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Indian Journal of Animal Sciences 71 (3): 195-197, March 2001

Isolation and identification of *Yersinia pseudotuberculosis* from rodent species *Tatera indica*

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Received: 7 February 2000; Accepted: 20 September 2000

ABSTRACT

Tissue specimens of *Tatera indica* rodents examined during plague surveillance in the Deccan plateau yielded 4 isolates of *Yersinia pseudotuberculosis* confirmed on the basis of conventional biochemical testing and by bacteriophage lysis test. These isolates when subjected to SDS-PAGE analysis could be easily distinguished from other yersiniae and non-yersinae species of family enterobacteriacae. Two thick prominent bands at 14-16kDa and 24-26kDa regions were characteristic identification markers for *Y. pseudotuberculosis*.

Key words: Bacteriophage lysis test, SDS PAGE, *Yersinia pseudotuberculosis*

Yersinia pseudotuberculosis was found associated with numerous outbreaks and sporadic infections of gastero-enteritis, ilietis, lymphadenitis and lymphangitis both in man and animals from several parts of the world (Sato 1987, Brown and Davies 1989 and Toyakawa *et al.* 1993). Infections due to this organism have occasionally been reported from cattle and swine in our country (Jayaramman and Sethumadavan 1973, Srivastava *et al.* 1978 and Behra *et al.* 1984). Wild animals and birds are often considered as reservoirs (Fukushima and Gomyoda 1991).

Antigenically and genetically, *Y. pseudotuberculosis* is very close to *Y. pestis* the causative agent of plague and shares at least 13 antigenic components (Chen and Meyer 1966) and 90% DNA sequence homology with this organism (Bercovier *et al.* 1980). The clinical polymorphism of *Y. pseudotuberculosis* is also comparable to monomorphic aspects of plague and the anatomopathology of both infections is similar (Ravangangan and Chiesa 1995). The recent re-emergence of plague in the country (Batra *et al.* 1996) was a pointer to strengthen effective surveillance for these organisms. The isolation of *Y. pseudotuberculosis* from pericommensal rodent species, *Tatera indica* examined during the plague surveillance work is being documented.

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MATERIALS AND METHODS

Isolation of organism

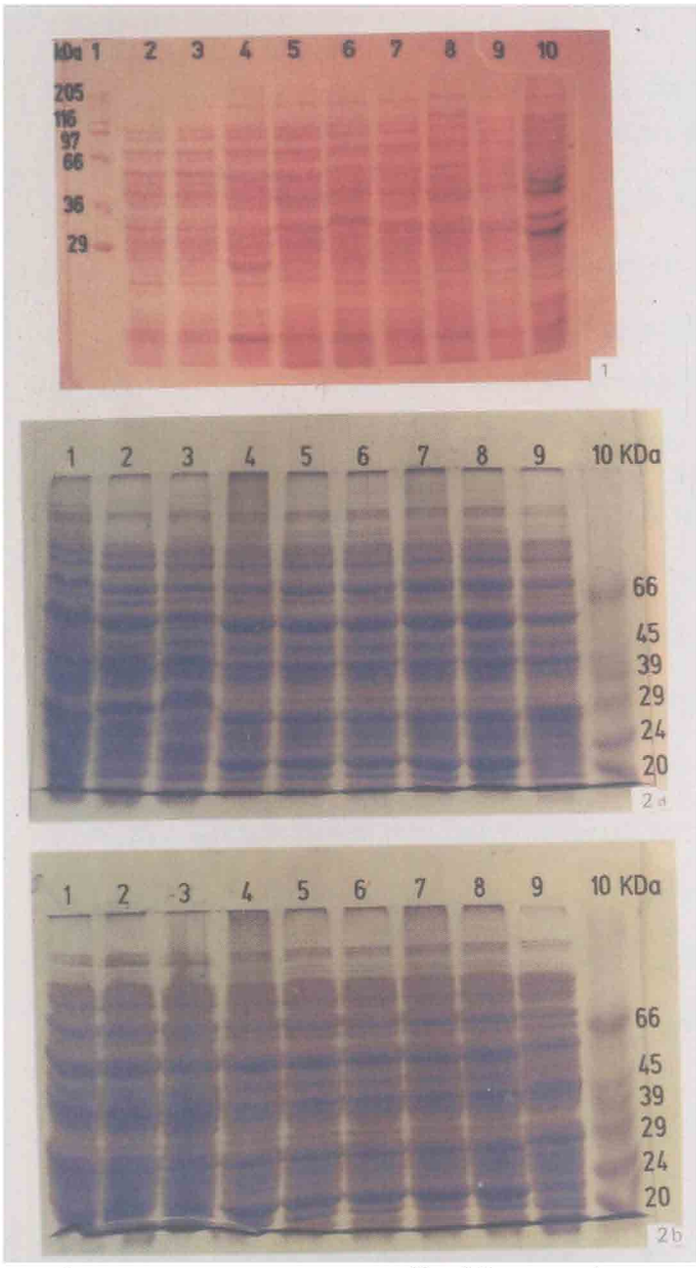
Spleen and liver tissue samples from 40 rodents of the species *Tatera indica* trapped from border states of Karnataka, Tamil Nadu and Andhra Pradesh were processed for isolation of *Y. pseudotuberculosis*. Briefly, all the samples were inoculated in nutrient broth and incubated at 4°C for a week. Loopful of broth cultures were than inoculated onto blood agar plates containing 5% sheep blood. Bacterial colonies suspected for *Yersinia* species were tested for biochemical reactions.

Bacteriophage lysis test

Organisms exhibiting typical biochemical reactions of *Y. pseudotuberculosis* were subjected to bacteriophage lysis test at 28° and 37°C with standard strains of *Y. pseudotuberculosis* 1A and *Y. pestis* A1122 obtained from Dr May C. Chu of CDC Fort Collins, USA, as controls.

SDS-PAGE

Yersinia frederiksenii, Y. kristensenii, Y. intermedia and *Y. enterocolitica* 0:9 obtained from a laboratory in Norway and standard strains of *E. coli* and *S. typhi* maintained in our laboratory were grown on brain-heart -infusion agar. The harvested growth was sonicated at 4 micron output using Microson sonicator for 15 min to prepare whole cell lysate antigens. These lysate antigens were then resolved on 10% SDS-PAGE (Laemmli 1970) using Mini gel electrophoresis apparatus.



Figs 1-2. **1.** SDS-PAGE protein profile of *Yersinia* and other bacterial species. 1=Molecular weights, 2=*Y. enterocolitica* O: 8; 3=*Y. enterocolitica* O: 9; 4=*Y. pseudotuberculosis;* 5=*Y. kristensenii;* 6=*Y. intermedia;* 7=*Y. frederiksenii;* 8=*S. typhi;* 9=*K. pneumoniae;* 10=*E. coli.* **2a.** SDS-PAGE protein profile of *Yersinia pseudotuberculosis* isolates and other *Yersinia* species 1=*Y. kristensenii;* 2=*Y. frederiksenii;* 3=*Y. intermedia;* 4=*Y. pseudotuberculosis* 1 A; 5=*Y. pseudotuberculosis* isolate No.1; 6=*Y. pseudotuberculosis* isolate No.2; 7=*Y. pseudotuberculosis* isolate No.3; 8=*Y. pseudotuberculosis* isolate No.4; 9=*Y.pestis* A1122; 10=Molecular weights. **2b.** SDS-PAGE protein profile of *Yersinia pseudotuberculosis* isolates and other *Yersinia* species 1=*Y. pestis* isolate; 2=*Y. pestis* A1122; 3=*Y. pestis* isolate; 4=*Y. pseudotuberculosis* 1A; 5=*Y. pseudotuberculosis* isolate No.1; 6=*Y. pseudotuberculosis* isolate No.2; 7=*Y. pseudotuberculosis* isolate No.3; 8=*Y. pseudotuberculosis* isolate No.4; 9 *Y. enterocolitica* O: 9; 10 molecular weights.

RESULTS AND DISCUSSION

Four isolates were confirmed biochemically to be *Y. pseudotuberculosis* as these were positive for catalase, methyl red, urea, reduced nitrates to nitrites and were negative for Voges-Proskauer, citrate utilization and indole production. All the isolates fermented glucose, maltose, mannitol, trehalose, L-arabinose, rhamnose and melibiose with acid production and were unable to ferment lactose, sucrose, sorbose and sorbitol. These 4 isolates like the standard *Y. pseudotuberculosis* 1A strain exhibited bacteriophage lysis at 37°C but not at 28°C.

In SDS-PAGE, the whole cell lysate antigens of yersiniae and non-yersiniae organisms could be resolved into 40 to 50 protein bands with majority of them appearing to be common among different organisms (Fig.1). All the *Y. pseudotuberculosis* isolates and the standard strain of *Y. pseudotuberculosis* 1A were clearly distinguishable from other organisms owing to their unique banding pattern with 2 thick prominent bands at 14-16 kDa and 24-26 kDa region (Figs 2a,b).

Epizootic outbreaks of enteritis and sporadic cases of abortion and mesentric lymphadenitis have been frequently encountered in recent years in animals including ruminants, swine, rodents and birds throughout the world (Furowicz 1996). Only scanty reports of *Y. pseudotuberculosis* diseases in animals have been reported from our country. Absence of human infections of *Y. pseudotuberculosis* from India and other developing countries could partly be due to low awareness of its etiological identity, as the agent is not specifically looked for in clinical samples during routine microbiological investigations.

