirel *et al.* 1995).

Sodium aluminosilicate supplementation markedly affected P utilization (Monstaghian *et al.* 1991) and increased egg specific gravity in hens (Monstaghian *et al.* 1991, Roland 1990, Roland 1991). High ion-exchange capacity and pH of bentonite has positive effect on egg specific gravity (Roland *et al.* 1990). Negative effect on utilization of phosphorus in gastro-intestinal tract may also be related to formation of a complex between aluminium and feed phosphorus (Monstaghian *et al*, 1991, Roland 1990, Roland 1991).

Spent bentonite that is used in bleaching didn't significantly affect the egg production, feed consumption, feed efficiency, and egg weight in hens (Al-Zubaidy 1992)

In a study used a commercial bentonite, supplementation of 2.5% bentonite considerably increased the egg production, and decreased 13% feed intake per unit egg (Vasil'ev and Mirzaliev 1990).

Present address: [1,2]Assistant Professor, [4]Assistant, Department of Animal Nutrition.

Also, aliminocilicates have a positive effect to diminish of mycotoxicosis (Kubena *et al.* 1990). Voss *et al.* (1993) reported that bentonite was nontoxic and provided significant protection against aflatoxicosis, and safely added to animal feed up to 2.5% for this purpose.

In addition, bentonite is used for broiler feeds. It is reported that bentonite that have used refining of canola oil, added 7.5% level of broiler ration; feed intake, growth rate and feed efficiency did not have negative effect (Blair *et al.* 1987).

On the other hand bentonites is a important pellet binding and is used at 1–2% level for bird ration (Al-Zubaidy 1992). This study was conducted to evaluate the effects of an activated bentonite on egg yield performance in hens.

MATERIALS AND METHODS

Babcock B-380 brown laying hens (320), 32 week old were randomly divided into 4 equal groups each with 8 replicates of 10 hens per treatment and housed in individual cages (45cm × 55cm × 50cm). The egg production were recorded during 7-days and ensured similar preproduction values of treatments.

Feed and water supplied for *ad lib.* throughout the 94-day experimental period. The dietary treatments were the basal diet (Table 1) and the basal diet plus 1.5, 2.5, or 3.5 supplementary dietary activated bentonite. The diets contained 16% protein, ME of 11.38 MJ/kg.

Egg production was determined daily and feed consumption and feed efficiency at 14 day intervals. Also damaged (broken plus abnormal size) egg rates in total egg production was recorded. Egg weight and egg specific gravity

Table 1. Ingredients and chemical composition of basal diet

Ingredients	%
Corn	59.5
Soybean-meal	9.6
Sunflower-meal	13.5
Fish meal	0.8
Meat and bone-meal	2.5
Full fat soybean	4.7
Limestone	8.4
Dicalcium phosphate	0.4
Salt	0.26
Vitamin/mineral mix[1]	0.25
Kemizym	0.05
Methionine	0.04
Chemical analysis	
Dry matter	92.17
Ash	11.28
Crude protein	16.67
Ether extract	3.26
Crude fiber	5.49

[1]Provided per kg of diet: vitamin A, 10 million IU; cholecalciferol, 1,200 ICU; vitamin E, 35 mg; vitamin K_3, 5mg; vitamin B_1, 3mg; vitamin B_1, 3mg; vitamin B_2, 7mg; niacin, 20mg; ca-D-panthotenate, 10mg; vitamin B_6, 5mg; vitamin B_{12}, 0.015mg; folic acid, 1mg; D-Biotin, 0.045mg; choline chloride, 125mg; vitamin C, 50mg; charophyll red, 25mg; charophyll yellow 5mg; Mn, 80mg; Fe, 30mg; Zn, 60mg; Cu, 5mg; Co, 0.5mg; I, 2mg; $CaCO_3$, 236mg.

(Hempe *et al.* 1988) were determined monthly. Dry matter, ash, ether extract, crude protein and crude fibre values were determined (Table 1) by chemical analysis (Akkiliç and Surmen 1979).

Data obtained were subjected to analysis of variance using one-day ANOVA procedures (SPSS 1993).

RESULTS AND DISCUSSION

Egg production was not affected by bentonite used at the *levels of 1.5, 2.5, and 3.5% throughout present experiment* (Table 2). In the groups, means of egg production were 85.72, 85.25, 85.50, and 85.21% respectively (P<0.05). Spent bentonite did not influence the egg yield in laying hens (Al-Zubaidy 1992). However, 2 different bentonite increased egg production, especially at the level of 2.5%. (Vasil'ev and Mirzaliev 1990).

During 30–60 days of research, damaged egg rate were significantly less in the groups fed 1.5% and 2.5% bentonite than control (P<0.05). The inclusion of 1.5% bentonite significantly reduced damaged egg percentages throughout this study (P<0.05). Egg shell quality improved due to bentonite's high mineral content. Also, sodium aluminosilicate had a high affinity for Ca and high ion exchange capacity (Roland 1990, 1991).

In this study, the damaged egg percentages increased related to bentonite levels (Table 3). Among the bentonite

Table 2. Saleable and damaged egg percentages

Period	Control	Bentonite		
		1.5%	2.5%	3.5%
Saleable egg, pre cent				
0–30 day	92.13	92.33	91.88	91.46
30–60 day	88.61	88.51	89.92	88.75
60–94 day	76.43	74.89	74.71	75.42
Damaged egg pre cent				
0–30 day	0.37	0.18	0.41	0.55
30–60	1.50[a]	0.66[b]	0.38[b]	1.14[ab]
60–94 day	1.19	0.64	0.96	1.25

[a,b]Means with a different superscripts within a row are significantly different (P<0.05).

fed groups lowest damaged egg percentage was noted with 1.5%, while highest was with 3.5%. In the control it was 1.02%. In another research, a bentonite source used at level of 2.5% improved the egg specific gravity, but others decreased the egg shell thickness (Vasil'ev and Mirzaliev 1990, Al-Zubaidy 1992).

During whole period egg weight and specific gravity were not statistically significant (P>0.05) (Table 3). Egg weights were almost similar among experimental groups. Bleaching bentonite did not affect egg weight either (Al-Zubaidy 1992), but another bentonite decreased egg weights (Vasil'ev and Mirzaliev 1990). There is a conflict between results of this study and other researchers (Monstaghion *et al.* 1991, Roland 1990, 1991).

Supplementation of bentonite did not affect feed efficiency (Table 3). In the other research, bentonite did not influence feed efficiency either (Al-Zubaidy 1992). This study is in research, bentonite did not influence feed efficiency either (Al-Zubaidy 1992). This study is in disagreement with those reported that bentonite decreased feed per unit egg (Vasil'ev

Table 3. Some performance parameters

Paramcter	Control	Bentonite		
		1.5%	2.5%	3.5%
Egg Production, pre cent	*85.72*	*85.25*	*85.50*	*85.21*
Damaged egg, pre cent	1.02[a]	0.49[b]	0.58[ab]	0.98[ab]
Egg weight, g	*61.42*	*61.15*	*61.21*	*60.41*
Egg specific gravity, g/cm^3	1.089	1.088	1.089	1.090
Feed consumption, g/day	129.31	126.19	125.85	131.99
Feed efficiency, kg feed/kg egg	2.47	2.45	2.43	2.59
Mortality, pre cent	2.50	3.75	1.25	2.50
Live weight change, g	+8.61	+8.89	+14.58	+25.62
Number of saleable egg/ tonne feed	6550.15	6716.86	6747.72	6381.54
Saleable egg change, number	0	+166.71	+197.57	−168.61
Number of saleable egg, pre cent	100	102.55	103.02	97.43

[a,b] Means with a different superscripts within a row are significantly different (P<0.05).

and Mirzaliev 1990).

The inclusion of 1.5, 2.5, and 3.5% bentonite into laying diet did not influence egg yield, egg weight, egg specific gravity, and feed efficiency. However, the supplementation of 1.5 and 2.5% bentonite significantly decreased damaged egg percentage (broken and abnormal size). Addition of bentonite in levels of 1.5 and 2.5% increased number (Table 3) of saleable eggs (167 and 198, respectively, per tonne feed consumed). According to the results of this research, bentonite may be add into the diets of laying hens up to 2.5% without negative effects.

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Indian Journal of Animal Sciences 70 (2): 197-199, February 2000

Effect of individual and combined supplementation of vitamin E and selenium on the performance of broiler chicks fed ochratoxin – A

K PRAKASH[1], B UMAKANTHA[2], G DEVEGOWDA[3], C V GOWDH[4] and G KRISHNAPPA[5]

University of Agricultural Sciences, Banglore, Karnataka 560 024

Received: 11 February 1999; Accepted: 17 August 1999

ABSTRACT

An experiment with a completely randomized block design was used to evaluate the effects of ochratoxin (2 mg/kg of diet), vitamin E (400 mg/kg) and selenium (1 mg/kg), both individually and in combination, in 6 week old broilers. Commercial broilers (240) were randomly placed in battery brooder with 10 birds/pen. Each treatment was replicated 3 times. A significant ($P< 0.05$) drop in body weight, total protein and increase in mortality, relative weights of kidney, liver, serum uric acid and gamma glutamyl transferase activity were noticed. Mortality was highest among the broiler fed OA. Addition of selenium alone did not alter any of the parameters evaluated. Vitamin E alone and in combination with selenium significantly ($P < 0.05$) diminished the growth inhibitory effect of OA, reduced the liver weight, severity of hepatic lesions and reduced the activity of GGT to control level. The increase in uric acid and decrease in total serum protein values caused by OA were significantly diminished to differing degree by vitamin E plus selenium. These data suggest that vitamin E alone or in combination with selenium can modulate the toxicity of OA in broilers.

Key words: Body weight, Broilers, Clinical chemistry, Ochratoxin – A, Organ weight, Selenium, Vitamin E

The toxic effects of ochratoxin A (OA), a mycotoxin produced by *Aspergillus ochraceus* are well established (Huff *et al.* 1974, Kubena *et al.* 1997).

Lipid peroxidation is one of the manifestation of cellular damage in the toxicity of several mycotoxins including ochratoxin (Rizzo *et al.* 1974, Shen *et al.* 1994). *In vitro* studies (Rahimtula *et al.* 1988) showed that ochratoxin-A, when added to rat liver micro-somes, increased the rate of NADPH ascorbate dependent lipid peroxidation resulting in changes in metallic activity within the cell and ultimately leading to cell necrosis. Few reports have indicated that dietary addition of antioxidants, vitamins E and C can modulate the severity of toxicosis and was able to ameliorate the oxidative stress caused by ochratoxin-A (Hazzele *et al.* 1993). Vitamins E and C, when used as free radical scavenging agents, provided protection against toxicosis caused by OA in rats and chicken. Selenium is a potent nutritional agent and plays central role in maintenance of cell membrane integrity and function. Nutritional interrelationship and interdependence exist for selenium and vitamin E.

Dietary addition of selenium (Burguera *et al.* 1983) or in combination with amino acid and a-tocopherol (Rizzo *et al.* 1994 and Shen *et al.* 1994) diminished the harmful efforts of aflatoxin in rats. Hoehler and Marquardt (1996) showed that the dietary use of vitamin E significantly reduced lipid peroxidation as it tended to counteract the OA induced increase uric acid in the plasma of chicks. Based on the current knowledge of mechanisms of OA toxicity, a suitable dietary modification may help in counteracting the adverse effect of OA. Therefore, the present trial was conducted to evaluate the effect of selenium and vitamin E alone and in combination for protection against the toxicity of OA in broiler chicks.

MATERIALS AND METHODS

Day-old broiler chicks (240) were wing banded and randomly assigned to 24 compartments of 2 battery brooders. Each compartments had 10 chicks with equal number of males and females, each treatment was replicated 3 times. The chicks were given *ad lib.* feed and water. Their diet consisted of unmedicated chicks starter for first 3 weeks and there after they received finisher diets, prepared as per BIS (1992). Ochratoxin A was produced through the fermentation of wheat by *Aspergillus ochraceus* NRRL 3174 (Trenk *et al.* 1971). The contaminated wheat was steamed and ground to a powder. The OA content was determined by TLC analysis (AOAC 1995). The wheat powder was then incorporated into basal diets to provide desire level (2 ppm) of OA. Vitamin E (400

Present address: [1]M.V.Sc. Scholar, [2] Associate Professor, [3]Professor and Head; [4] Professor; Department of Poultry Sciences, Veterinary College, Bangalore 560 024.

[5] Professor and Head, Department of Microbiology, Veterinary College, Bangalore 560 024.