Recovery of 4 isolates from 40 rodents suggests a fairly high prevalence of this agent in rodent species in southern states of the country. Probability of this organism's involvement in disease causation therefore, exists both in man and animal. The yersiniae overall being slow growing and fastidious are often not recovered in routine microbiology laboratory. Clinical samples are required to be specifically processed for these organisms. Characteristic banding pattern of the organism with 2 thick bands at 14-16 kDa and 24-26 kDa regions in SDS-PAGE as observed in this study can help provide a rapid identification marker. Moreover, in the plague surveillance work this procedure will be useful to differentiate *Y. pestis* and *Y. pseudotuberculosis*, both of which are closely related morphologically, biochemically and genetically.

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Cell-mediated immune response in goats vaccinated with killed *Mycoplasma mycoides* subsp. *capri* saponin vaccines

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Received: 21 March 2000; Accepted: 20 September 2000

ABSTRACT

The cell-mediated immune response of sonicated *M.capri* saponin vaccines was studied in goats. Vaccines, viz. vaccine A (1.0 mg protein/dose) and vaccine B (1.5 mg protein/dose) were prepared and inoculated with 1.0 ml of sterile saponin solution (3.0 mg/ml) in 2 different groups of animals comprising 17 in each group. Vaccine A was better than that of vaccine B as it elicited higher per cent leukocyte migration inhibition (LMI) values while consisting of lesser protein concentration.

Key words: Cell-mediated immune response, *Mycoplasma mycoides* subsp *capri*, Saponin, Vaccine

Mycoplasmas are considered as both intracellular and extracellular parasites. The organism after entering the host elicits both humoral and cellular immune responses. In goats, lot of work has been conducted on humoral immune response however, the information on cellular immunity is meagre (Barari and Srivastava 1990, Rana and Srivastava 1993). Therefore, the present study was conducted to monitor the CMI response in goats against *Mycoplasma mycoides* subsp *capri* (*M.capri*) and also to investigate its further role as an immunogen.

MATERIALS AND METHODS

Antigen preparation

The strain *M.capri* isolated from goats by Uppal and Kumar (1981) was used. The organism was grown in bulk in Hank's Balanced salt solution (HBSS-L) as per Carmichael *et al.* (1972) adopted by Srivastava (1982). The growth was centrifuged @10 000 rpm for 20 min in Sorvall RC-5B refrigerated centrifuge and was given 3 washings in PBS (ice cold). The pellet was harvested in normal saline and sonicated in MSE-Soniprep 150 by applying 10 jerks @10 μ of 2 min each with an alternate gap. The protein concentration of sonicated antigen was estimated as per Lowry *et al.* (1951) and was adjusted to 1.0 mg/dose for vaccine A and 1.5 mg/dose for vaccine B. These vaccines were freeze-dried in ampoules and were kept at 4°C for further use. Similarly, saponin solution was also prepared (conc. 3mg/ml) in normal saline and was kept under refrigeration.

Prior to inoculation, sterility of both the vaccines and of saponin solution were checked on Blood agar, Robertson's cooked meat and Sabouraud dextrose agar media.

Animals

Healthy goats (36) of 9-12 months of age and of either sex were used. These animals were divided in 3 groups of 17,17 and 2, and were pre-screened by indirect haemagglutination (IHA) and slide agglutination (SA) tests for the absence of mycoplasmal antibodies in their sera and mycoplasmal growth on HBSS- solid medium.

Inoculation of immunogen

The ampoules of vaccine A and B were opened and mixed individually with 1.0 ml of sterile saponin solution. The preparations were mixed thoroughly and inoculated as 1.0 mg/dose in first group of 17 animals, 1.5 mg/dose in second group of 17 animals and pure saponin solution @ 1 ml in third group of 2 animals. All the preparations were inoculated subcutaneously at neck region.

Leukocyte migration inhibition test

The cell-mediated immune response of all the goats was assessed by leukocyte migration inhibition test. Blood was collected on day 0 and 3 and week 1,2,3,4,6,8,10,12,14 and 16 post inoculation, in sterilized flasks having EDTA (2.7%). The method of Boyam (1968) with minor modification was adopted for leukocyte separation.

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The migration index (MI) (Cohen 1980) and per cent migration inhibition (Hyunh and Chubb 1987) were calculated as follows:

$$MI = \frac{\text{Av. migration of antigen containing culture}}{\text{Av. migration of normal culture}}$$

% migration inhibition = (1–MI) ×100

RESULTS AND DISCUSSION

All the animals were seronegative for mycoplasmal antibodies and no mycoplasmal growth could be detected on HBSS agar medium.Both vaccines A and B were free from any aerobic, anaerobic and fungal contamination. No untoward reaction was observed in any one of the inoculated animals. In third group of goats, no LMI response was observed up to third week PI therefore, collection of blood was discontinued from this group.

On 0 day, the mean per cent LMI values were 4.0 and 3.0

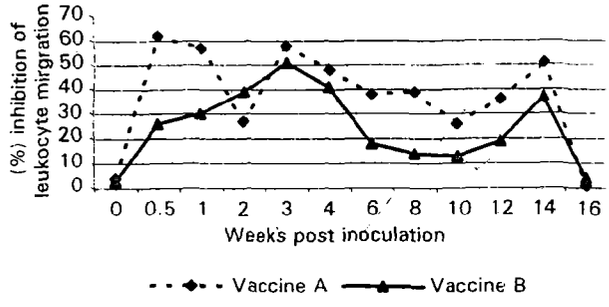


Fig 1. Leukocyte migration inhibition per cent in vaccinated goats

for vaccine A and B group, respectively. A biphasic peak response of 62.0 and 58.0 was observed at third day and third week PI in vaccine A group while in vaccine B group, a peak response of 51.0 was observed at third week PI. The mean per cent LMI responses of 1.0 and 4.0 were observed at 16th week PI in vaccine A and B group respectively (Fig. 1).

On the basis of above results, it can be concluded that LMI response reaches to its peak by third week of inoculation while using sonicated *M.capri* along with saponin as an adjuvant. The finding disagrees with that of observations recorded by Barari and Srivastava (1990). They inoculated formaline-killed *M. capri* with aluminium hydroxide gel and observed peak (39.27) LMI response at fifth week PI. The reason might be due to use of aluminium hydroxide gel as adjuvant which forms depot when compared to saponin.

Our finding of peak LMI response by third week of PI was in agreement with that of Rana and Srivastava (1993) which might be because of similar antigen and adjuvant. Besides, they recorded higher LMI value (71) than ours (62 with 1.0 mg protein dose and 51 with 1.5 mg protein dose) which might be because of the higher protein concentration (2.5 mg/dose) used by them.

As far as the role of vaccine A and vaccine B is concerned in eliciting LMI response in goats, vaccine A proves to be better than that of vaccine B. The reasons are- lesser protein concentration used (1.0 mg/dose) and higher average per cent LMI values observed in vaccine A group of animals.

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A comparative study of outer membrane proteins of selected *Brucella* strains

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Received: 8 February 2000; Accepted: 17 September 2000

ABSTRACT

Outer membrane proteins (OMPs) of *Brucella abortus* strains (99, 544 and 19), *Brucella melitensis* 16M and *Brucella suis* 1330 were isolated by differential centrifugation method. Based on sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE) profiles, *Brucella* OMPs were classified into 3 distinct molecular weight (MW) ranges, viz. low MW (14-30 kDa), moderate MW (39-60 kDa) and high MW (68-93 kDa). Polypeptides above 60 kDa were not observed in *B. abortus* 544. The moderate MW bands were more varied in their migration and relative quantity. Silver staining confirmed the presence of polysaccharide moieties in major bands at position 39.2-47.3 kDa.The subtle differences observed in SDS-PAGE analysis of *Brucella* OMPs did not represent reliable marker for identification of *Brucella* species.

Key words: *Brucella*, Outer membrane proteins, SDS-PAGE

Brucellosis, a widely spread (Anonymous 1994) important reproductive disease of livestock, is caused predominantly by *B.abortus* in bovines, *B.melitensis* in sheep and goats, and *B.suis* in pigs. Their host specificity is not so often absolute (Mathur 1985, Hemashettar *et al.*1987, Bhaskar Rao *et al.*1998). In view of their interhost transmission potential, the disease has assumed public heath importance. Therefore it is an epidemiological necessity to differentiate *Brucella* species / strains.

Structurally, *Brucellae* have cell wall and envelope, and the latter consists of cytoplasmic and outer membranes. The outer membrane proteins (OMPs) differ in their physical, chemical, immunological and biological properties. OMPs are antigenic and play an important contributory role in conferring protective immunity. The OMPs of *Brucellae* were studied by SDS-PAGE (Verstreate *et al.*1982, Santos *et al.* 1984, Hill and Cook 1994). The present study is an attempt to determine whether or not SDS-PAGE profiles of OMP could be used to differentiate strains of *Brucella* for identification purposes.

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MATERIALS AND METHODS

The *Brucella abortus* strains (99, 544 and 19) and *Brucella melitensis* 16 M and *Brucella suis* 1330 were procured from the Indian Veterinary Research Institute, Izatnagar. The cultures were routinely maintained and grown on tryptose agar at 37°C. *B. abortus* 544 was supplemented with 5% carbon dioxide atmosphere (Alton *et al.*1975).

Isolation of OMPs from Brucella *strains*

Brucella OMPs were isolated by differential centrifugation method (Sowa *et al.* 1991). After resuspending 1 part in 50 parts of Lutkenhaus buffer, the protein content in OMPG was estimated (Lowry *et al.* 1951) and 5% SDS solution was added maintaining a 1: 10 (w/w) ratio of protein to SDS. The preparation was boiled for 5 min to remove the insoluble peptidoglycan sacculi and then centrifuged at 134 000 g for 1 hr and 40 min to isolate soluble proteins. Finally, the protein content in the OMP preparation was reestimated and the lyophilized product was stored at —20°C.

SDS-PAGE analysis of OMPs

The OMP polypeptide profiles of *Brucella* strains were analysed by SDS-PAGE using an adjustable vertical gel system, with an automatic power pack (Laemmli 1970). The 40 µl of each sample containing 25 µg of protein and MW marker, prepared in sample buffer (2 mercapto ethanol—0.5 ml, 20% SDS—1 ml, tris-HCl, 1.5 M, pH 6.8-0.16—6 ml, glycerol—2 ml, 0.2% bromophenol blue—60 µl and distilled water up to 10 ml) were boiled in a water-bath for 5 min

before being loaded into each well of the 10% polyacrylamide gel. The electrophoresis was carried out under reducing conditions at a constant current of 10 mA with tris -glycine (pH 8.3) as running buffer for 10 hr. Prestained MW marker protein standards were run on each gel. The electrophoresed gels were stained using coomassie blue.

Electrotransfer of OMPs from SDS-PAGE gel to polyvinyldifluoride (PVDF) membrane

The electrophoresed gels were also subjected to electrotransfer and *Brucella* OMPs electroblotted on PVDF membrane were stained using ponceau S as described by Sambrook *et al.* (1989).

Silver staining of SDS-PAGE gels for detection of polysaccharide in OMPs

Silver staining of periodic acid treated SDS-PAGE gels was performed to detect the presence of polysaccharide in *Brucella* OMP preparations (Tsai and Frasch 1982). The relative mobility (Rf) value of the marker proteins was plotted against their MW to calculate the size of peptide bands of various proteins obtained in the study.

RESULTS AND DISCUSSION

SDS-PAGE analysis of OMPs

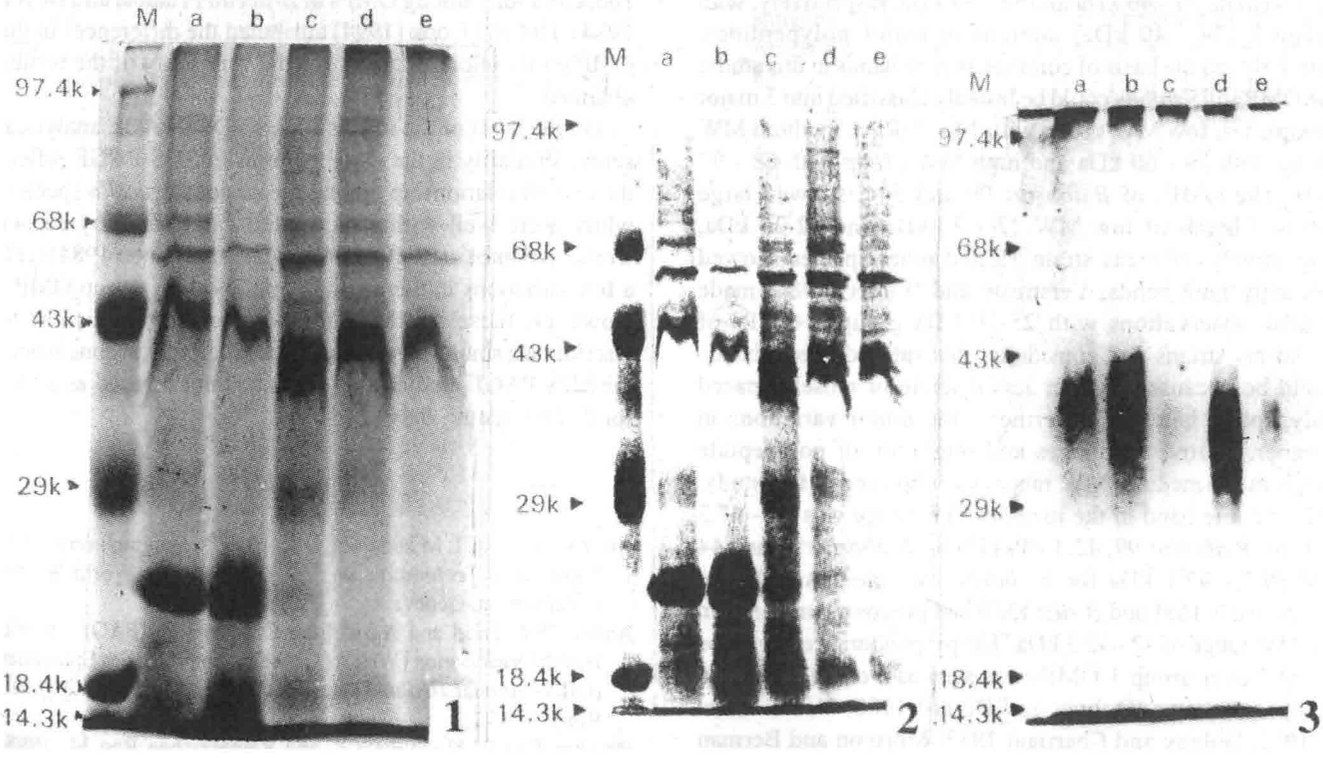
Coomassie brilliant blue staining of the polypeptides

In our initial study of OMPs of *Brucella* species, carried out with coomassie blue staining of the SDS - PAGE gels, several distinct and faint polypeptide bands were observed which indicated dissimilarities in the OMPs of 5 strains of *Brucella.*

A critical analysis of the banding pattern showed that OMPs of *B.abortus* 99 revealed 2 diffused bands of 22 - 25 kDa and 44 - 47.3 kDa, and distinct bands of 59.5 and 68.7 kDa with several less intense bands ranging between 68.7 and 93 kDa (Fig. 1, Lane a), whereas, *B.abortus* 544 showed 3 major bands of 22 - 26 kDa, 42.3 - 44 kDa and 60 kDa without showing polypeptide bands above 60 kDa (Fig. 1, Lane b). The OMPs of *B.abortus* 19 revealed 3 bands of 39.2 - 42.1 kDa, 42.2 - 42.8 kDa and 59.5 kDa and demonstrated several high MW bands in the 68.7 - 93 kDa region. Furthermore, *B. abortus* 19 did not show the presence of diffused band in the range 22 - 26 kDa (Fig. 1, Lane c) that was prominent in *B.abortus* 99 and 544. Polypeptide profiles of *B. melitensis* 16M showed bands of 42 - 42.3 kDa (trailing), 42.4 - 43 kDa and 59.5 kDa along with several bands in 68.7 - 93 kDa region but did not reveal the presence of 22 - 26 kDa low MW bands (Fig. 1, Lane d). In *B. suis* 1330, bands of 42 - 42.3 kDa (trailing), 42.4 - 43 kDa and 59.5 kDa but not 68.7 - 93 kDa were observed (Fig. 1, Lane e).

Identification of polypeptide bands by ponceau staining

The presence of faint bands in coomassie blue stained gel led to explore the use of PVDF membrane for electro -



Figs 1-3. **1.** Coomassie blue stained SDS-PAGE gel of *Brucella* OMPs. **2.** Ponceau stained PVDF membrane showing SDS-PAGE profile of *Brucella* OMPs. **3.** Silver stained SDS-PAGE gel showing polysaccharide moities in *Brucella* OMPs. Lane a-*B.abortus* 99, b-*B.abortus* 544, c-*B abortus* 19, d- *B.melitensis* 16M, e- *B suis* 1330 and M-MW markers.MW (kDa).

transferring of OMPs and subsequent ponceau staining to define the undefined bands. It is inferred that the ponceau staining detected all those polypeptides revealed by coomassie blue method. However, the former was superior in that it recognized additional polypeptide bands (Fig. 2). Therefore the SDS - PAGE patterns of OMPs as detected by ponceau staining are detailed below.

The pattern of the OMP polypeptides and their MW were divergent (Fig. 2) as evidenced by 19, 8, 22, 22 and 21 polypeptide bands noticed in *B.abortus* 99, 544, 19, *B.melitensis* 16M and *B.suis* 1330 strains respectively.

Ponceau staining detected several additional polypeptide bands. *B. abortus* 99 showed diffused polypeptide bands in 14.4 -18.6 kDa and 29.2 - 32.3 kDa ranges. In contrast, the bands in 68.7 - 93 kDa range were discrete (Fig. 2, Lane a). However in *B.abortus* 544, an intensely stained diffused band in 14.4 - 18.6 kDa region was recorded (Fig. 2, Lane b) but bands in 29.2 - 32.3 kDa and 68.7-93kDa range could not be demonstrated. Furthermore, *B.abortus* S19 produced diffused bands from 14.4 to 18.6 kDa, 22 to 25 kDa, and 29.2 to 32.3 kDa and discrete bands in 68.7-93 kDa range, similar to *B.melitensis* 16 M and *B. suis* 1330. However, in *B.suis* 1330, excepting 42 - 42.3, 42.4 - 43, 47.3 and 59.5 kDa bands, other bands were faint.