Table 1. Effect of vitamin E and selenium both individually and in combination on body weight, feed efficiency, mortality relative organ weight and certain biochemicals paramaeters of broiler fed Ochratoxin at 6th week of age

Treatment	Ochratoxin A (ppm)	Vatamin E (mg/kg)	Selenium (mg/kg)	Body weight (g)	Feed efficiency	Mortality (%)	Kidney	Liver	TSP (g/l)	GGT (IU/l)	Uric acid
T_1	0	0	0	1240±32^{a}	2.10±0.01	0	0.7005±0.01^{a}	1.95±0.08^{a}	29.52±0.8^{de}	16.00±1.0^{ab}	5.42±0.06^{d}
T_2	2	0	0	784±32^{d}	2.24±0.01	6.67	0.9485±0.04^{d}	2.90±0.15^{d}	24.80±0.3^{ab}	20.83±0.7^{c}	8.24±0.16^{d}
T_3	0	400	0	1237±31^{a}	2.04±0.03	0	0.7555±0.02^{ab}	2.29±0.10^{ab}	31.10±0.2^{ef}	17.67±0.2^{abc}	5.43±0.14^{a}
T_4	2	400	0	915±24^{b}	2.10±0.02	0	0.8920±0.05^{cd}	2.29±0.12^{ab}	26.33±1.3^{bc}	17.50±1.8^{ab}	6.78±0.15^{c}
T_5	0	0	1.0	1229±38^{a}	2.12±0.01	0	0.7612±0.01^{ab}	2.43±0.18^{bc}	31.58±0.9^{f}	18.67±1.1^{bc}	6.17±0.02^{b}
T_6	2	0	1.0	851±21^{ab}	2.19±0.02	0	0.8986±0.35^{cd}	2.73±0.04^{cd}	24.10±0.98^{d}	14.83±0.7^{a}	8.06±0.17^{d}
T_7	0	400	1.0	1239±27^{a}	2.08±0.01	0	0.82540±0.01^{bc}	2.31±0.15^{b}	30.53±0.5^{ef}	1730±0.7^{ab}	7.02±0.33^{c}
T_8	2	400	1.0	850±31^{ab}	2.16±0.02	0	0.9645±0.04^{d}	2.30±0.07^{d}	27.65±0.9^{cd}	18.17±1.9^{bc}	7.30±0.34^{c}

Note: Means with in each column bearing atleast one common superscript do not differ significantly (P<0.05).

mg/kg) and selenium (1 mg/kg) was added to the OA contaminated feed and fed to chicks for 6 weeks. Care was taken to include appropriate controls.

The chicks were weighed individually on a weekly basis, feed consumption was recorded weekly, and mortality was recorded as it occurred. At the end of experiment 12 broilers from each treatment were bled by puncturing brachial vein for serum biochemical analysis. Six broilers (2 birds from each replicate) from each treatment were killed by cervical dislocation and the liver, and kidney were removed and weighed on a digital top pan electronic balance (0.1 g accuracy). Serum concentration of uric acid, total protein and activities of gamma glutamyl transferase were determine on automatic analyzer, according to the manufacturers recommended procedure. Experimental data was subjected to statistical analysis under randomized complete block design (Snedecor and Cochran 1967) and the interaction means were compared to Duncan's multiple range 'F' test (Duncan 1955).

RESULTS AND DISCUSSION

Chicks fed OA contaminated feed had a significantly ($P < 0.05$) lower mean body weight than the control. The body weights of chicks fed vitamin E and selenium, alone and in combination were identical to the chicks fed ochratoxin – A free diets. When vitamin E, was given with contaminated diet, body weight increased significantly ($P<0.05$) from 784 g to 915 g, a difference of 131g, as compared to control indicating the reversal of adverse effects of ochratoxin on body weight. When compared with control, there was no significant treatment effects on the efficiency of feed utilization.

Mortality average 6.67% when OA was fed to birds. When protectants were added to OA containing diets no birds died.

The OA induced growth depression was consistent with findings of Huff *et al.* (1974), Kubena *et al.* (1997) and Devegowda *et al.* (1998). The efficacy of various agents to counteract the adverse effects of OA is of great therapeutic interest. Lipid peroxidation is one of the mechanisms of OA induced injury. Thus use of free radical scavenging agents, which will counteract the lipid peroxides, is one of promising method of diminishing the toxic effects of OA. Vitamin E and selenium are an effective antioxidant. In this study, vitamin E (400 mg/kg) added to OA contaminated diet proved to be partially effective in ameliorating the growth depressing effects of OA. These results are in confirmation to be findings of Hoehler and Marquardt (1996). Feeding OA caused significant increase in the relative weights of the kidney and liver as compared with the control (Table 1). Significant ($P < 0.05$) increase in relative kidney weights of broiler chicks fed dietary OA were reported by Huff *et al.* (1974), Kubena *et al.* (1997) and Devegowda *et al.* (1998). Adding vitamin E and selenium, both individually and in combination, to the diets diminished the effects of OA on relative weights of the liver but not of the kidney. Grossly, kidneys and livers from broilers fed 2 ppm OA containing diet were enlarged, friable, congested and pale in appearance. Dwivedi and Burns (1984) reported a similar gross lesions in broilers chicks. Addition of dietary supplements did not alter the severity of OA induced kidney lesions, whereas livers from broilers consuming vitamin E plus 2 ppm OA diets were morphologically similar to those of control birds.

The concentration of total serum protein, indicator of protein synthesis (Swamy and Devegowda 1998) was reduced in chicks fed OA compared with control.

Addition of vitamin E plus selenium to OA diet significantly ($P<0.05$) improved the total serum protein level and diminished the adverse effects of OA on uric acid indicating the protecting effects against OA induced toxicity on the above parameters, whereas vitamin E or selenium did not alter the response of OA on total serum protein.

Feeding vitamin E alone resulted in a significant ($P < 0.05$) reduction only in uric acid concentration. A similar reduction in uric acid concentration in the plasma of the chicks by feeding 1 000 IU of vitamin E during ochratoxicosis was reported by Hoechler and Marquardt (1996).

The activity of GGT was significantly ($P<0.05$) increased by OA and vitamin E or selenium reduced this effect of OA to the level of control values.

The present study defined the toxicity of OA in broiler chicks. The current data indicate that all the negative effects

of OA on body weight, gross lesions, clinical chemistry parameters were either partially counteracted or reversed by dietary addition of vitamin E (400 mg/kg). The protective effects produced by vitamin E may be due to scavenging of free radicals by vitamin E there by inhibiting lipid peroxidation as suggested by Hoehler and Marquardt (1996). Selenium had shown interrelationship with vitamin E in counteracting the adverse effects of OA. The results also indicate that dietary compounds, when used in conjunction with other mycotoxin preventive practices, may prove to be useful tool in mitigating the adverse effects of ochratoxin A in chicken.

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Indian Journal of Animal Sciences 70 (2): 200-202, February 2000

Evaluation of kids rearing system for meat production

A K SHINDE[1], N P SINGH[2], A R SEN[3] and D L VERMA[4]

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

Received: 9 February 1999; Accepted: 17 August 1999

ABSTRACT

Marwari goats (60; 3-4-years old) were randomly distributed into 3 groups of 20 each and maintained under intensive, semi-intensive and extensive system of feeding. The kids born from these animals were also maintained under the 3 respective systems. Birth and slaughter weights (8 month) of kids were found higher under semi-intensive than those were under extensive and intensive systems. Dressing yield on live weight basis was higher in semi-intensive compared to intensive and extensive systems. Intensive feeding with minimum movement increased caul and kidney fat in the kids of intensive than extensive system. Expenditure on rearing of kids including cost of milk suckled, concentrate mixture, green fodder, labour and treatment was higher in intensive (Rs 797/ kid) followed by semi-intensive (Rs 521/ kid) and extensive (Rs 416/kid) system. Total receipt from sale of meat produce and manure were higher in semi-intensive (Rs 981/kid) than intensive (Rs 899/kid) and extensive (Rs 855/kid) system. Net profit per kid in intensive, semi-intensive and extensive system was Rs 102, 459 and 439 respectively.

It can be safely concluded that extensive and semi-intensive system of kid rearing are more remunerative provided adequate grazing lands are available for grazing / browsing of the kids.

Key words: Kid, Meat production, Rearing system

During to shrinkage of grazing lands, sharp increase in livestock population and controversy over role of goats under extensive system is threatened. Performance of the kids under intensive, semi-intensive and extensive system was reasonably well (Singh 1992). However, adaptation of any particular system for kid rearing depends not only upon animal performance but also on its economic viability. The present study was, therefore carried out to identify suitable system of kid rearing based on production performance and economics of different feeding system.

MATERIALS AND METHODS

Marwari does (3 to 4-year-old and 2-3 lactations), 60, were randomly distributed into 3 groups of 20 each and maintained under intensive, semi-intensive and extensive feeding management system. The kids were born in November 1997 and attained 8 months of age in the June 1998. They were also maintained in the respective systems. Kids in all the system were allowed to suckle their dams, twice daily during 0-3 months of age. The kids in intensive system were offered concentrate mixture in mash form *ad lib.* from 0-3 months of age and, therefore, same mixture @ 300g during 3-8 months of age with green lucerne *ad lib.* Under stalled condition. Whereas, in semi-intensive system, they were offered same concentrate mixture *ad lib.* during 0-3 months of age and @ 200g during 3-8 months of age with 6-8 hr of grazing on silvipasture. The predominant vegetation cover of silvipasture was *Cenchrus ciliaris* as grass, *Zizyphus nummularia* as bush and *Prosopis cineraria* as fodder tree. The kids in extensive system were also maintained on same pasture alone with kids of semi-intensive system except that they were not provided any concentrate supplement. Milk consumption of kids was determined twice daily at weekly interval by weighing the kid before and after the suckling their dams. In the mid-experiment, a digestion trial on 6 kids from each group was conducted by double indicator method (Krishna *et al.* 1981). Samples of forage consumed by the kids of semi-intensive and extensive systems were collected by following the animals in the pasture. Predominant vegetation in the diet of the kids was *Zizyphus numshularia* and *Prosopis cineraria* leaves. Samples of pasture forage, green lucerne and concentrate mixture were analysed for crude protein (AOAC 1984), neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin (Goering and Van Soest 1970). All the kids were weighed by salter balance at birth, weaning (3 months), and slaughter (8 months) age. Male kids (6) of each group were slaughtered following the standard procedure. Expenditure on feed (milk, concentrate and green fodder) was calculated

Present address: [1]Scientist, Senior Scale (Livestock Production and Management), [2]Principal Scientist (Animal Nutrition), [3]Scientist (Livestock Product Technology), [4]Technical Officer.

Table 1. Ingredients and chemical composition of concentrate mixture, green fodder and pasture forage consumed by kids

Ingredients	Parts
Physical composition	
Barley	58
Groundnut-cake	30
Wheat bran	10
Mineral mixture	01
Salt	01

Chemical composition (on % DM basis)

	DM	CP	NDF	ADF	Cellulose	Lignin	GE
Concentrate mixture	93.2	16.6	50.7	17.8	20.9	6.1	4.0
Green lucerne	20.5	26.5	64.7	38.9	25.6	8.6	3.9
Pasture	35.1	12.2	58.9	31.5	16.6	11.2	3.6

by considering the market price of the feeds and amount consumed from birth to slaughter age. Labour charges for grazing and management of kids were calculated as per the existing rate contract of the Institute (Rs 21.75/ animal / month). Whereas, in intensive system the charges were calculated by considering the hours of labour involvement in management of these animals. The cost of prophylactic (vaccination, drenching and dipping) and curative treatments as and when required were also calculated. Slaughter produce of kids was sold as per the prevailing market price. The skin, stomach and intestine, head and hooves, liver, lungs and heart were sold @ Rs 93.50, 30.00, 52.50 and 31.50 per head respectively. Meat was sold @ Rs 80 per kg.

Data on growth performance and carcass characteristics were tested by analysis of variance and group difference was compared (Snedecor and Cochran 1967). Expenditure incurred on individual component and receipt from sale of produce were present as mean.

RESULTS AND DISCUSSION

The chemical composition of pasture forage, green lucerne and concentrate mixture is presented in Table 1. Average concentrate mixture intake of kids during 0-3 months of age was 99 and 101 g/head/day in intensive and semi-intensive systems respectively. Kids on an average consumed 512, 441 and 302 g DM/head/day in intensive, semi-intensive and extensive systems respectively.

The birth weights of kids in intensive and extensive systems were almost similar and slightly higher in semi-intensive. While weaning weights were significantly ($P<0.05$) higher in extensive than in semi-intensive and intensive systems (Table 2). Slaughter weights at 8 months of age were higher in semi-intensive than under the other 2 systems. Lower slaughter weight in intensive system despite highly nutritious diet were due to higher incidence of diarrhea cases as a result of poor exercise in confinement. Parthsarthy *et al.* (1984) and Shinde *et al.* (1995) also reported similar observation in kids of intensive system. Overall average daily gain in kids

Table 2. Growth performance of kids under different systems

Growth performance	Different systems		
	Intensive	Semi-intensive	Extensive
No. of kids	16	18	18
Birth weight (kg)	2.44±0.13	2.57±0.08	2.45±0.12
Weaning weight (kg)	9.92^{a}±0.69	9.46^{a}±0.10	10.58^{b}±0.56
Slaughter weight (kg)	19.71^{a}±0.13	23.90^{b}±0.18	21.77^{a} 0.17
ADG (g)			
(0–3 months)	83^{b}±2.62	76^{a}±5.52	90^{b}±4.62
(3–8 months)	71^{a}±2.67	102^{b}±3.87	81^{a}±2.75
(0–8 months)	76^{a}±3.11	93^{b}±2.62	84^{a}±4.31

Unlike superscript in a row differ significantly ($P<0.05$).

during 0-8 months of age was higher in semi-intensive (93g) than extensive (84g) and intensive (76g) systems. Contrary to this finding, higher growth rate of kids under intensive than semi-intensive system was reported by Nagpaul *et al.* (1995).

Carcass characteristics of kids (Table 3) revealed that dressing percentage on live weight basis was higher in semi-intensive (44.78%) than that in intensive (43.63%) and extensive (41.86%) systems. Rearing of kids under intensive system increased the caul and kidney fat (1.27 and 0.61%) as compared to semi-intensive (0.86 and 0.41%) and extensive (0.53 and 0.35%). High proportions of caul and kidney fat for intensively fed kids were probably brought about by high intake of protein and energy (Mtenga and Kitaly 1990) and is not considered as desirable parameter of carcass characteristics. It is possible that high dietary energy concentrations when fed to confined kids leads to less utilization of energy for tissue synthesis due to lack of sufficient exercise, resulting in more energy deposited as fat.

Table 3. Carcass characteristics of kids slaughtered under different systems

Carcass characteristics	Different systems		
	Intensive	Semi-intensive	Extensive
Weight (kg)			
Slaughter	20.24^{a}±0.13	24.99^{c}±0.14	22.35^{b}0.37
Empty life	17.12^{a}±0.92	20.49^{b}±1.19	18.30^{a}±0.17
Hot carcass	9.06^{a}±0.54	11.20^{b}±0.71	9.53^{a}±0.47
Per cent (%)			
Dressing (on live wt. basis)	43.63^{b}±0.78	44.78^{b}±0.87	41.86±0.69
Dressing (on empty live wt. basis	52.93^{a}±0.66	54.41^{b}±0.87	52.04^{a}±0.58
Caul fat	1.27^{c}±0.14	0.86^{b}±0.08	0.53^{a}±0.11
Kidney fat	0.61^{b}±0.07	0.41^{a}±0.09	0.35^{a}0.06
Total separation			
Lean	71.04^{a}±1.17	74.38^{b}±2.26	76.39^{b}±1.51
Fat	6.74^{b}±0.49	6.71^{b}±0.65	5.20^{a}±0.43
Bone	23.69^{b}±1.38	18.88^{a}±1.83	18.37^{a}±1.22

Unlike superscripts in a row differ significantly ($P<0.05$).

Table 4. Economics of kid rearing under different systems

Economics of kid rearing	Kid rearing systems		
	Intensive	Semi-intensive	Extensive
Cost of			
Milk @ Rs 6.00/kg	180.00	168.00	187.00
Concentrate @ Rs 5.50/kg	387.00	124.00	–
Dry roughage @ Rs 2.50/kg	90.75	–	–
Green roughage @ Rs 0.50/kg	30.50	–	–
Pasture forage	–	–	–
Total feed cost (Rs/head/8 month)	690.25	332.0	227.00
Labour @ Rs 21.75/ *animal/month*	91.00	174.00	174.00
Treatment (Preventive and curative)	15.78	15.22	15.02
Total expenditure	797.03	521.22	416.02
Meat/offels	633.60	757.20	631.20
Skin	93.50	93.50	93.50
Stomach and intestine	30.00	30.00	30.00
Head and hooves	52.50	52.50	52.50
Liver, heart and lung	31.50	31.50	31.50
Manure	48.50	16.50	16.50
Total receipt	899.60	981.20	855.20
Net profit	102.57	459.98	439.18

The separation of carcass into lean, fat and bone indicated that intensive feeding under confinement increased the fat (6.74%) and bone (23.69%) and decreased lean (71.04%). On the contrary rearing of kids on grazing alone (extensive system) increased lean (76.39%) and decreased the fat (5.2%) and bone (18.37%) (Table 3). Singh and Sahu (1997) have also reported higher bone and lean percentage in semi-intensive and fat percentage in intensive system of feeding of kids.

Feed cost constituted 86, 63 and 54% of the total expenditure incurred on maintenance of kids in intensive, semi-intensive and extensive systems (Table 4). Higher feed cost in intensive system was obvious as the kids were maintained on stall feeding, while in other 2 systems feed requirement was partly or fully met out from the grazing land at nominal charges (Saini *et al.* 1988). Labour involvement in grazing of flock is a major labour costing activity, constituting 90% of the total labour cost in management of the flock. Labour charges in semi-intensive and extensive systems were similar and higher than intensive system. Preventive and curative treatments in all the systems were almost similar except slightly higher charges of curative treatments in intensive due to higher incidence of non-specific diarrhoea cases.

Total expenditure on rearing of kids up to slaughter age (8 months) in extensive system was Rs 416.02 and lowest among the 3 systems, while it was 91 and 25% higher in intensive and semi-intensive over extensive systems. Expenditure in semi-intensive was 34% less than that in intensive system (Table 4).

Receipt from sale of skin, stomach and intestine, head and hooves, liver, lung and heart were similar in all the system, as these were sold on per head basis rather than per kg basis. Sale receipts from boned – meat/ offal's and dissected meat were higher in semi-intensive than the other 2 systems because of higher slaughter weights and carcass yield in this group. Total receipt in extensive system was Rs 855.20 and lowest among the 3 systems, while it was 14 and 5% higher in semi-intensive and intensive over extensive systems. Net profit from kid rearing for meat production under intensive, semi-intensive and extensive systems was Rs 102.57, 459.98 and 439.18/ kid respectively.

It can be concluded from the study that kid rearing for meat production under extensive and semi-intensive systems are more remunerative as long as grazing/browsing resources are available free and /or at nominal cost as was the case with most of the farmers who meet out the forage requirement of their goat flocks on no cost basis from public grazing lands.

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Indian Journal of Animal Sciences 70 (2): 203-204, February 2000

Gir crossbreds for reproductive traits

W K NAGARE[1] and M B KULKARNI[2]

Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra 413 722

Received: 13 July 1998; Accepted: 13 August 1999

Key words: Crossbred cow, Gir, Reproductive traits

Performance regarding age and weight at first service (AFS, WFC), age at first conception (AFC), service period (SP), calving interval (CI) were evaluated amongst F-1 and F-2 of Gir crosses with Holstein Friesian (HF), Jersey (J) and Brown-Swiss (BS) on the basis of overall performance. It is recommended that Jersey should be the choice temperated dairy breed followed by Friesian for cross breeding of indigenous cattle in Maharashtra.

A few reports are available on the performance of Gir crossbred in tropical region for selection of superior germplasm for higher milk production. Present investigation was undertaken to study the effect of different factors on various reproductive traits of different genetic groups of Gir crossbreds with Friesian × Jersey and Brown-Swiss raised in Cattle Project, Mahatma Phule Krishi Vidyapeeth, Rahuri (MS).

The data on 3 235 records from 1 072 crossbred animals during 1972-1991 raised at Cattle Project, MPKV, Rahuri, were collected. The Gir females were bred at random with the bulls of Holstein Friesian (HF), Jersey (J) and Brown-Swiss (BS) by artificial insemination (AI), Gir females large in number were bred to Friesian followed by Jersey and Brown Swiss. The crossbred calves were weaned at birth and raised on standard nutritional regime.

The management of calves, heifers and cows was uniform for all the catagories of genetic groups. The heifers were restricted to be inseminated for the first time when they reached 15 months of age or 250 kg body weight in HF and Brown Swiss crosses and 200 kg body weight in Jersey crosses. The cows were milked twice a day.

The data were classified according to genetic groups and season of calving. The constants of these effects were fitted by the method of least-squares (Harvey 1990). The differences among least-squares means for different traits were tested for their significance by Duncan's multiple range as modified by Kramer (1957).

The overall mean for age at first service (AFS) for Gir crossbreds ranged from 498 to 582 days (16.60 to 19.10 month) (Table 1). Though FG heifers bred later 6-7 days than JG heifers but the difference was nonsignificant. Thus the FG also did well for AFS. Amongst triple crossbred, BFG heifers bred late by 19 days than FJG and 16 days than JFG. However, differences were nonsignificant. The averages and their trends in the halfbreds and 3 breed groups were similar to findings of Bhat (1980), Narayanswamy (1981) and Navale (1991). Tropical cattle show first oestrus at around 22 months and AFS at 30 months, whereas in crossbreds of local cattle (*Bos indicus*), particularly, with Jersey both age at first heat and AFS (16-17 month) were reduced to about 10 to 15 months (Galina and Arthur 1989). The age at first service (AFS) was significantly ($P<0.01$) influenced by genetic groups.

The body weight at first service (WFS) in FG was higher by 40 kg than that in the JG heifers. The WFS were 285.73, 268.24 and 297.98 kg in FJG, JFG and BFG triple crosses were significantly ($P<0.01$) lower in interse groups than their parents. FG and BFG heifers were heavier than Jersey heifers. Similar results were reported by Chopra (1990) for Hariana crossbreds. Thus, amongst crossbreds studied, FG, FJG and JG group showed a better performance than the rest.

The overall mean for age at first conception for Gir crossbreds was 587.32 ± 4.74 days (19.32 month). The halfbreds has an average age at first conception of 553 days whereas triple crosses had 568 days; thus halfbred conceived earlier by 15 days in spite of the fact that AFS was the similar.

The overall means for age at first calving (AFC) for Gir crossbreds was 858.72 ± 4.59 days (28.30 months). A substantial reduction for AFC in Gir crossbreds was observed to be 15.30 months over Gir (43.60 month) as reported by Taneja and Bhat (1989). The genetic groups had highly significant effect on AFC (Table 1). Similar findings were reported by Kale (1984) for Gir crossbreds. In general Jersey crossbreds, halfbreds and triple crossbred calved earlier than HF crossbred. However, the FJG and JFG heifers calved at the same age.

Present address: [1]Ph.D. Thesis by first author, Subject Matter Specialist Dairy, Directorate of Extension, MPKV, Rahuri.

[2]Head, Department of Animal Science and Dairy Science, MPKV, Rahuri.

Table 1. Least-squares means of reproductive traits for Gir crossbreds and seasons

Traits effects	n	AFS (days)	WFS (days)	AFCon (days)	AFC (days)	WFC (kg)	SP (days)	CI (days)
Overall mean	1072	534.94 ± 3.84	277.44 ± 2.18	587.32 ± 4.74	858.72 ± 4.99	382.76 ± 2.68	123 24 ± 2.62	413.83 ± 2.33
Genetic group								
FG	168	505.37e ± 10.50	291.41ab ± 5.86	568.43cd ± 13.54	839.36cd ± 14.11	421.85a ± 7.32	123.02cd ± 5.13	414.77bcd ± 4.31
FG-I	87	578.68a ± 12.83	281.12c ± 7.73	645.61a ± 13.48	923.27a ± 14.83	371.63de ± 9.81	130.78abc ± 7.56	413.57bcd ± 7.24
FJG	120	516 88cde ± 9.60	285.73bc ± 5.32	556.98 ± 12.58	821.50de ± 13.06	389.40bc ± 6.70	120.32cde ± 5.71	409.65cd ± 5.21
FJG-I	196	536.61bc ± 8.20	277.66c ± 4.80	582.47bcd ± 9.83	857.78bc ± 10.10	380.82cd ± 5.75	140.43ab ± 5.69	428.99ab ± 4.71
JFG	88	508.71de ± 9.68	268.24d ± 5.29	557.68cde ± 12.98	827.23cde ± 13.41	366.39e ± 6.74	106.48 ef ± 6.23	398.92de ± 5.98
FJG-I	92	544.10b ± 10.15	253.81e ± 5.81	605.99b ± 12.37	884.23b ± 13.06	349.19f ± 7.10	125.81bcd ± 7.31	420.02abc ± 7.04
BFG	94	525.66bcd ± 18.25	297.98a ± 5.78	588.33bc ± 12.93	855.55bc ± 13.54	422.24a ± 7.10	114.66def ± 5.93	410.33cd ± 5.00
BFG-I	124	581.80a ± 9.19	291.85ab ± 5.20	642.39a ± 11.50	917.63a ± 12.07	397.39b ± 6.42	145.95a ± 6.67	435.42a ± 6.88
JG	183	498.67cde ± 11.79	249.76e ± 6.39	538.85e ± 16.04	801.54e ± 16.52	345.94f ± 8.21	101.72f ± 7.46	392.85e ± 6.71
Season of calving								
Summer	123	533.54 ± 5.71	278.03 ± 2.76	585.56 ± 7019	859.10 ± 7.37	383.62 ± 3.59	125.60 ± 3.19	420.10 ± 3.33
Rainy	368	534.65 ± 4.95	279.55 ± 2.67	586.37 ± 6.82	856.37 ± 7.00	382.00 ± 3.44	120.62 ± 3.38	409.10 ± 3.32
Winter	381	530.60 ± 4.91	274.74 ± 2.65	590.64 ± 6.74	860.69 ± 6.92	383.65 ± 3.42	123.51 ± 3.27	412.25 ± 3.18

*Means with similar superscripts differ significantly (P<0.01).