Santos *et al.* (1984) and Verstreate and Winter (1984) designated the OMPs of *B.abortus* and *B.melitensis* strains as 1, 2 and 3 groups containing polypeptides of MW ranging 25 - 30 kDa, 34 - 40 kDa and 88 - 94 kDa, respectively, with group 2 (34 - 40 kDa) containing major polypeptides. Similarly, on the basis of commonality of bands in this study, the OMPs of 5 strains could be broadly classified into 3 major groups, viz. low MW group with 14 - 30 kDa, medium MW group with 39 - 60 kDa and high MW group with 68 - 93 kDa. The OMPs of *B.abortus* 99 and 544, showed large diffused bands of low MW 22 - 25 kDa and 22-26 kDa, respectively, whereas strain 19 and other species showed distinctly faint bands. Verstreate and Winter (1984) made similar observations with 25-30 kDa group 3 OMPs of *B.abortus* strains and considered that these diffused bands could be because of either less discrete or closely spaced polypeptide bands. Furthermore, the minor variations in number, relative intensities and migration of polypeptide bands in the medium MW range was observed in this study. The variable band in the medium MW range was 44 - 47.3 kDa for *B.abortus* 99, 42.3 - 44 kDa for *B.abortus* strain 544 and 39.2 - 42.1 kDa for *B.abortus* vaccine strain19. The *B.melitensis* 16M and *B.suis* 1330 had predominant bands in the MW range of 42 - 42.3 kDa. The preponderance of groups 2 and 3 over group 1 OMPs was seen as a characteristic in *B.abortus* strains (Dubray and Bezard 1980, Verstreate *et al.*1982, Dubray and Charriaut 1983, Moriyon and Berman 1982). The 39 kDa band recorded in *B.melitensis* 16M by Santos *et al.* (1984) closely matched to 42 kDa peptide band observed in this study.

Demonstration of glycoproteins by silver staining

The SDS - PAGE profiles of *Brucella* OMPs, in both coomassie blue stained gel and ponceau stained PVDF membrane revealed smeared polypeptide bands in the MW range 39.2 - 47.3 kDa. The special silver staining of these OMP gels confirmed the presence of polysaccharide moiety linked to polypeptides in the medium MW group OMPs. The polysaccharides varied from 29 to 43 kDa (Fig. 3). *B.abortus* 99 and 544 demonstrated moderately intense bands (Fig. 3, Lanes a, b), whereas, *B.abortus* 19 and *B.suis* 1330 showed faint diffused bands suggestive of low amount of polysaccharide (Fig. 3, Lanes c, e). On the other hand, *B.melitensis* 16M revealed an intense diffused band (Fig. 3, Lane d). Similar observations were made by Moreno (1983) while Santos *et al.* (1984) recorded the appearance of 2 broad smears in the MW range of 35 - 90 kDa, because of the presence of polysaccharide.

The variability in the reported MW of OMPs of *Brucella* by several workers appear to be because of the different purification protocols adopted. Dubray and Charriaut (1983) found 40 discrete bands in their SDS soluble cell wall fraction of *B.abortus*, with a major band at 43 kDa. On the other hand, Moriyon and Berman (1982) showed 38 kDa polypeptides as major by extracting OMPs with SDS-B mercaptoethanol at 100°C. However, it may be possible that polypeptides are masked to varying degrees by substances such as lipids or sugar and hence failed to be detected at very low concentrations among OMPs of *Brucella* (Tatabai and Deyoe 1984). Hill and Cook (1994) attributed the differences in the OMP purification protocols for the variability of the results obtained.

On the basis of the results of this SDS - PAGE analytical study, similarity in the OMP patterns in SDS-PAGE reflect the overall relationship among the smooth *Brucella* species, which were well within the patterns described by earlier workers (Santos *et al.* 1984, Verstreate and Winter 1984) with a few variations in the medium and low MW group OMPs. However, these subtle differences are not sufficient to discriminate smooth *Brucella* strains. Therefore in conclusion, the SDS-PAGE analysis of OMPs con not be used as a tool for discriminating *Brucella* strains.

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Morphohistogenesis and mode of growth of spleen in goat (*Capra hircus*)

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ABSTRACT

Spleen was measured in 18 goat foeti of 4.00 - 35.00 cm CR length and 6 neonatal goats. The foetuses were divided into 3 groups on the basis of CR length. The spleen was irregularly triangular in group 1 and elongated elliptical in group 2 onward. It shifted caudad and laterad with the advancement of age. The spleen weighed 16.17 ± 2.242 mg in group 1 and increased to 536.67 ± 35.277 and 2 081.17 ± 231.144 mg in groups 3 and 4 respectively. The length, width and thickness (4.38 ± 0.472, 2.83 ± 0.115 and 1.32 ± 0.083 mm) in group 1 increased to 23.83 ± 2.522, 19.33 ± 2.679 and 4.00 ± 0.500 mm respectively in group 3. The weight of the spleen revealed maximum (3218%) total increase (groups 1 - 3) and surface area (3142%) was next in order. However, all the parameters showed 51 - 80% of their respective total increase in early stage of gestation. Significant correlation coefficients among CR length and splenic parameters suggested symmetry between the growth of foetus and spleen. White pulp differentiated in group 2.

Key words: Goat foetuses, Growth spurt, Morphohistogenesis, Spleen

The present study was planned to investigate the development, mode of growth and histogenesis of spleen at different stages of development in prenatal and neonatal goats.

MATERIALS AND METHODS

Goat foetuses (18) of 4.00 to 35.00 cm CR length were procured from local animal slaughterhouse/market. The foetuses were divided into 3 groups (6 in each group) on the basis of CR length. Group 1, included foetuses up to 10 cm; group 2, 10+ to 20 cm and group 3, 20+ cm and above CR length. The neonates (newly born) formed fourth group. The specimens were fixed in 10% buffered formalin for 24 to 48 hr. The spleen was dissected out of the body and following measurements were recorded with the help of compass/scale/ nylon thread and vernier calliper:

Table 1. Range, mean and SE of splenic parameters in prenatal and neonatal goats

Parameter		Groups			
		1	2	3	4
Foetus					
CR length (cm)	Range	4.00–10.00	13.00–20.00	21.00–35.00	37.00–45.00
	Mean±SE	6.83±1.046	16.00±1.176	26.92±2.051	40.33±1.174
Spleen					
Weight (mg)	Range	10.00–25.00	200.00–400.00	400.00–650.00	1540.00–3000.00
	Mean±SE	16.17±2.242	308.00±30.047	536.57±35.277	2081.17±231.144
Length (mm)	Range	3.00-6.00	12.00–25.00	14.00–30.00	30.00–45.00
	Mean±SE	4.38±0.473	20.00±2.129	23.83±2.522	38.17±2.386
Width (mm)	Range	2.00–3.20	8.00–18.00	10.00–28.00	25.00–30.00
	Mean±SE	2.83±0.115	14.33±1.520	19.33±2.679	27.17±0.873
Thickness (mm)	Range	1.00–1.50	1.50–3.50	2.00–5.50	6.00–8.50
	Mean±SE	1.32±0.820	2.750±0.337	4.00±0.500	7.08±0.396
Surface area (mm^2)	Range	4.00–8.00	65.00–170.00	120.00–250.00	400.00–50.000
	Mean±SE	6.08±0.663	105.00±15.384	197.17±19.460	456.67±19.437

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Table 2. correlation coefficient of splenic parametres with CR length of foetus and weight of spleen

Group	Spleen				
	Weight	Length	Width	Thickness	Surface area
CR length	0.926**	0.884*	0.986**	0.851*	0.940**
Weight of spleen	-	0.938**	0.908**	0.822*	0.838*
CR length	0.967**	0.892*	0.913**	0.908**	0.893*
Weight of spleen	-	0.964**	0.973**	0.951**	0.865
CR length	0.946**	0.937**	0.956**	0.910**	0.905**
Weight of spleen	-	0.977**	0.990**	0.992**	0.984**
CR length	0.985**	0.877*	0.933**	0.956**	0.828*
Weight of spleen	-	0.891	0.957**	0.953**	0.814*

Table 3. Regression coefficient of splenic parameters with CR length of foetus and weight of spleen

Group	Spleen				
	Weight	Length	Width	Thickness	Surface area
CR length	1.985	0.400	0.111	0.064	0.596
Weight of spleen	-	0.198	0.048	0.029	0.248
CR length	24.699	1.614	1.181	0.259	11.687
Weight of spleen	-	0.068	0.049	0.011	0.443
CR length	16.269	1.152	1.247	0.222	8.582
Weight of spleen	-	0.070	0.075	0.014	0.543
CR length	194.121	1.782	0.694	0.323	13.710
Weight of spleen	-	0.009	0.004	0.002	0.068

Foetus: CR length, spleen: greatest length, width, thickness and visceral surface area. For surface area outline sketch of the viseral surface were made on graph paper and the area was calculated in millimeters. The spleen was weighed to the nearest milligram by electronic digital monopan balance. After taking the measurements the spleens were fixed in 10%

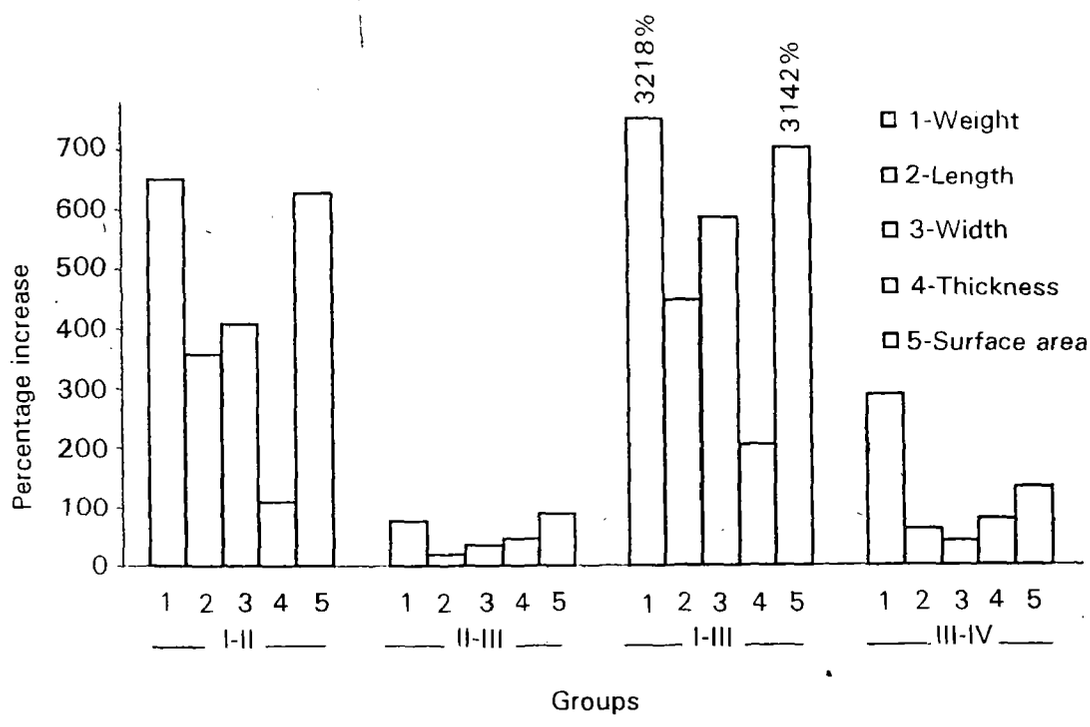


Fig 1. Growth spurt of splenic parameters.