The overall mean body weight at first calving (WFC) of Gir crossbreds irrespective of exotic donar breeds and level or genetic inheritance was significantly higher by 18% than those of JG heifers. The triple bred ranges from 346 to 457 kg. BFG and FG had higher body WFC and differ significantly from all other genetic groups. It may be on account of HF and BS are heavy breeds.

The overall least squares mean for service period (SP) in Gir crossbreds was 12 days shorter than Gir cows regressing more towards exotic breeds. Nonsignificant effects of season of calving on SP was observed. Similar results were reported by Kale (1984).

The overall calving interval (CI) was 13.61 months (Table 1) which is an ideal for crossbreds under Indian condition. The CI was significantly affected by genetic groups and nonsignificant differences for season of calving were observed in all the genetic groups. Kale (1984) and Navale (1991) reported similar results.

The lower averages for AFS, WFS, AFC-on, AFC, WFC, SP and CI in Gir crossbreds in this study were at variance with the findings of Taneja and Bhat (1989) and Katpatal (1977).

For reproductive traits, year showed significant variation reflecting differences in herd structure, feed and fodder supply and management. Season of calving did not show significant effect on reproductive traits; signifying the minimum differences in feeding and management as well as other stressful conditions to the animals.

Consequently, reproductive performance of the halfbreds and triple bred Gir crossbred groups revealed that halfbred of HF (FG) and Friesian × Jersey × Gir (FJG) breeds were ideally suited as dairy animals than Brown Swiss crosses in reproductive performance. Jersey seems to be the breed of choice for crossing local zebu for improvement in reproductive traits followed by Friesian.

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Indian Journal of Animal Sciences 70 (2): 205-206, February 2000

Effect of dietary copper and molybdenum levels on animal growth*

O P SINGH[1] and B L SARASWAT[2]

Udai Pratap Autonomous College, Varanasi, Uttar Pradesh

Received: 11 November 1998; Accepted: 17 August 1999

Key words: Animal growth, Dietary copper, Moly bdenum

The present study was carried out to determine the extent of accumulation of Cu and Mo in animal organs and to assess their impact on animal growth.

Experimental animals comprised of 15 guinea pigs (*Cavia porcellus*) of identical physiological specifications, divided into 5 groups of 3 each and assigned to Cu and Mo treatments following a randomized block design. The experiments were continued over 40 days under identical conditions of feeding and management with *Cynodon dactylon* grass as basal feed.

Treatments were T0 (basal feed), TC1 (basal feed ± 5 mg Cu), TC2 (basal feed ± 10 mg Cu), TM1(basal feed ± 5 mg Mo) and TM2 (basal feed ± 10 mg Mo). While Cu was supplemented as copper sulphate ($CuSO_4 5H_2O$), Mo was given as ammonium molybdate $(NH_4)Mo_7O_{24}.4H_2O$ satisfying 99% of the requirement. At the end of experiment, animals were slaughtered to assess deposition of metals in blood, muscle, liver and kidneys. The samples of feed, faeces, urine and body organs were analysed for Cu and Mo concentrations using atomic absorption spectrophotometer after digestion of freeze-dried in the presence of nitric and perchloric acids (1: 3V/V).

Retention of Cu and Mo

Dietary intake, excretion and retention rates of Cu and Mo in different treated groups of *Cavia porcellus* are shown in Table 1. Copper retention (mg/day/head) was 1.096 in T0 (control) which increased 5 and 8 times in TC1 and TC2 respectively. At the other stream, only 0.80 mg Mo was retained in T0 enhancing by 2.88 mg in TM1 and 4.05 mg/day/head in TM2.

Further, observations revealed that both the excretion and retention rates (mg/day/head) of Cu or Mo in the animal body were significantly ($P < 0.01$) higher with 10 mg supplemented diet than with 5 mg or the control. The results suggested that metal retention enhanced in accordance with the increase in dietary intake. On per day basis, retention of Cu was recorded at a higher rate than Mo, though animals stored double amount of latter in their body than the former during 40 days which is in agreement with the earlier findings (Moshtaghi-Nia *et al.* 1989).

Table 1. Excretion and retention rate of Cu and Mo (mg/day/head) in *Cavia porcellus*

Treatment	Intake	Excreted	Retained	Retention /day/head (%)
		Copper treated groups		
T0	3.84	2.74a	1.10 a	28.57
T C1	8.79	3.52	5.27 b	59.92
T C2	13.74	5.31 b	8.43 c	61.34
Mean ± SE	8.79 ±3.27	3.86 ±1.39	4.93 ± 2.26	56.09
		Molybdenum treated groups		
T0	1.01	0.94 a	0.08 a	7.50
T M1	5.96	3.09 b	2.88 b	48.23
T M2	10.91	6.86 c	4.05 b	37.12
Mean ±SE	5.96 ±4.62	3.63 ±3.29	2.33 ±1.98	39.09

The values bearing different superscripts in a column differ significantly ($P < 0.01$).

Relative accumulation of Cu and Mo

The maximum quantity of Cu (35.50 ±2.76 mg/head) was accumulated in liver followed by muscles, kidney and blood (Table 2). Charmely and Ivam (1989) also recorded significantly ($P < 0.01$) high deposition of Cu in liver than the kidney in crossbred wethers.

Amongst Mo treated groups, its major fraction (33.06±3.32 mg/head) was found to get stored in kidneys, minimum (2.17±0.089 mg/head) in the blood and almost equal amount was observed in muscles and the liver (Table 2). Total accumulation rate of Mo, irrespective of treatments, was more (91.82 mg/head) than the Cu (52.11 mg/head). Significantly ($P < 0.01$) more Mo was stored in TM2 than the TM1 and T0 groups.

Influence on body weight of cavia porcellus

Levels of Cu/Mo in the body affect the growth rate of animals. Average daily gain was markedly lower in T0 as compared to T C1 (Table 3). Charmely and Ivan (1989) also

*A part of Ph. D. Thesis submitted by the first author.

Present address: [1]Senior Lecturer, [2]Senior Reader, Department of Animal Husbandry and Dairying.

Table 2. Relative accumulation of Cu and Mo (mg/head) in vital body organs of *Cavia porcellus* (in 40 days)

Treatment	Blood	Muscles	Liver	Kidney	Total accumuulation in body
Copper treated groups					
T0	0.58[a]	5.03[a]	28.87[a]	3.25[a]	37.73[a]
$T C_1$	0.86	9.75 b	35.68	5.25	51.5
$T C_2$	2.13[b]	12.98[c]	41.96[b]	10.00[b]	67.07[b]
Mean ±SE	1.19± 1.07	9.25± 3.26	35.50± 2.76	6.17± 2.68	52.11
Molybdenum treated groups					
T0	1.68	21.06[a]	24.03[a]	29.15[a]	75.92[a]
$T M_1$	2.22	27.56[b]	28.12	33.02	90.92[b]
$T M_2$	2.61	34.97[c]	34.01[b]	37.01[b]	108.62[c]
Mean ±SE	2.17± 0.89	27.86± 3.12	28.72± 2.41	33.06± 3.32	91.82

The values bearing different superscripts in a column differ significantly ($P < 0.01$).

reported more gain in body weight of sheep fed 29 mg Cu/kg DM supplemented diet as against the control with intake of 6.7 mg/kg DM. It may be retained that during the course of present investigation animals lost their weights by 4.33% after 40 days with the increased level (10 mg) of copper (T C2) in diet.

While dietary supplement of Mo @5 mg/day/head (T M1) did not result in any satisfactory gain, the weight of animals in T M2 declined considerably (8.25%) indicating that additional intake of Mo produced adverse effect on the growth of animal.

Incorporation of 5 mg/Cu/head/day in the diet of *Cavia porcellus* was found beneficial as against 10 mg. Additional fortification of Mo, on the other hand, may lead to adverse effect. Maximum Cu accumulation was recorded in the liver, however, additional feeding of both the metals accelerated their rate of excretion through urine.

Table 3. Body weight of *Cavia porcellus* affected by Cu and Mo dietary intake

Treat-ment	Initial body weight (g)	Final weight (g)	Weight gain or loss in 40 days	Weight gain or loss/day	Per cent weight gain or loss in (g) 40 days
T_0	345.00 (±33.42)	365.67 (±35.67)	±20.67	±0.52	±5.99
$T C_1$	335.00 (±25.49)	368.33 (±25.95)	±33.33	±0.83	±9.95
$T C_2$	315.67 (±4.92)	302.00 (±5.09)	−13.67	−0.34	−4.33
$T M_1$	298.33 (±13.12)	300.67 (±13.96)	±2.34	±0.05	±0.78
$T M_2$	315.00 (±10.80)	289.00 (±9.63)	−26.00	−0.65	−8.25

Figures within parentheses indicate standard error.

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Indian Journal of Animal Sciences **70** (2): 207-208, February 2000

Efficacy of cabbage waste incorporated diet on the performance of layers*

KAMLESH SINGH[1] and B L SARASWAT[2]

Udai Pratap Autonomous College, Varanasi, Uttar Pradesh

Received: 11 November1998; Accepted: 13 August 1999

Key words: Cabbage waste, Feed, Layers

About 25 000 tonnes of fruit and vegetable wastes are accumulated each year in India and an estimated 275 million rupees are lost annually. The Energy Survey Committee of India reported that about 30 million tonnes of vegetable wastes are burnt every year in India. The incorporation of vegetable wastes in poultry feed is one of the ways to reduce feed cost and pollution may be controlled to some extent.

Day-old White Leghorn chicks (180) were divided into 9 groups of 20 each. First group was fed control ration (T0) and replaced with cabbage waste as green @ 20% (T1) and 30% (T2), as meal @ 30% (T3), 40% (T4) and 50% (T5) and as silage @ 30% (T6), 40% (T7) and 50% (T8). The diets of all groups were made isonitrogenous and isocaloric fortified with required amount of nitrogen or energy rich ingradients. Cabbage waste in the form of green, meal and silage was offered in separate containers to the birds for 50 weeks. Chemical composition of different feeds used in the study is given in Table 1. Meal of waste was prepared after properly drying and grinding of cabbage waste and its silage was prepared (Ranjhan 1980). Feed samples were analysed as per AOAC (1980). Data were subjected to analysis of variance (Snedecor and Cochran 1968).

Dry matter consumption enhanced significantly ($P < 0.01$) by increasing the level of silage in layer ration only except all groups under study and highest (92.13 g/day/chick) was recorded when cabbage waste silage was incorporated at 50% level. T1 and T6 gained highest body weight and almost equal response. T0 (control) and 40 and 50% of silage groups (T1 and T8) gave equal response representing identical value (1500 g) at 20th week of age. A significantly ($P < 0.01$) highest hen day average (54.14) was recorded in T7 (40% silage) followed by T0, T2 and T4 and T6 groups between 26th to 50th week of age. Hen day average was lowest during starting of egg production in all treated groups and it increased thereafter (up to 50 weeks).

Layers of T1 and T2 groups required significantly ($P <$ 0.01) greater amount of feed to produce 10 eggs than the layer of other groups under study (Table 2). It may probably be due to high moisture content in green cabbage waste which should be required comparatively in much amount to fulfil D M need of the layers. It was significantly low in control group (T0). When layers were maintained on meal incorporated diets the feed conversion ratio was comparable to the control group. Cost of feed to produce per 10 eggs was lowest when meal was incorporated in layer ration as compared to other feed treatments under study.

Hen day average was recorded highest (54.14%) in T7 and T2 and T4 showed equal response. Feed conversion ratio

Table 1. Composition of experimental ration (% on DM basis)

	Usual ration		Cabbage waste		
Constituents	Grower	Layer	Fresh	Meal	Silage
D M	91.00 (±0.06)	90.50 (±0.33)	12.00 (±0.42)	91.00 (±0.32)	40.00 (±3.05)
C P	18.00 (±0.57)	17.00 (±0.32)	21.00 (±0.31)	19.00 (±0.42)	15.00 (±0.90)
E E	2.00 (±0.06)	2.50 (±0.18)	2.50 (±0.16)	3.08 (±0.19)	3.50 (±0.16)
N F E	58.00 (±0.75)	56.00 (±0.84)	43.50 (±0.84)	42.62 (±0.67)	47.50 (±1.12)
C F	9.00 (±0.21)	10.20 (±0.50)	10.00 (±0.31)	10.20 (±0.28)	12.00 (±0.38)
T C	67.00 (±0.47)	66.25 (±0.59)	53.50 (±0.72)	52.82 (±0.70)	59.50 (±0.78)
Total ash	13.00 (±0.31)	14.25 (±0.38)	23.00 (±0.68)	25.10 (±0.30)	22.00 (±0.69)
Solu. ash	7.50 (±0.29)	8.25 (±0.68)	19.60 (±0.54)	21.24 (±0.25)	18.10 (±0.36)
Insolu. ash	5.50 (±0.12)	6.00 (±0.27)	3.40 (±0.17)	3.86 (±0.16)	3.90 (±0.36)
Ca	1.20 (±0.063)	3.00 (±0.016)	3.00 (±0.24)	3.40 (±0.11)	2.80 (±0.19)
P	0.64 (±0.045)	0.65 (±0.044)	0.50 (±0.031)	0.44 (±0.017)	0.45 (±0.04)
Fe	0.020 (±0.0019)	0.020 (±0.0019)	0.018 (±0.0008)	0.011 (±0.0006)	0.015 (±0.0015)

Figures within parentheses indicate standard error.

*Part of Ph. D. Thesis submitted by first author.

Present address: [1]Senior Research Fellow, [2]Senior Reader, Department of Animal Husbandry and Dairying.

Table 2. Performance of chicks and layers fed different level of cabbage waste

Treatments	Av. DM intake (g/d) (0-20 wks)	Body wt. gain (g) (at 20th wks)	Av. Egg production (% hen day)	Egg mass (wt. of 10 eggs in g.)	Av. Feed efficiency (kg feed/ 10 eggs) (26 to 50 wks)	Av. cost of feed (Rs/10 eggs)
T_0 (Control)	77.73[a]	1500[a]	53.17[b]	590[a]	1.66[a]	6.29[b]
T_1 (20% green)	85.59[c]	1520[a]	52.16[c]	510[b]	3.70[c]	4.97[b]
T_2 (30% green)	83.10[c]	1450[b]	53.58[b]	505[b]	3.91[c]	4.12
T_3 (30% meal)	79.44[a]	1450[b]	50.83[c]	584[a]	1.76[a]	4.70
T_4 (40% meal)	79.00[a]	1380[b]	53.57[b]	570[a]	1.77[a]	3.73[a]
T_5 (50% meal)	76.62[a]	1320[b]	50.73[c]	550	2.04	3.47[a]
T_6 (30% silage)	89.18[b]	1520[a]	53.17[b]	605[a]	2.35[b]	5.05[b]
T_7 (40% silage)	90.81[b]	1500[a]	54.14[a]	595[a]	2.46[b]	4.78
T_8 (50% silage)	92.13[b]	1500[a]	52.16[c]	602[a]	2.67[c]	4.71

The values bearing different superscripts in a column differ significantly ($P < 0.01$).

(kg feed/10 eggs) was recorded minimum when birds were reared with control ration (T0). Inclusion of cabbage waste either in the form of silage or meal lowered the cost of feeding and enhanced egg production.

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Indian Journal of Animal Sciences **70** (2): 209-210, February 2000

Udder measurements in Jersey × Red Sindhi crossbred cows

SANTOSH KUMAR[1], S KATOCH[2], Y P THAKUR[3] and N K MANUJA[4]

Himachal Pradesh Krishi Vishvavidalaya, Palampur, Himachal Pradesh 176 062

Received: 17 December 1998; Accepted: 6 August 1999

Key words: Jersey, Milk yield, Red Sindhi, Udder measurements

The present study was carried on to estimate different udder measurements in Jersey ×Red Sindhi cows and find out their relationship with milk yield.

Udder measurements (udder height, udder length and udder rear attachments) were measured on 95 Jersey ×Red Sindhi crossbred cows maintained at 2 organized dairy herds in Himachal Pradesh (Himachal Pradesh Krishi Vishvavidalaya dairy farm, Palampur and Government Jersey Cattle Breeding farm, Palampur). Udder measurements were recorded with the help of measuring tape (Mangurkar and Desai 1981) after morning milking. The data on their milk yield were taken from the production records maintained at respective farms. The entire data was analysed for various effects such as farm, parity of calving, season of birth, year of birth and stage of lactation by least squares analysis method using the LSMLMW PC-2 version computer programme (Harvey 1990).

The overall least-squares means estimates for udder height, udder rear attachment and udder length in Jersey × Red Sindhi crossbred cows were 52.89±5.19cm, 27.16±3.85cm and 36.39±4.57 cm respectively (Table 1). The estimated means for these traits were lower than the reported means for udder length in Jersey (Sharma *et al.* 1983, Bhadauria and Johar 1985), as well as in Jersey crosses with different Indian breeds (Mali *et al.* 1986, Saiyed and Patel 1989, Baruah *et al.* 1991).

Effects of various non-genetic factors on udder measurements

The analysis of variance for different factors affecting udder measurements revealed significant effects of sire, parity and year of birth on all the traits considered. The farm effect was observed significant for udder height whereas season of birth significantly influenced only the udder length. The stage of lactation significantly influenced only the udder height. Similarly significant effects of parity on the udder height and udder length in Jersey × Hariana crossbred and Hariana cows were reported by Sharma *et al.* (1983) whereas nonsignificant

Present address: [1] Research Scholar; [2, 3] Associate Professor, [4]Professor and Head, Department of Animal Breeding, Genetics and Biostatistics, COVAS.

Table1. Least-squares means ± SE of udder measurement traits in Jersey × Red Sindhi crossbred

Source of variation	No. of observation	Udder height (cm)	Udder rear attachment (cm)	Udder length (cm)
Overall mean	095	52.89±5.19	27.16±3.85	36.39±4.57
Farms				
HPKV Dairy farm	84	45.25±5.36a	25.79±3.97a	39.33±4.76a
GJCBF Palampur	11	60.52±5.44b	28.52±4.03a	33.46±4.85a
Parity				
1st	3	51.23±6.34b	22.12±4.67a	30.31±5.83a
2nd	25	51.27±5.45b	22.37±4.03a	35.04±4.86b
3rd	28	50.96±5.33ab	26.33±3.94b	31.98±4.72ab
4th	18	50.53±5.26ab	25.92±3.90b	36.91±4.66b
5th	18	49.31±5.61b	24.99±4.14b	35.65±5.03b
6th	3	48.03±7.01a	23.23±5.15ab	48.47±6.54c
Season of birth				
Winter	23	53.10±5.35a	26.53±3.96a	32.21±4.75a
Summer	32	52.73±5.26a	25.18±3.90a	38.06±4.65c
Rainy	18	52.42±5.36a	29.12±3.97a	39.83±4.76d
Autumn	22	53.31±5.30a	27.82±3.93a	35.47±4.70b
Year of birth				
1988	6	57.91±6.20d	29.15±4.57c	26.69±5.68a
1989	14	54.21±5.89c	28.64±4.35d	25.18±5.35a
1990	21	56.21±5.91cd	30.14±4.36e	33.49±5.37b
1991	15	51.63±5.50b	31.79±4.07f	40.00±4.92c
1992	27	58.21±5.84d	21.40±4.31a	36.05±5.29bc
1993	9	42.85±5.89a	23.68±4.35b	47.21±5.36d
1994	3	49.20±6.61b	25.30±4.86c	46.13±6.11d
Stage of lactation				
Ist	47	62.02±5.49b	29.51±4.06a	33.48±4.90a
2nd	15	49.05±5.46a	24.59±4.04a	40.40±4.87a
3rd	9	50.73±5.42a	27.52±4.01a	34.10±4.83a
4th	10	49.38±5.47a	27.56±4.04a	37.15±4.88a
5th	14	49.26±5.51a	26.62±4.08a	36.83±4.93a
Reg. on AFC				
Linear		0.010±0.004	0.005±0.003	0.003±.004
Quad		0.002±0.0007	−0.0002±0.001	0.0008±.0007

Least-squares means with same superscripts do not differ ($P<0.05$) significantly.

effect of parity on udder rear attachment but significant on udder length and udder height in buffaloes was reported by Mangurkar and Desai (1981). Increasing trend in udder length was observed with advancing parity similar to the finding of Mali *et al.* (1986). The udder height was observed highest during the first trimester of lactation, which declined during second trimester and remained uniform thereafter.

Heritability estimates of udder measurements

The heritability for the udder height was observed very low (0.018±0.065), whereas the estimates for other 2 traits were moderate in magnitude (0.205±0.148 and 0.205±0.148).

Relationship with milk yield

The genetic and phenotypic correlation's of different udder measurements with milk production revealed significant positive genetic correlation of udder height, udder rear attachment and udder length with 305-day-milk-yield with values of 0.859±0.123, 0.100±0.554 and 1.047±0.487, while positive phenotypic correlation of udder rear attachment with milk yield up to 305-day-lactation and negative phenotypic correlation of udder height and udder length were observed with 305-day-milk-yield. The correlation of these udder measurements with complete lactation milk yield were mostly nonsignificant with udder height having negative genetic and phenotypic correlation with complete lactation milk yield and remaining 2 udder measurements showing lov positive and nonsignificant correlation's. Similar findings were also reported by Naidu (1972) in Red Sindhi and Hariana cattle. Baruah *et al.* (1991) also reported significant positive genetic correlation in Jersey × local (Assam) crossbred cows with the value ranging between 0.37 and 0.56.

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Indian Journal of Animal Sciences 70 (2): 211-212, February 2000

Hair quality attributes of *Camelus dromedarius*

BANAMALI YADAV[1], B P MISHRA[2], C BHAKAT[3] and M S SAHANI[4]

National Research Centre on Camel, Post Box-07, Jorbeer, Bikaner, Rajasthan 334 001

Received: 30 June 1999; Accepted: 10 August 1999

Key words: Hair quality, Single humped camel

Although the hair production trait in camel (*Camelus dromedarius*) is of secondary importance as compared to draught power, but the fibre especially from younger age group is soft and fine quality (Singh 1966) and is widely used in rural cottage industry of Rajasthan and Gujarat for preparation of daries, bags, bhaklas etc. (Sahani and Khanna 1993). The preliminary results of camel hair blends with wool, silk waste and polyester have shown encouraging results (Gupta *et al.* 1987, 1989). Thus the assessment of fibre quality attributes of various age groups, body sites of camels and breeds is of primary importance in exploiting the hair production potential and quality of fibre for the future utility of camel fibres in the form of blends with other fibres and its uses.