buffered formalin for 24 hr. In groups 1 and 2 whole spleen and in groups 3 and 4 half of the spleen were taken and processed. The sections of 5 - 7 μm thickness were cut and stained with haematoxylin and eosin stain. The thickness of the capsule was measured with the help of occular micrometer which was standardized with stage micrometer. The data were analysed statistically (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

The spleen was irregularly triangular in group 1 and elongated elliptical in groups 2 onward. It shifted caudad and laterad with the advancement of age. However, base/dorsal part of the spleen was wider as reported in sheep by Getty (1975). The caudad and laterad shift of the spleen with the advancement of age in this study is probably dependent on relative increase in the size of the abdominal cavity and its viscera, especially stomach to which it is attached. Change in orientation, moulding and shift of the organs with relative increase in size appears a normal phenomenon during development as reported by Patten and Carlson (1977).

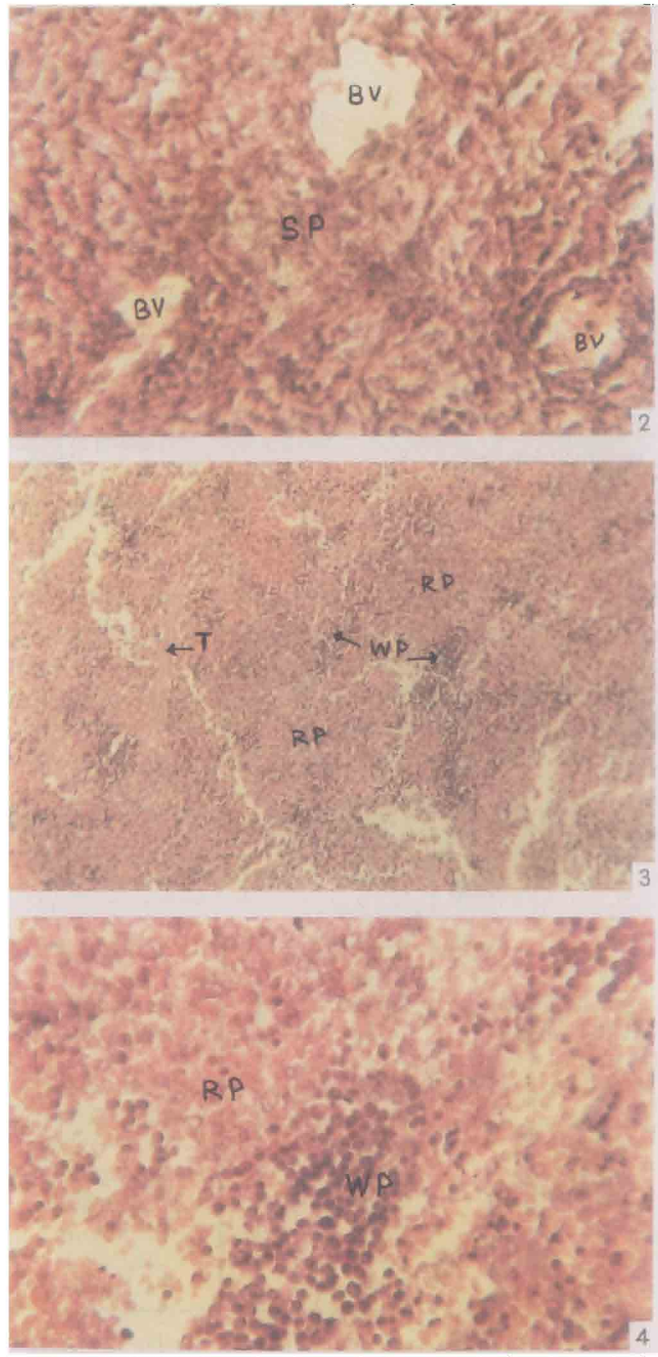
The measurements of different parameters of spleen have been given in Table 1. All the parameters of spleen in neonatal goat increased approximately one-and-half-times to their corresponding value in group 3 except weight and area of visceral surface which increased about 4-and-2-times respectively. The thickness of the spleen increased more in group 4 from group 3 in comparison to its length and width.

Correlation coefficients revealed that all the parameters of spleen had significant association with CR length of the foetus and weight of the spleen in almost all stages of gestation (Table 2). However, the extent of association in general increased with the advancement of age. Highly significant correlations between CR length of foetus and parameters of spleen is suggestive of growth symmetry between the two and a definite growth ratio to mould the spleen in a definite shape and size characteristics to specific genotypic configuration of an individual.

The values of regression coefficients (Table 3) of different parameters of spleen revealed maximum change in the weight of spleen due to 1 cm change in the CR length. It increased with the advancement of age and was maximum in neonatal goat (group 4). The visceral surface area was next in order and revealed maximum change (13.710 mm^2) in group 4.

Growth spurt

The growth spurt of different parameters of spleen revealed variable increase in different age-groups (Fig.1). However, weight showed maximum (3218%) total increase. The area of visceral surface was next in order (3142%). All the parameters of the spleen revealed markedly greater increase in early (group 1 - 2) stage of gestation than late. The increase in groups 1 - 2 ranged between 51 to 81% of respective total increase for different splenic parameters. Greater increase in early stage of gestation than late in this study is in support of quick embryogenesis and increase reported by Patten and Carlson (1977) in animals. Malik *et al.* (1992, 1995) also reported greater increase in early stage of gestation in prenatal growth index and histogenesis and mode of growth of scrotum in goat respectively. The increase from groups 3-4 was more



Figs 2-4. Section of spleen of: **2**. group 1 showing splenic parenchyma (SP), blood vessel (BV) H&E.×100; **3**. group 2 showing trabeculae (T), red pulp (RP) and white pulp (WP), H & E.×100; **4**. group 2 showing red pulp (RP) and white pulp (WP), H & E.× 400.

than the corresponding increase from groups 2-3 but very less in comparison to early stage of gestation. However, it was maximum (287%) for weight and area of visceral surface (13.1%) was next in order.

Histogenesis

The connective tissue capsule covering the spleen was thin (9.30μm), condensed and fibrous with serous lining superficially. The trabeculae extending from it into the substance of the spleen were not identified in group 1. However, the blood vessels entered into the splenic parenchyma indicating preformation of trabecular network (Fig.2). The smooth muscle fibers in the capsule could not be seen. In group 2 the capsule got thickened (40.00μm), condensed and fibrous with appearance of smooth muscle cells. The distinct trabeculae extended into the interior of the spleen and showed fibromuscular character (Fig.3). The capsular part of the hilus of spleen was made up of loose, highly cellular connective tissue and contained thin walled blood vessels with large lumen. In group 3 the capsule still thickened (64.00μm) and became more fibromuscular with increased trabecular network. The muscular component increased in the trabeculae with the advancement of age. However, 2 distinct layers of smooth muscle fibers in the capsule as described in ruminants by Dellmann and Brown (1987), were not conspicuous in this study. Most probably the layers may be formed in later development.

The splenic parenchyma was diffuse and made up of delicate connective tissue fibers and prominent connective tissue cells (Fig.2). The arteries and veins penetrated profusely throughout the connective tissue framework. Along the wall of the blood vessels the lymphoid cells had begun to conglomerate in the tunica adventitia. The wall of the blood vessels had endothelium over which the adventitious tissue enriched with lymphoid cells. However, the formation of the splenic corpuscles was not distinct. In group 2 the parenchyma of the spleen showed the presence of red pulp and white pulp distributed diffusely without distinct demarcation (Figs 3 and 4). The splenic parenchyma showed diffuse distribution of the white pulp in the loose areas of the red pulp. However, differentiation to the extent of circumscribed lymphoid areas was seen in certain areas. In group 3 the spheroid character of the lymphoid tissue of the splenic corpuscles increased. The density of the lymphoid cells was also more. Artery was placed more centrally in the corpuscle. Relatively the diffuse area of the red pulp decreased on account of the lymphoid tissue in it.