Hair samples (127) from 17 yearling camel calves and 15 camels of 3-4 years of age from Bikaneri, Jaisalmeri and Kachchhi maintained at the NRC on Camel, Bikaner were collected. The hair samples of annual clip were collected from

Table 1.Breed, sex, site and age group-wise LSQ means of hair attributes of dromedary camels

	Staple length (cm)	Staple diameter μ ± SE (n)	Mean fibre Pure	Least square Hetero	Mean fibre diameter (μ) Hairy	Kemp	Pure	Percentage of fibre Hetero	Hairy	Kemp
Breed	**	**	**	**	**	NS	NS	NS	*	**
Bikaneri	6.89±0.20 (68)	27.22±0.37 (68)	20.41±0.41 (68)	26.48±0.37 (68)	37.31±0.69 (68)	67.54±1.08 (66)	41.50±0.31 (68)	36.84±0.58 (68)	18.14±0.62 (68)	3.61±0.27 (66)
Jaisalmeri	6.29±0.23 (51)	27.45±0.43 (51)	22.06±0.47 (51)	26.04±0.43 (51)	40.03±0.79 (51)	68.25±2.53 (15)	40.62±0.82 (51)	37.84±0.67 (51)	20.56±0.72 (51)	2.22±0.41 (15)
Kachchhi	4.97±0.64 (8)	30.99±1.16 (8)	23.53±1.27 (8)	3.30±1.17 (8)	45.65±2.15 (8)	–	43.49±2.22 (8)	38.60±1.82 (8)	17.68±1.94 (8)	-
Sex	*	**	**	**	NS	NS	*	NS	NS	NS
Male	5.72±0.25 (68)	27.81±0.45 (68)	21.14±0.49 (68)	27.57±0.45 (68)	40.37±0.83 (68)	67.99±1.53 (42)	40.67±0.86 (68)	38.58±0.70 (68)	19.01±0.75 (68)	3.06±0.25 (42)
Female	6.38±0.31 (59)	29.30±0.56 (59)	22.86±0.62 (59)	29.64±0.57 (59)	41.63±1.24 (59)	67.80±1.89 (39)	43.07±1.08 (59)	36.94±0.88 (59)	18.57±0.94 (59)	2.77±0.31 (39)
Site	**	**	**	**	*	NS	NS	NS	NS	NS
Shoulder	4.69±0.35 (32)	27.85±0.63 (32)	21.80±0.69 (32)	27.29±0.63 (32)	40.23±1.17 (32)	68.17±2.16 (21)	41.97±1.21 (32)	37.30±0.99 (32)	19.56±1.06 (32)	2.38±0.35 (21)
Mid-side	4.11±0.35 (32)	26.73±0.63 (32)	20.74±0.69 (32)	26.30±0.63 (32)	39.04±1.17 (32)	67.82±2.33 (19)	41.81±1.21 (32)	37.25±0.99 (32)	19.65±1.06 (32)	2.71±0.38 (19)
Hump	11.60±0.35 (31)	31.18±0.64 (31)	24.41±0.70 (31)	31.70±0.64 (31)	42.15±1.19 (31)	68.86±2.05 (22)	40.22±1.23 (31)	38.34±1.00 (31)	19.29±1.07 (31)	3.22±0.33 (22)
Neck	3.80±0.35 (32)	28.45±0.63 (32)	21.06±0.69 (32)	29.14±0.64 (32)	42.56±1.17 (32)	66.73±2.20 (19)	43.48±1.21 (32)	38.14±0.99 (32)	16.66±1.06 (32)	3.36±0.36 (19)
Age	**	**	**	**	**	**	**	**	N.S.	*
1 Year	5.56±0.30 (67)	25.66±0.55 (67)	20.08±0.61 (67)	26.16±0.55 (67)	36.27±1.02 (67)	58.50±1.36 (52)	45.18±1.06 (67)	34.57±0.87 (67)	18.29±0.92 (67)	3.33±0.22 (52)
4 Year	6.55±0.25 (60)	31.45±0.46 (60)	23.93±0.51 (60)	31.05±0.47 (60)	45.72±0.86 (60)	77.29±2.09 (29)	38.56±0.89 (60)	40.95±0.73 (60)	19.29±0 78 (60)	2.50±0.34 (29)
Over all	6.05±0.23 (127)	28.55±0.42 (127)	22.00±0.47 (127)	28.61±0.43 (127)	41.00±0.79 (127)	67.90±1.40 (81)	41.87±0.81 (127)	37.76±0.66 (127)	18.79±0.71 (127)	2.91±0.23 (81)

N.B. : * P<0.05**P<0.01 N.S.= Not significant.

Present address: [1]Research Associate, [2,3] Scientist, [4]Director.

4 major body sites, viz. shoulder, mid-side, hump and neck region during March. The samples were analyzed for fibre diameter, staple length and percentage of fibre types, viz. pure, hetero, hairy and kemp. The staple length was measured before washing of the hair samples. The hair samples were washed thoroughly in petrol, dried and further processed for evaluating mean fibre diameter and percentage of hair types. The hair samples were mixed thoroughly to prepare a representative sample of each and then slides were prepared after proper sectioning. The slides were examined under the Ermascope at 500X magnification for measuring fibre diameter and assessing the fibre types based on medullation percentage. In each slide 300 observations were recorded to minimize the error. The data were analyzed using mixed model least square and maximum likely hood programme (Harvey 1987).

The fibre quality attributes, viz. staple length, mean fibre diameter and percentage of fibre types are presented in Table 1. Bikaneri camels indicated higher staple length followed by Jaisalmeri and Kachchhi and the breed, site and age effect were significant ($P<0.01$). Sex effect was also significant ($P<0.05$). The hump region had the highest staple length of 11.60 cm followed by shoulder, mid-side and neck region. The overall staple length in indigenous dromedary camel was 6.05 cm. The average fibre diameter of camel hair ranged from 27.22μ to 31.45μ in 1 to 4 years age groups. Gupta *et al.* (1989) reported large variability in mean fibre diameter of camel hair ranging from 26-38μ. Least-square analysis indicated significant effect of breed, sex, site and age for mean fibre diameter ($P<0.01$). The lowest fibre diameter was observed in yearling camel calves indicating fine quality of fibre, while highest fibre diameter of 30.99μ was observed in Kachchhi breed.

The mean fibre diameter values were also estimated for different fibre types, viz. pure, hetero, hairy and kemp and compared for breed, sex, site and age effect. The lowest fibre diameter was observed for pure fibres (20.08μ to 24.41μ) while highest for kemp. Least-square analysis indicated significant effect ($P<0.01$) of breed, sex, site and age for mean fibre diameter of pure and hetero fibres while for hairy fibres mean fibre diameter had significant effect on breed, age ($P<0.01$) and site ($P<0.05$). Similarly for kempy fibres mean fibre diameter was nonsignificant among breed, sex and sites while age had significant effect ($P<0.01$) as expected. Thus mean fibre diameter of pure and hetero types indicated good prospect for preparation of blends with natural or synthetic fibres. The variability in mean fibre diameter among various breeds indicated scope for further improvement through selection.

Based on medullation the fibres were graded into different types and thereafter the percentage for different types were calculated. Least-square analysis for percentage of fibre types indicated age having significant effect on pure, hetero and kempy types. For percentage of hairy fibres breed effect was significant. The percentage of kemp fibres revealed significant effect of breed and age. Comparison of per cent of fibre types indicated maximum percentage of pure fibres among one year camel calves (45.18%) which revealed superior fine quality fibres from yearling camel calves. The result also indicated overall percentage of fibre types pure, hetero, hairy and kemp to be 41.87, 37.76, 18.79 and 2.91% respectively.

Fibre quality results indicated scope for preparation of blends of camel hair with other natural and synthetic fibres and also production of fabrics. The data indicated scope for further improvement of hair quality attributes and a wide scope for its utility in addition to its traditional uses under village conditions.

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Indian Journal of Animal Sciences 70 (2): 213-221, February 2000

Chromosome manipulation in fish-A review

TARIQ A KHAN[1], MINAL P BHISE[2] and W S LAKRA[2]

Central Institute of Fisheries Education, Versova, Mumbai, Maharashtra 400 61

Received: 13 January 1999; Accepted: 2 August 1999

ABSTRACT

Chromosome manipulations can be carried out easily in fishes due to their external fertilization and embryogenesis. In recent years a great deal of research has been conducted on chromosome set manipulation (polyploidy, gynogenesis and androgenesis) in a number of species. The rationale for this work has been to produce sterile progeny for culture; for sex manipulation; and to produce highly inbred or cloned lines for crossbreeding for research purpose. Haploid, gynogenetic diploid, triploid and tetraploid genotypes have been produced in a number of species and yet this research has been of little or no discernible benefit to the world-wide culture industry. Techniques for induction and verification of chromosome set manipulation are reviewed and the potential applications of these are discussed.

Key words: Chromosome manipulation, Polyploidy, Gynogenesis, Andragenisis

Genetic engineering provides the latest tools for the improvement of fish stocks through chromosome manipulation techniques gynogenesis (all maternal), androgenesis (all paternal) and polyploidy (triploidy and tetraploidy). Fishes unlike commercially important higher vertebrates have certain reproductional and biological properties (eg. external fertilization and embryogenesis) allowing easy manipulation of genetic and physiological processes in the early stages of development, for producing an altered phenotype. Introduction of exotic fishes for aquaculture or water resources management may result in establishment with the consequent adverse impact on native fauna, thus reproductively limited populations can be developed so as to avoid this problem. Sterile or monosex fish has considerable potential in exotic fish management and for aquaculture.

Gynogenesis

Gynogenesis is the production of embryos from female gametes without genetic contribution from male parent. Gynogenesis can be induced artificially by using various treatments at fertilization and at early development stages. The treatment has to solve two problems; first, to inactivate the genetic material of the male gametes, and second, to restore diploidy to the zygote. Artificial destruction of the paternal genome can be achieved by treating the sperms with chemical mutagens such as dimethyl sulphate, toludine blue, and ethylene urea, Gamma rays from a 60 Co source (Chourrout, 1984), X-rays (Golovinskaya 1966) or UV-light (Stanley and Sneed, 1974). UV-rays have the disadvantage that they are not adequately penetrative, and some sperms may accidentally escape from their effects, leading to the contamination of gynogenetic lines. A promising alternative is to use the irradiated sperm of a foreign species and this technique has been tested in grass carp with common carp sperms (Stanley and Jones 1976), in rainbow trout with coho salmon sperms (Chourrout and Quillet 1982).In order to restore viability, diploidy must be induced artificially by retention of 1st or the 2nd polar body (meiotic gynogenesis), or by suppression of the 1st or the 2nd embryonal mitosis (mitotic gynogenesis). The widely used treatments for restoring the diploid constitution are based on the two physical methods: Temperature shock (heat or cold) and by hydrostatic pressure shock. Thermal shock treatment is the preferred method to induce diploidy because large batches of eggs can be easily treated.

From the aquaculture point of view, gynogenesis can be useful in production of monosex population in case of female homogametic species. Besides, the other applications include gene mapping, generation of homozygous lines which could be used for selective breeding programe and in the generation of fish with two maternal chromosome sets plus chromosome fragments from the male parent if the sperms are not completely irradiated.

Artificial gynogenesis was first observed in frogs by

Present address: [1, 2]Centre for Environmental Management, University of Ballarat, PO Box 663, Ballarat 3353, Victoria, Australia.

[3]Division of Fish Genetics and Biotechnology, Central Institute of Fisheries Education, Versova, Andheri (W) Mumbai 400 061.

Hertwig (1911). The first evidence that gynogenesis may be induced artificially in fish was presented by Opperman (1913). Consequently, positive results were reported in experiments with the loach. The systematic study in this field was laid down by the work with the loach and the carp (Golovinskaya *et al.* 1963). Gynogenesis has now been induced in several species (Table 1) of the sturgeon, rainbow trout, flat fishes, grass carp and catfish (Romashov *et al.* 1963, Stanley and Sneed 1974, Purdom 1976, Nagy *et al.* 1978, Lakra and Das 1998, Pandian and Koteeswaran 1998).Some authors have used the sperm donor from the same species (homozygous) for dominant genetic markers to confirm that the paternal genes are not transmitted (Nagy *et al.* 1978). The sperm donor from a foreign or different species can also be used to confirm transmission of the paternal genes (Sugama *et al.*1988). Komen *et al.* (1988) reviewed the incubation temperatures, shock timing after fertilization and shock duration in gynogenesis of common carp. In 1965, Romashov and Belyaeva reported an increased yield of diploid gynogenetic animals induced by temperature shock in loach.Purdom (1969) reported that gynogenetic yield can be increased from 1% to 60% by cold shock treatment in plaice *(Pleuronectus platessa* L.) and flounder *(Platichthys flesus* L.).Regarding growth, there is an evidence of reduced growth rate in gynogenetic coho salmon (Refstie *et al.* 1982) but satisfactory growth rate has been reported in gynogenetic grass carp (Stanley and Sneed 1974) and common carp (Cherfas 1975). In common carp one of the gynogenetic lines grew faster than the best normal cross (Nagy *et al.* 1984). Quillet (1994) collected over a period of three years on growth of meiotic and mitotic gynogens of *Paralichthys olivaceus* and *Oncorhynchus mykiss*. Irrespective of meiotic or mitotic gynogenesis, representative salmonid and cyprinid species suffered significant growth depression. Conversely, Siluridae and Paralichthyidae grew faster, hence induction of gynogenesis in these fish may prove to be a profitable venture. The gynogens of *Paralichthys olivaceus* displayed significantly faster growth than their diploid counterparts. Ploidy-induced, slow growing strains may be a welcome character for ornamental fish species (Pandian and Koteeswaran 1998).