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Morphohistogenesis of trachea in goats (*Capra hircus*)

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ABSTRACT

The trachea was measured in 18 goat foeti of 4.00 - 35.00 cm CR length. The foetuses were divided into 3 groups on the basis of CR length. The length of trachea measured 25.00 ± 3.406 mm in group 1 and increased to 108.33 ± 7.149 mm in group 3. It was wider (5.66 ± 0.248 mm) than its height (5.25 ± 0.311 mm) in all the age groups. The wall-thickness of trachea (0.050 ± 0.012 mm) in group 1 increased to approximately double (1.06 ± 0.058 mm) in group 2. The extent of association of tracheal parameters with CR length decreased with the advancement of age. Regression coefficient revealed that the circumference was influenced maximum (4.050 mm) due to change in CR length in group 1. In the wall of the trachea the fibrocellular plates transformed into cartilaginous plates in right, left and ventral positions in group 1. These matured and attained typical cartilaginous structure in group 2. The mucosa lined by pseudostratified epithelium was smooth in group 1, got folded in group 2 and folds enlarged and epithelium well differentiated and organized in group 3. The glands in lamina propria/submucosa could not be seen in any of the groups.

Key words: Cartilage, Epithelium,Parameters,Trachea

The present study was planned to investigate the development, mode of growth and histogenesis of trachea at different stages of development in prenatal goat.

MATERIALS AND METHODS

The study was conducted in 18 goat foetuses of 4.00 - 35.00 cm CR length procured from local animal slaughter house. The foetuses were divided into 3 groups (6 foetuses in each group) on the basis of CR length. Group 1, included foetuses up to 10 cm; group 2, 10+ to 20 cm; and group 3, 20+ cm and above CR length. The specimens were fixed in 10% buffered formalin for 48 hr. The trachea was dissected out of the body and following measurements were recorded with the help of compass/scale/nylon thread and vernier calliper:

Foetus: CR length. Treahea: greatest length, width, thickness, circumference, wall-thickness and cross sectional (CS) area. For CS area the trachea was transsected in the middle and stam-pad-ink impressions of cut surface were made on graph paper and the area was calculated in millimeters. The tissue pieces from middle of the trachea in groups 1, 2 and 3 were taken, fixed in 10% buffered formalin and processed for paraffin sections in cedarwood oil-alcohol and acetone-benzene sequence. 5 -7 µm thick sections were cut and stained with haematoxylin and eosin stain. The data were analysed statistically (Snedecor and Cochran 1967).

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RESULTS AND DISCUSSION

The trachea consisted of 55 - 58 cartilaginous rings in group 3. Hare (1975) reported variable number of cartilaginous rings in ruminants (48 - 60), dogs (42 - 46), cats (38 - 43) and pigs (32 - 36). It was marked by dorsal median longitudinal ridge in groups 1 and 2, which converted into groove in group 3. The additional bronchus for right apical lobe was given against third intercostal space, 4 - 9 mm rostral to the bifurcation of the trachea in groups 1 - 3. The measurements of trachea in different age groups of goat foetuses are given in Table 1. The trachea measured 25.00 ± 3.406, 1.68 ± 0.173 and 2.53 ± 0.274 mm in its length, height and width, respectively, in group 1 which increased to 108.33 ± 7.149, 5.25 ± 0.311 and 5.66 ± 0.248 mm in group 3. Width of trachea was greater than its height in groups 1 and 2 but in approximation in group 3. The circumference was 8.33 ± 1.116 mm in group 1 and increased about one-and-half- and two-and-half-times in groups 2 and 3 respectively. The wall-thickness measured 0.50 ± 0.012 mm in group 1 which increased to 1.28 ± 0.041 mm in group 3.

Highly significant values of correlation coefficients of all the parameters of trachea with CR length and length of trachea (Table 2) revealed strong and intense association between

Table 1.Range, mean and standard error of parameters of trachea in prenatal goat

Parameter		Group		
		1	2	3
Length (mm)	Range	15.00–35.00	50.00–85.00	90.00–130.00
	Mean±SE	25.00±3.406	70.27±5.375	108.33±7.149
Height (mm)	Range	1.00-2.10	3.50–5.00	4.00–6.00
	Mean±SE	1.68±0.173	4.25±0.252	5.25±0.311
Width (mm)	Range	1.50-3.20	4.40–6.50	5.00–6.50
	Mean±SE	2.53±0.274	5.32±0.316	5.66±0.248
Circumferance range (mm)	Range	5.00-12.00	10.00-14.00	15.00-27.00
	Mean±SE	8.83±1.116	11.67±1.126	20.08±2.123
CS area (mm^2)	Range	1.00–3.00	5.00–6.50	5.66–7.33
	Mean±SE	2.17±0.334	5.80±0.238	6.47±0.294
Wall thickness range (mm)	Range	0.30–0.80	0.85–1.25	1.08–1.40
	Mean±SE	0.50±0.012	1.06±0.058	1.28±0.041

Table 2. Correlation coefficient of tracheal parameters with CR length and length of trachea

Group		Trachea				
		Length	Height	Width	Circumference	CS area
I	CR length	0.977**	0.075	0.951**	0.991**	0.973**
	Length of trachea	-	-0.045	0.929**	0.954**	0.925**
II	CR length	0.874*	0.425	0.873*	0.965**	-0.526
	Length of trachea	-	0.258	0.592	0.948**	-0.415
III	CR length	0.203	-0.011	-0.284	0.380	0.810*
	Length of trachea	-	-0.226	-0.534	0.952*	0.709

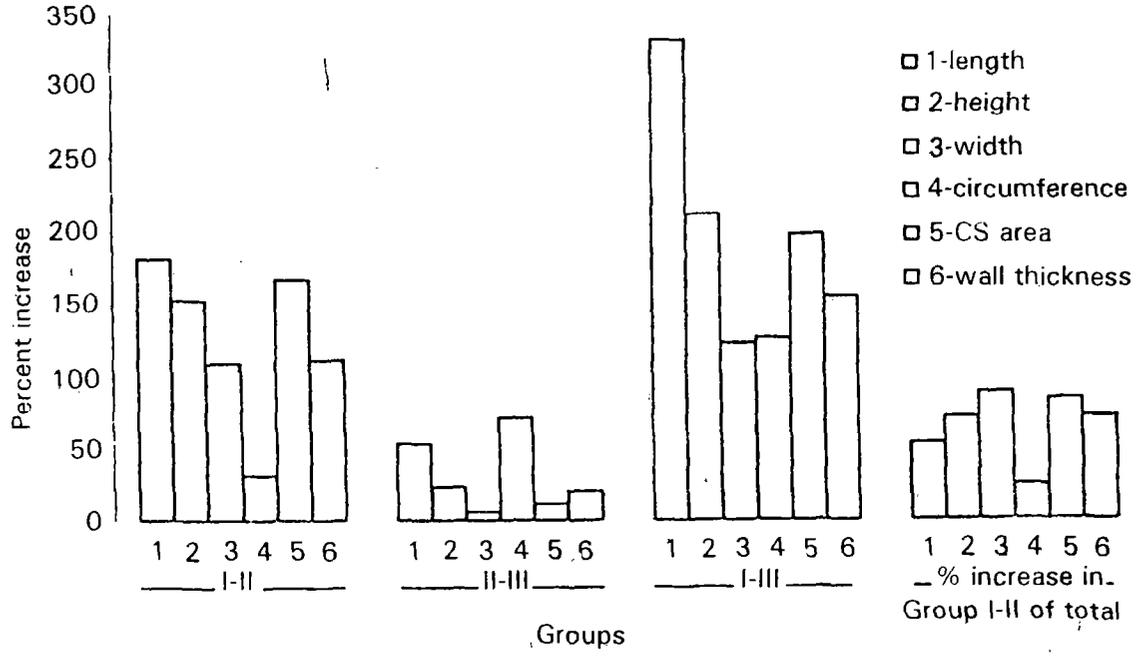
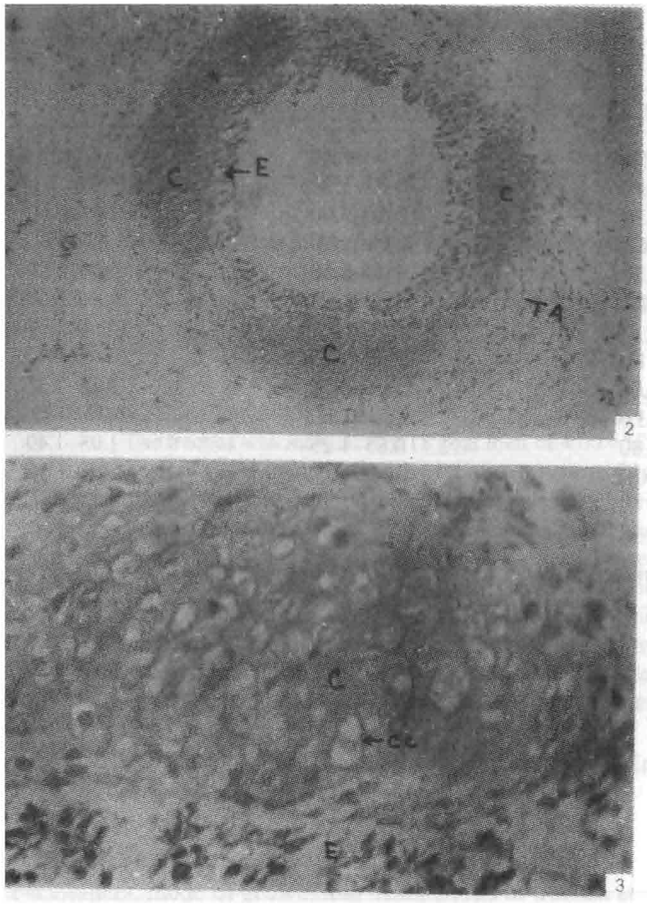


Fig. 1. Growth spurt (percentage increase) of tracheal parameters in prenatal goat.

parameters of trachea and CR length except height which showed nonsignificant but positive correlation. However, the extent of association decreased with the advancement of age. Decrease in the association of tracheal parameters with CR length and length of trachea may be due to (i) trachea being tubular organ revealed greater length-oriented increase, and



Figs 2-3. **2.** Cross section of trachea of group 1 showing epithelium (E), cartilage (C) and tunica adventitia (TA). H & E. × 80. **3.** Cross section of trachea of group 1 showing epithelium (E), cartilage (C) and cartilage cell (CC). H & E. × 320.