Komen *et al.* (1991) have reported the production of homozygous diploid gynogenetic clones and F1 hybrids in common carp. A consistent yield of 5-15% viable, gynogenetic fry were obtained when eggs were heat shocked at 40°C for 2 min, 28-30 min after fertilization. Cherfas *et al.* (1993) induced diploid gynogenesis and polyploidy in ornamental (koi) carp, by studying the timing of heat shock during the first cleavage in order to induce diploidy and up to 15% diplod gynogens were obtained. Linhart *et al.* (1995) induced the gynogenesis in tench by inactivating the sperm genome by gamma irradiation (60 CO; 1400 Gy dose). The non participation of paternal genome in cold shocked groups was confirmed morphologically, karyologically and biochemically. Pangnathana *et al.* (1995) induced the diploid gynogenesis in the silver barb (*Puntius gonionotus*) and confirmed the female homogamety. Smoker *et al.* (1995) induced gynogenesis in pink salmon by thermal shock, a gynogenetic yield of 32% was obtained when heat shock temperature was 26°C beginning 10 min after fertilization. Successful mitotic gynogenesis in zebra fish was reported by Streisinger *et al.* (1981). Nagy (1987) produced mitotic gynogenetic carp by applying heat shock at 40 min after fertilization. Komen *et al.* (1990) produced 5-12% of fry of carp by mitotic gynogenesis. Goudle *et al.*(1995) induced gynogenesis and polyploidy in the channel catfish (*Ictalurus punctatus*) by pressure shock. Channel catfish eggs were fertilized with sperm from blue catfish (*I.furcatus*)or channel catfish (60 or 90s UV-irradiation)and were subjected to early hydrostatic pressure(5 min post fertilization; 8000 psi, 3 min duration) to produce meiotic gynogens or late hydrostatic pressure (90 min post fertilization) to produce mitotic gynogens. Relative survival at 1.5 months was 2% for meiotic and 0.2% for mitotic gynogens. Lin *et al.* (1996) reported effects of sperm irradiation and heat shock on induction of gynogenesis in muskellunge (*Esox masquinongy*) and optimal conditions for induction of gynogenesis in muskellunge included the application of 30°C for 8-10 min beginning 20 min post fertilization with a yield of gynogens 5.8% (hatched normal larvae). Volckaert *et al.* (1997) induced the gynogenesis in the African catfish, (*Clarias gariepinus*) and optimized the induction of polar body gynogenesis with combined pressure and temperature shock. Highest survival rate were attained at a pressure between 40 and 45 Mpa, regardless of temperature at which the shocks were applied.

Very little work has been done on the chromosome manipulations in India. John *et al.* (1984) first reported the gynogenesis in Indian major carps (*Labeo rohita* and *Catla catla*). Subsequently, gynogenetic mrigal was also achieved by John *et al.* (1988). Pandian and Varadaraj (1990) used a combination of selective breeding, hormonal sex reversal and gynogenesis in conjunction with tilapia production. The authors induced gynogenesis and later subjected gynogenetic offsprings to hormonal treatment to create males with female chromosome (XX). Earlier, the same authors used similar techniques to produce what they referred as "Super male tilapia" which has only male (YY) chromosomes (Varadaraj and Pandian 1989).

There are still several unanswered questions concerning the utilization of gynogenesis in aquaculture. Several authors have reported remarkable results achieved by meiotic gynogenesis. while success in mitotic gynogenesis has been reported by only a few. This is because the production of meiotic and mitotic gynogens are heterozygous and homozygous respectively.

Since cytological detection of sex chromosomes remains difficult (Rishi 1989), induction of gynogens and the subsequent observation of sex ratio proved to be a reliable

Table 1. Information available on induction of gynogenesis in some important fish species

Species	Method	Success (%)	References
Cyprinus carpio	Cold	31	Nagy *et al.* (1978)
Salmo salar	Heat	2	Refstie (1983)
Catla catla	Heat	10	John *et al.* (1984)
Oncorhynchus mykiss	Pressure	60	Chourrout *et al.* (1984)
Labeo rohita	Cold	36	John *et al.* (1984)
Cyprinus carpio	Heat	50	Hollebecq *et al.* (1986)
O.niloticus	Pressure	27	Hussain *et al.* (1993)
Onorhynchus gorbuscha	Heat	32	Smoker *et al.* 1995
Esox masquinongy	Heat	5.8	Lin *et al.* 1996
Clarias gariepinus	Pressure and temperature	–	Volckaert *et al.* 1997

method to fix the heterogamety with the male or female. In female homogametic species, induction of gynogenesis is expected to result in 100% females. However, a number of publications have reported 2 to 100% deviations from the expected all-female gynogens. This could be due to (i) parental genetic contamination, and (ii) assumed presence of minor sex genes, (iii) environmental factor such as changes in habitat temperature and population density.

In studies of Varadaraj (1990) and Cherfas *et al.* (1994) are interesting with regard to performance, fertility and survival of gynogenetic line in *Oreochromis mossambicus* and *Carassius auratus gibelio* respectively. In *Oreochromis mossambicus*, the survival of G0 mitotic progeny was lower (22%) than that of meiotic progeny (47%) and the control (89%). Mitotic gynogens suffered high mortality due to the expression of recessive deleterious genes in homozygous diploid state. However, survival increased to 51% and 64% in F1 and F2 progenies, indicating a progressive elimination of deleterious genes from the population. Cherfas *et al.* (1994) produced gynogen in the hybrids of *Carassius auratus gibelio* (female) and *Cyprinus carpio* (male). It was found that 22% of these were fertile females and the proportion of fertile females increased to 83% and 97% in F1 and F2.

Androgenesis

Androgenesis involves the development of an organism which has chromosomes only of paternal origin. Androgens can be produced in two ways, most commonly used method is to fertilize irradiated eggs with normal sperm. This produces a haploid androgen and at first cleavage a shock is given to prevent cell division, and the two haploid (N) nuclei fuse to form a diploid (2N) nucleus. Androgenetic rainbow trout (Parsons and Thorgaard 1985, Thorgaard *et al.* 1990), brook trout (May *et al.* 1988), and grass carp (Stanley *et al.* 1976), have been produced by this technique and are highly inbred. The second technique that can be used to produce androgens is to fertilize eggs whose genetic material has been destroyed by irradiation with sperm from tetraploid male. Consequently, the sperm pronucleus is diploid rather than haploid, which means the ensuing zygote will also be diploid. Thorgaard *et al.* (1990) produced androgenetic rainbow trout using this technique, these androgens are far less inbred. Survival of androgenetic diploids produced by using sperms from tetraploid males is much better than that of androgenetic diploids produced using haploid sperm which suggests that egg irradiation is not a problem and that cleavage suppression treatment and /or homozygosity are responsible for poor survival.

The production of viable diploid progeny by androgenesis is much more difficult than through gynogenesis because, of two reasons (a) It is quite difficult to perform the elimination of female pronucleus and polar bodies without damaging the cytoplasm and radiation's may adversely affect the mitochondrial DNA, messenger RNA (mRNA) and other constituents besides chromosomal DNA. (b) The production of diploid progeny is a difficult process, since there is no partner genome integrating, like polar bodies in gynogenesis. This is the reason that first mitotic division has to be inhibited in androgenesis.

Androgenesis like gynogenesis has number of application, one of the application is to produce monosex population in species with male homogamety, Gillespie and Armstrong (1981) produced androgenetic diploid males in azolott *(Ambystoma mexicanum) a male homogametic* Salmander, by suppressing first cleavage after UV-inactivation of egg chromosomes. It also allows the possibility of rapid development of inbred lines for domesticated hatchery brood stock, but the potential is difficult to realise because androgens produced though 100% homozygous, suffer high abnormalities and mortalities. Androgenesis has very interesting and important application in the area of germplasm maintenance and the conservation of endangered species (Thorgaard *et al.* 1990). Androgenesis could be used to recover genes from "stored" populations of cryopreserved sperm that exist in germplasm resource centres. Cryopreservation and storage of diploid spermatozoa from tetraploid males would be a very useful tool in the conservation of genetic resources. Such a practice would enable the recovery of heterozygous diploid genotypes, even in the event of extinction of species or strain through the use of androgenesis. Another application of androgenesis lies in monitoring the effects of mitochondrial genotype on performance, because mitochondrial DNA in animals is maternally inherited (Avise and Lansman 1983) and this is the case with androgenetic individuals inspite of egg genome inactivation. By using androgenesis it would be possible to compare the performance of fish with identical nuclear genotypes and different mitochondrial genotypes.

In amphibians, UV-light or pressure treatments have been used to inactivate the maternal genetic material (Gillespie and Armstrong 1980).UV-light being mild is advantageous but

because large size and opacity of fish eggs in some cases, makes it difficult for use. This may be overcome by incorporating more than one UV-sources operating from different angles. The haploid androgenesis has been induced in several teleost species using 60 CO irradiation of unfertilized eggs (Parson and Thorgaard 1984, May *et al.* 1988). The doses used were between 10^4 and10^5 roentgen (R). By using 60 CO source, inheritance of chromosome fragments from the irradiated eggs has been observed (Parsons and Thorgaard 1984), although this does not seem to occur in all cases (Parsons and Thorgaard 1985). Thorgaard *et al.* (1990) produced diploid androgenetic rainbow trout by the normal route and also by fertilizing ganma ray inactivated eggs with diploid spermatozoa from previously generated tetraploid males. The comparison of two methods showed clearly that fertilization by haploid spermatozoa followed by pressure shock at first mitosis was followed by much lower survival to hatching and to first feeding than the method using diploid spermatozoa. He opined low survival of androgenetic diploids was a feature of the shock treatment, rather than inactivation of the egg genetic material. Haploid androgens have been produced by UV-irradiation (430-540 Jm^{-2}) of eggs in *Oreochromis niloticus* with an average yield, at the pigmented stage of embryo, in excess of 30%. Diploidization of the haploid genome was achieved by suppression of first mitosis by giving a pressure shock. In a series of experiments only 0.5% diploid androgens were produced (Myers *et al.* 1995). Androgenetic rainbow trout *(Onchorhynchus mykiss)* were produced by gamma ray inactivation of the female genome, followed by diploidization of the female genetic material by a pressure shock (Parsons and Thorgaard 1985). Successful androgenesis in carp was first reported by Grunina *et al.* (1990) by applying 25-30 KR of X-ray irradiation to the eggs prior to and 2-3 min of heat shock at 40.5-41°C at 1.7-1.9 TO following fertilization (T0 = 21 min at 22.5°C or 20 min at 23°C). Mutation in the colour of males (b1b2 orange) was used as marker, since they exhibited poor pigmentation at the larval stage. (6-9%) reated eggs of the developed into larvae without pigmentation, representing androgenetic diploids. Bongers *et al.* (1994) have further improved and simplified the procedure of producing androgens of carp. Eggs were UV-irradiated (150-300 mJ/ m^2) and later heat shocked (40°C, 2 min and 26-30 min after fertilization) to restore diploidy. In order to facilitate a more homogenous distribution, the irradiation of the egg was done while stirring in synthetic ovarian fluid, which also prevented them from being activated. The ratio of androgenetic diploids identified by the absence of dominant black colour of the females ranged from 7.2 to 18.3%. While no biparental diploids exhibiting a black colour were produced by applying the optimum UV-dose (250mJ/ m^2) followed by the heat shock. Preliminary investigations on androgenesis in common carp have been carried in India and putative androgens were observed in some experiments with a very low yield of 2.3 ± 1.2% viable larvae (Ponniah *et al.* 1995). Recently, Nagoya *et al.* (1996) have induced androgenesis using super 60 CO gamma irradiation and hydrostatic pressure shock in amago salmon *Oncorhynchus masou*, *Androgenetic haploids* were produced when eggs were irradiated prior to fertilization, with an optimal dose of 450 Gray. The optimal timing of the hydrostatic pressure shock (650 kgf/cm super(2), 6 min) was determined to be 7.5 hr after insemination at 10°C and later DNA fingerprinting was used to confirm the clonal nature of androgenic diploids.

Table 2. Information available on induction of triploidy in some important fish species

Species	Method	Success (%)	References
Cyprinus carpio	Cold shock	60	Gervai *et al.* 1980
Oncorhynchus mykiss	Heat shock	70	Thorgaard *et al.* 1982
Oreochormis mossambicus	Heat	–	Penman *et al.* 1987
O. mossambicus	Heat	100	Pandian and vardaraj 1988
O.mossambicus × Red tilapia	Heat	100	Vardaraj and Pandian 1989
Brachydanio rerio	Heat	100	Kavumpurath and Pandian 1990
Betta splendens	Heat and Pressure	86	Kavumpurath and Pandian 1992
Cyprinus carpio	Heat	80–100	Cherfas *et al.* 1993
Penaeus chinensis	Cold and cytochalasin B	70 48	Lin *et al.* 1996
Dicentrarchus labrax	Cold	100	Felip, *et al.* 1997

Androgenesis may prove useful for the production of (i) viable (YY) supermales in male-heterogametic species, (ii) inbred isogenic lines and (iii) conservation of germplasm. When crossed with a normal female, a supermale can produce all-male progenies. Androgenesis has successfully induced viable YY males in a few cyprinids, cichlids and salmonids. However, the technology has been successful in only few species as compared to gynogenesis. A major obstacle to use androgenesis for generation of inbred lines and for "gene banking" is the extremely low survival expected in androgenetic progenies, because of homozygosity for deleterious recessive genes.