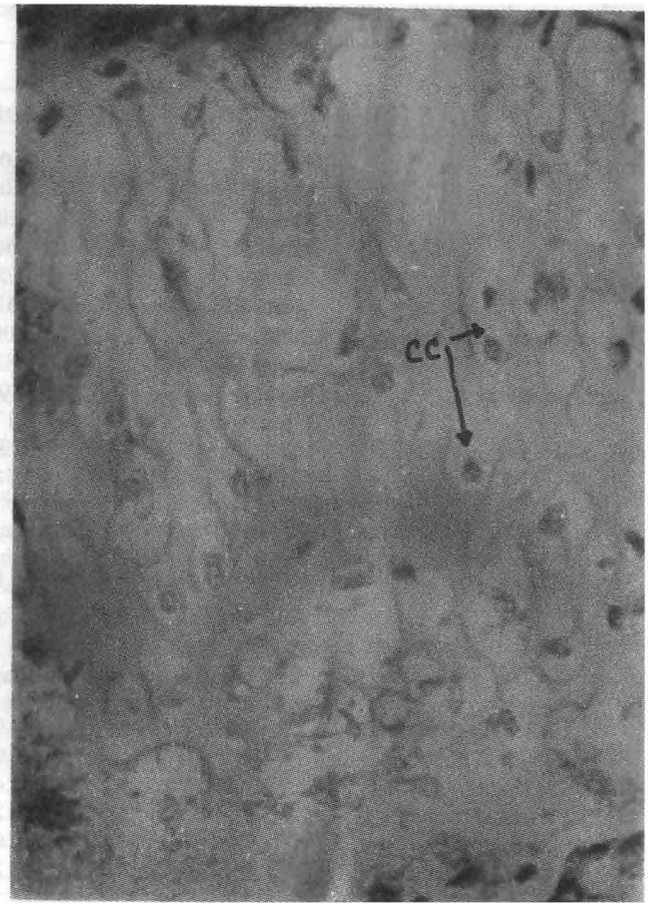


Fig. 4. Cross section of trachea of group 2 showing cartilage cell (CC) in radial arrangement. H & E. × 400.

(ii) wall of the trachea contained greater amount of dense connective tissue in comparison to the body as a whole in later stage of development. Moreover, in later stage of development the change in the growth and difference in association is likely due to greater increase of viscera, connective tissue and muscular tissue in the body in comparison to the trachea.

The values of regression coefficient of tracheal parameters (Table 3) revealed variable extent of influence of each on CR length and length of trachea. However, circumference of the trachea influenced the CR length to maximum (4.050 mm) extent in group 1 whereas in groups 2 and 3 length of trachea influenced CR length maximum (3.995 mm and 0.408 mm). However, the extent of influence of each parameters on CR length and length of trachea decreased with the advancement of age.

Growth spurt

The values of growth spurts of different parameters varied in different age-groups (Fig.1). However, length showed

Table 3. Regression coefficient of tracheal parameters with CR length and length of trachea

Group		Trachea				
		Length	Height	Width	Circumference	CS area
I	CR length	3.63	0.014	0.283	4.050	0.354
	Length of trachea	-	-0.002	0.074	1.047	0.091
II	CR length	3.995	0.090	0.235	0.916	-0.106
	Length of trachea	-	0.012	0.035	0.197	-0.018
III	CR length	0.408	-0.001	-0.020	0.227	0.067
	Length of trachea	-	0.010	0.018	0.283	0.029

maximum (333.00%) total increase. Height (212.00%) and CS area (198.00%) were next in order. However, all the parameters revealed greater increase in early stage (groups 1 - 2) of gestation than late except circumference which showed 32.00% increase in groups 1 - 2 in contrast to 72.00% increase in late gestation. The increase in early stage of gestation ranged between 25 - 89% of the total increase for different tracheal parameters. It was maximum for length (181.00%), CS area (167%) and height of the trachea were next in order. Greater increase in early stage of gestation also have been reported in histogenesis and mode of growth of scrotum (Malik *et al.* 1995), prenatal growth changes of heart (Malik *et al.* 1996) and growth spurts of ventricular walls and interventricular septum (Malik *et al.* 1996) in prenatal goat.

Histogenesis

Loose mesenchymal tissue formed the oesophagotracheal part of the fibroprimordia of the foregut as also has been described in the text books (Patten and Carlson 1977). In the tracheal part the mesenchymal tissue differentiated in the form of condensed fibrous plates. With greater differentiation the condensed fibrocellular plates transformed into cartilaginous plates in right, left and ventral positions in group 1(Fig.2). Right and left plates appeared similar in size and shape being elongated, while the mid ventral plate was small, ellipsoidal with differentiation of hyaline character. The matrix was homogeneous and purple (Fig.3). The small and dark cartilage cells (chondrocytes) in the centre occupied lightly stained larger cartilage nests. The cartilage cells at the periphery were small and immature (chondroblasts) (Figs 3 and 4) and resembled to the description given by Trautmann and Fiebiger (1957) in domestic animals.

In the specimen of group 1 mucosa was smooth and in group 2 it was folded and lined by unorganised pseudostratified epithelium (Fig.2). The cartilage matured and attained the typical C shaped cartilaginous structure. Dellmann and Brown (1987) also reported 'C' or 'U' shaped pieces of hyaline cartilage that are incomplete dorsally. The number of the cartilages in the wall of the trachea varied not only among different species but among different individuals of the same species. In group 3 the mucosa became more folded and lining pseudostratified columnar epithelium was well differentiated and organised. The epithelial tissue was diffuse, purple and more condensed in the outer half. Short and small smooth muscle bundles extended in both the sides from mid dorsal position to a variable extent between the cartilages and mucous membrane. The glands reported by Trautmann and Fiebiger (1957) and Dellmann and Brown (1987) were not seen in any of the groups in the lamina propria or submucosa. Probably they might develop late as the sweat glands in goat appear before term as reported by Malik *et al.* (1997).

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Histomorphological study on the ovary of prenatal Indian buffalo *(Bubalus bubalis)*

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ABSTRACT

Histomorphological study was conducted on the ovary of 8 prenatal buffaloes. The surface epithelium of the ovaries was simple squamous to simple cuboidal which was thrown into folds at locations. It was invaginated into ovarian cortex. The average height of epithelium was 4.77±0.68μm. The average thickness of the tunica albuginea was 75.90±4.87μm. The per cent distribution of cortex and medulla were 78.96±1.16 and 21.04±1.16 respectively. Ovarian stroma was fibrocellular structure. It comprised mesenchymal cells differentiating into fibroblasts with collagen fibres and reticular fibres. No elastic fibres could be seen except at perivascular area. Smooth muscle cells were also observed. All types of follicles were observed in the ovary. Polynuclear ova were also observed. The interstitial gland cells were seen at locations. The mast cells were seen in perivascular area. The rete ovarii and mesonephric tubules were also present. They were lined with cuboidal to low columnar epithelium. Blood vessels of different caliber were seen in medulla.

Key words: Buffalo, Histomorphology, Ovary, Prenatal buffalo

The buffalo is known for the poor reproductive performance such as silent heat, low conception rate and long calving interval, thus causing heavy economical losses to the farmers. The embryo transfer technique in buffalo has not proved to be much successful, mainly due to very poor superovulatory response and poor recovery of viable embryo (Taneja *et al.* 1991). The main constraints encountered in superovulation are the lack of histological and endocrinological information on the buffalo ovaries. Therefore the study was conducted to record the status of ovary in full term fetus.

MATERIALS AND METHODS

The study was conducted on the 8 female genitalia of prenatal (full term pregnancy) buffaloes, collected from veterinary clinics, dairy complex Ludhiana and Saharanpur abattoir. The ovaries were collected and fixed in the 10% neutral buffered formalin. The ovarian samples were processed by Cedar wood oil method and paraffin sections at 5-7μm were obtained on glass slides for histological studies. The histological stains used are given in Table 1.

Table 1. Historical stains used in the experiment

Purpose	Method/stain	Reference
Histomorphology	Haematoxylin and Eosin	Luna (1968)
Collagen fibres	Masson's Trichrome	Luna (1968)
Elastic fibres	Weigert and Van Gieson	Sheehan and Hrapchak (1968)
Reticular fibres	Gridley's silver impregnation	Sheehan and Hrapchak (1973)
Mast cell	Unna's	Luna (1973)
Muscle	Heidenhain's Anilne blue	Luna (1968)
Neuronal elements	Holme's	Luna (1968)

RESULTS AND DISCUSSION

The ovaries of full term prenatal buffalo foetuses were distinctly divided into outer cortex and inner medulla (Fig.1). It was covered by surface epithelium, followed by tunica albuginea ovarii. The cortex consisted of follicles at various stages of development and regression, viz. primordial, primary, secondary, Graafian and atretic follicles. In addition, there was corpus albicans, interstitial gland cells and cortical stroma. Large vessels, lymphatics, nerves, embryonic remnents, and medullary stroma characterized the medulla. The mean cortical area was greater (78.96±1.16%) than the medullary area (21.03±1.16%) in the ovaries of prenatal buffalo fetuses.