Polyploidy

Induced polyploidy refers to the production of individuals with extra set of chromosomes. This can be done by treating fertilized eggs with either temperature shock, hydrostatic pressure or chemical treatments. If the treatments are applied shortly after fertilization, triploids can be produced due to retention of the second polar body of the egg.if the treatments are applied shortly before the first cleavage, tetraploids can be produced.The most important applications of ploidy manipulation in aquaculture are induced triploidy and tetraploidy.

Triploidy

Triploids are fish that have 3 sets of chromosomes instead of normal 2 sets of chromosomes. Triploidy can be induced by 2 ways, first is when newly fertilize eggs are given a shock to retain the second polar body and the other is when diploids are crossed with tetraploids. There are several techniques available for production of triploids using heat shock, cold shock, hydrostatic pressure shock and their combinations. Temperature shock treatments are particularly advantageous for situations in which large volumes of eggs need to be treated. High proportions of triploid rainbow trout have been produced with high survival after heat-shock treatments (Chourrout and Quillet 1982). Hydrostatic pressure has been used for induction of both triploidy and tetraploidy. It has been particullarly useful for blocking first cleavage (Parsons and Thorgaard 1985). A limitation of using hydrostatic pressure is that appropriate pressure vessels may not accomodate large volume of eggs. Production of triploids by crossing tetraploids with diploids is another development that holds promise as a simple, economical method. It requires that viable, fertile tetraploids can be produced for the species of interest.

One of the main reasons for production of triploid fish is the control of sex. Triploid fish are supposed to be sterile, although the reason of the sterility is not exactly known and exceptions to this phenomenon have been described. Sterility is advantageous when control of reproduction is needed. Sterile fish usually grow better, their stocking excludes unwanted overpopulation of natural waters (triploid grass carp in aquatic weed control) and they are more adaptive than diploid hybrids, because of increased heterozygosity (Shelton 1986). Sterilization substantially reduces the risk of perpetuation, when exogenous fish species are introduced into different aquatic ecosystems (Horvath and Orban 1995). The advantage in growing of elderly triploid fish over their diploid siblings may be due to the lack of or decrease in sexual development of the gonads (Nagy 1987).

Triploids are expected to be sterile because the odd number of chromosome sets and will lead to disruption of meiosis and either failure of gonad development or production of aneuploid gametes. The failure of gonadal development in turn might prevent the appearance of undesirable side effects of sexual maturation, such as poor meat quality, slower growth and high mortality. It appears that triploid fish are indeed functionally sterile, but that secondary sexual characters are not always suppressed. Triploid males generally show more gonadal development than triploid females, probably because triploidy does not interfere with the many mitotic divisions involved in bringing the testis to its mature size. The testis of triploid channel catfish were slightly smaller than those of diploids at the age of 8 months and, unlike diploid testis, histologically showed no sperm production (Wolters *et al.* 1982). Triploidy apparently inhibits gonadal development more in females than males. Failure of meiosis may prevent oocyte development and the associated increase in size of the gonad. Triploid female rainbow trout at maturity had small, string like gonads with many cells arrested at the pachytene stage of meiosis (Thorgaard and Gall 1979).The induced triploids show better quality of flesh, the reason being the divergence of energy allocated to gonadal growth in normal fish to somatic growth in triploids (Benfey and Stutterlin 1984). Besides this there is a decrease in mortality as the triploids are sterile and thus there is reduced post spawning mortality. Accurate methods of assessing ploidy are important because treatments are seldom 100% effective and offspring from treated eggs are usually composed of mixture of polyploids and diploids.

Several studies have found that triploids may grow faster than diploids at sexual maturity. Triploid channel cat fish were significantly heavier than diploids at the age of 8 months and older (Wolters *et al.* 1982).In contrast some workers have reported that triploid Juveniles grow no faster than diploids. Growth of juvenile triploids was similar to that of diploids in sticklebacks (Swarup 1959), plaice X flounder hybrids (Purdom 1976), carp (Gervai *et al.* 1980)and channel cat fish (Wolters *et al.*1982). In the blue tilapia, juvenile triploids were larger than diploids (Valenti 1975). It is of interest that Gervai *et al.* (1980) did not find any difference in growth rate between young diploid and triploid carps, while Cherfas *et al.* (1995) observed that the growth rate and survival of diploid carps were superior to those of triploids and found no other advantage of triploidy. Recently Lin *et al.* (1996) have reported induced triploidy in *Penaeus chinensis* by using cold shock treatments and cytochalasin B treatments and achieved best result by using cold shock (at 4°C for 30 min administered 7 min after spawning) or with cytochalasin B at 0.25 mg/lit (administered 22-32 min after spawning).The triploidy percentage was 70% by cold shock and 48% by cytochalasin B treatment. Felip *et al.* (1997) have determined the optimal conditions for the induction of triploidy (100%) in the European sea bass *(Dicentrarchus labras* L.*)* by using cold shocks. The optimal treatment parameters for the induction of triplody in the sea bass were: time after fertilization, 5 min; duration of shock, 10 min; temperature of shock 0°C; pre shock incubation temperature 12-13°C in all cases.

In tilapia, the technique of induced triploidy has been standardized to the extent that 100% triploid yields have been achieved using cold, heat and pressure shock (Chourrout and Itskovich 1983, Pandian and Varadaraj 1988, Varadaraj and Pandian 1988, Hussain *et al.* 1991). In addition, allotriploids have been produced from *Oreochromis mossammbicus* females and red tilapia males (Varadaraj and Pandian 1989). Survival of triploids up to first feeding fry stage is usually lower than that of unshocked control. Survival of allotriploids was greater than autotriploids.

The successful induction of triploidy (Table 2) in many fish species (Swarup 1959, Purdom 1972, Valenti 1975, Chourrout 1980, Gervai *et al.* 1980, Thorgaard *et al.* 1981)

and findings of spontaneous triploids (Thorgaard and Gall 1979) supports the view that triploid fish have good viability. This is in contrast to the inviability of triploid mammals (Niebuhr 1974), but similar to the good survival observed in triploid amphibians (Fankhauser 1945).

Triploidy may lead to increased viability in inter species hybrids. The triploid grass carp × big head carp hybrid is healthier than the frequently deformed diploid hybrids (Allen and Stanley 1981). The increased viability of triploid interspecific hybrids may be related to a buffering of the genetic incompatability between species by altering the ratio of parental chromosome from 1: 1 to 2: 1. Interspecific triploid hybrids could prove useful in fish culture because hybrid vigour and desirable attributes of both species might be combined in a relatively healthy sterile hybrid (Allen and Stanley 1981). For example, Refstie *et al.* (1982) found that coho salmon × chinook salmon hybrids grew much faster, but had a high mortality rate than coho salmon. The hybrids also demonstrated high frequencies of deformities. Equal proportions of males and females are expected with induced triploidy in species with female homogamety and in many cases with female heterogametic species. Studies of the sex of induced triploid carp (Gervai *et al.* 1980), induced triploid channel cat fish (Wolters *et al.* 1982), have noted roughly equal proportion of males and females.

One feature of current method of triploid production in fish is that they are not equally effective in different species. Cold shock treatments, for example, range from completely effective to totally ineffective. However, Purdom (1972) reported 100% success in inducing triploidy in flat fish using cold shock.In carp, cold shock induced triploidy was also accompanied by the presence of haploids and survival rates were different (Gervai *et al.*1980) whilst in the Channel cat fish (*Ictalurus punctatus*), 100% triploidy was induced by cold shock with no deterimental effects(Wolters *et al.* 1981). In salmonids, cold shocks have been almost completely unsuccessful. Triploids have been produced successfully by heat shock in rainbow trout (Thorgaard *et al.* 1981).

Regarding growth of triploids, the following conclusions may be drawn: (i) during post maturation, triploids of almost all tested species grew about 10-30% faster than their respective diploid counterparts, (ii) the solitary rearing supports faster growth of triploids, both during pre and post-maturation, (iii) the herbivores triploids suffer relatively less during pre-maturation and sustain faster growth during post-maturation than their carnivorous counterparts, (iv) the cold shock treatment works better than the heat shock or pressure shock. The cold shocked males and females of *Oreochromis niloticus* grow significantly faster than their heat-shocked or pressure shocked counterparts.

To accommodate the increased amount of nuclear DNA in triploids, the cell size is correspondingly increased and the normal ratio of nuclear-cytoplasmic volume is maintained. On the other hand, cell number in triploids is reduced to maintain the normal organ size of individual organ or the fish.

A large number of studies show a decrease (22-42%) in the number of erythrocytes per unit volume of blood in triploids. However, the observed decrease in the total number of erythrocytes appear to be more or less compensated by an increase in haemoglobin content of individual RBC.

Although majority of the workers have revealed that triploids display comparable rates and efficiency of food utilization but Wiley and Wike (1986) have shown that hybrid triploid carp *(C. idella* female × *H. nobilis* male*)* is significantly less efficient in utilizing food.

Tetraploidy

Tetraploids are fish that have 4 sets of chromosomes instead of normal 2 sets. In the case of induced tetraploidy, inhibition of formation of the cell membrane between the daughter cells at mitotic division takes place so that the genome duplication takes place but the cell does not divide. The methods used to induce tetraploidy are the same as listed for triplody, except that the timing of shock is different. As opposed to sterile triploid individuals, tetraploid fish are fertile and suitable for production of triploids by crossing with diploids individuals.

The potential application of tetraploids are in their increased heterozygosity and growth potential, the possibility to generate large numbers of sterile triploid progeny in tetraploid × diploid matings, and in the production of diploid spermatozoa for cryopreservation (Chourrout *et al.* 1988). Studies in rainbow trout have shown that triploids produced from matings of tetraploid females with diploid males exhibited better growth and survival than triploids induced by retention of the second polar body (Chourrout and Nakayama 1987). Tetraploid rainbow trout embryos are frequently abnormal in appearance (Thorgaard *et al.* 1981, Chourrout 1982). However, Refstie (1981) reported successful rearing of tetraploid rainbow trout to maturity.If tetraploids are produced by suppression of the first mitotic division, equal proportion of males and females are expected. If the Y chromosome is dominant in sex determination then XXXY individuals, which might be produced by crossing XXYY and XXXX individuals, would be males in male heterogametic species.

Production of tetraploid carp individual has been reported by Rekoubratsky *et al.* (1989), Linhart *et al.* (1991) and Cherfas *et al.* (1995). However, these tetraploid individuals died during their first year. Attempts by Komen (1990) to produce tetraploid carp were also unsuccessful. On the basis of their results, it seems questionable whether the production of viable tetraploid carp is possible at all. It is interesting to note that Bidwell *et al.* (1985) succeeded in generating tetraploid channel catfish by heat shock, while Chourrout *et al.* (1987) reported successful production of tetraploid rainbow trout males and females. Crossing of tetraploid

salmonids with normal diploids resulted in triploid offspring. According to Chourrout *et al.* (1985), the low fertilization rate might be due to the larger nucleus of the spermatozoa, thus reducing the possibility of its penetration across the micropyle. Don and Avtalion (1988) reported 1.3-4% viable tetraploids in *Oreochromis aureus* using cold shock. In *Oreochromis mossambicus* tetraploids were successfully produced using heat or pressure shock (Myers 1986), but these possessed characteristics like deformities of body shape and eyes and failed to survive to the first feeding stage.

Some workers have attempted induction of chromosome-set manipulation (diploidy or tetraploidy) using chemical treatments (Mair 1988, Varadaraj and Pandian 1988). However, preliminary experiments with varying concentrations of the mitotic inhibitors failed to induce diploidization of gynogens in *Oreochromis niloticus* (Mair 1988).Treatment of fertilized eggs with colchicine or cytochalasin B did produce diploid-tetraploid mosaics in *Oreochromis mossambicus* (Varadaraj and Pandian 1988).

Conclusion

The applied use of chromosome manipulation is being realized in fish cultivation, especially in carp, trout, salmon and channel cat fish. In species where the mechanism of sex determination cannot be detected cytologically or genetically, it has been possible to determine male or female heterogamety through chromosome manipulations. Gynogensis, androgenesis and induced polyploidy (triploids and tetraploids) have attracted great scientific interest and will play an important role in modern fish breeding and aquaculture.

ACKNOWLEDGEMENT

The authors are grateful to Dr S A H Abidi, Director, CI FE; for providing necessary support and encouragement.

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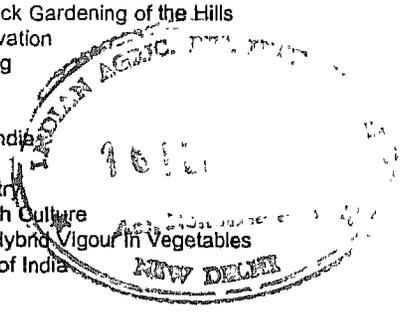

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