Simple squamous to simple cuboidal epithelium placed on basement membrane covered the ovaries of prenatal buffalo foetuses. Its cytoplasm was eosinophilic with spherical to oval nuclei as reported earlier in cow (Sharma *et al.* 1974) and buffalo (Goswami 1985). However, Prasad *et al.* (1979) and Goswami (1985) reported simple cuboidal epithelium

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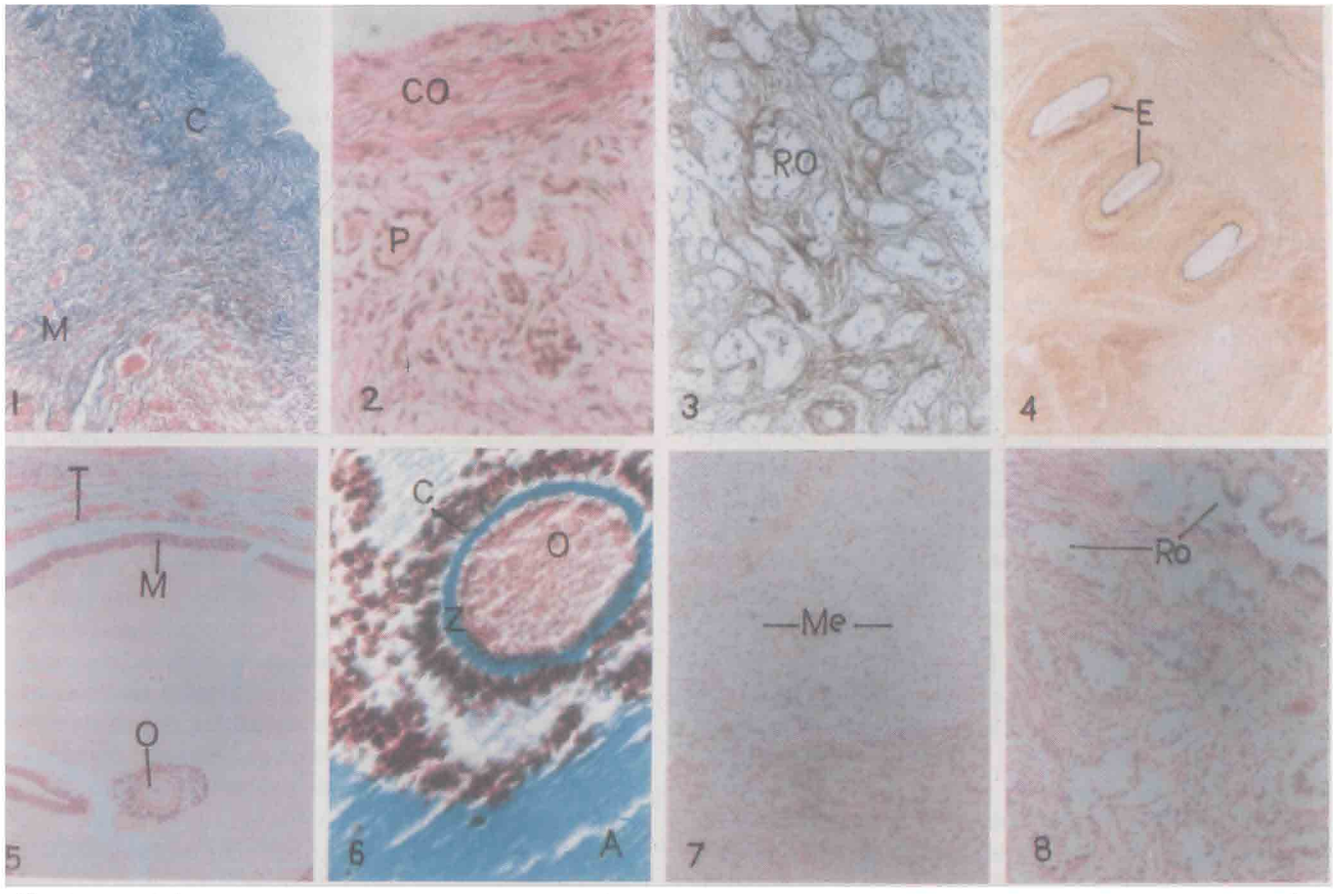
[2]Professor and Head.

with patches of stratified epithelium in buffalo. The stratification of surface epithelium as reported by latter authors might be the simple cuboidal epithelium cut at tangential level. The epithelium was placed on a distinct basement membrane made up of reticular fibres as reported earlier in cow (Moss *et al.* 1954), goat (Harrison 1962) and buffalo (Goswami 1985). This was in contrast to the description made on human ovaries (Bloom and Fawcett 1968, Leeson and Leeson 1976) where the distinct basement membrane has not been mentioned.

At locations the basement membrane along with surface epithlium bulged out particulary over the graafian follicles more so in the observations of Goswami (1985) in buffalo. The cell outlines were not clear. The mean height of surface epithelium was 4.77±0.68μm. It had infoldings in the form of deep fissures which invaginated into the cortical tissue (Fig.1). The epithelium was interrupted at the mesovarian attachement.

The folds and invaginations have been reported by Goswami (1985) in buffalo which were considered as age related morphological artifacts. Infoldings might be due to shrinkage of surface epithelium particularly in ovaries of valves. Which are more prone to such artifacts (Martinovic 1934) in ewe. In buffalo, Fadle *et al.* (1974) have reported a close relationship between surface epithelium and neofolliculogenesis, contrary to the present investigation. Similar implications could not be ascertained which was further supported by the ultrastructure study (Wischnitzer 1965, Weakly 1967) and histochemical evidences (O, Shea 1966). They proved that the surface epithelium does not contribute to the neofolliculogenesis.

Underlying the surface epithelium was tunica albuginea ovarii. It was made up of dense white fibrous connective tissue having lamellar configuration. The collagen fibres and reticular fibres were arranged parallel to surface epithelium with large number of fibroblasts and fibrocytes. No elastic fibres could be seen (Figs 2,3). The mean thickness of tunica albuginea was 75.90±4.87mm. There were some focal areas



Figs 1-8. **1.** Distinct cortex (C) and medulla (M). The cortex had higher proportion of collagen fibres stained green. Masson's Trichrome × 70. **2.** Dense collagen fibres (Co) in tunica albuginea and a group of primordial (P) follicles beneath it. Verhoeff × 280. **3.** Reticular fibres lining the basement membrane of reti ovarii (Ro) Gridley reticular × 140. **4.** Elastic fibres (E) mainly in and around the blood vessels in medulla. Verhoeff × 280. **5.** Graafian follicle with folded membrana granulosa (M), oocyte (O), the prethecal cell (T) around the follicle. Haematoxylin and eosin × 70. **6.** Graafian follicle at earliest stage of atresia, degenerating zona pellucida (Z), surrounding oocyte (O), corona radiata (c). The follicle filled with liquor follicle (A). Masson's Trichrome × 280. **7.** Mesenchymal cell (Me) in ovarian cortex. Haematoxylin and eosin × 140. **8.** Cortico-Medullary junction having reti ovarri (Ro) lined by epithelium. Haematoxylin and eosin × 140.

where the tunica albuginea was thinner particularly those covering the large follicles. This confirms the findings of Goswami (1985) in buffaloes. The small blood vessels and capillaries were also observed in this layer. Whereas Sinha (1980) and Singh (1983) observed large number of lymphatics and blood vessels, more in the cyclic and pregnant animals which might be related with hormonal influence and age related changes. This was complete layer except at hilar region. The trabeculae descended down into the cortical stroma to which it merged. The elastic fibres were found only in vascular walls (Banks 1981, Priedkalns 1987) in domestic animals.

Cortex

The cortical stroma enclosed within tunica albuginea comprised mesenchymal like cells maturing to fibroblasts. The collagen fibres constitute the main fibre type along with few reticular fibres. The elastic fibres could not be seen in the stroma except in the walls of blood vessels (Figs. 2, 4). A few smooth musle cells were observed in stromal tissue. The mesenchymal tissue have been responsible for the formation of various cell types of developing ovary (Guraya 1977). The mean cortical area in the prenatal animals was 78.96±1.16%. This reflects better development of cortical area due to large number of ovarian follicles in prenatal buffalo ovaries. Clusters of oocytes were observed just underneath the tunica albuginea ovarii (Fig. 2). They were seen as cord, clusters and isolated as reported in goat (Joshi *et al.* 1976) and cow (Sharma *et al.* 1974). They were usually seen at the dictyate stage as reported by Guraya (1985). In the process of folliculogenesis the oocytes breakdown from cord or clusters and undergo further development into developing follicles (Guraya 1985, Gosden and Telfer 1987). Different types of follicles, viz. primordial, primary, secondary, Graafian and ateretic follicles and polyovular and polynuclear ova were observed in the prenatal buffalo ovary (Figs 2, 5, 6). Such polyovular follicles and polynuclear ova/oocyte has been observed in the ovaries of number of domestic animals like cat, dog, pig and sheep (Hartman 1926, Hadek 1958, Hadek and Getty 1959, Bal 1966, Scott 1970, Talukdar *et al.* 1993). The occurrence of polyovular follicle and polynuclear ova has been related with age and sexual cycle of goat (Joshi *et al.* 1976).

Medulla

The central part of the ovaries comprised large blood vessels, lymphatics, nerve fibres, interstitial gland cells and embryonic remnants. These structures were continuous in the medulla through hilus as reported in human ovary (Copenhaver *et al.* 1978). The medullary stroma was identical to cortical stroma comprising maturing fibroblast cells (Fig.7). The collagen and reticular fibres constitued the fibrous elements. Elastic fibres were found to be absent, though present in the wall of blood vessels and perivascular stroma (Fig.4). The connective tissue fibres were much finer and fewer in number. In addition to arterial network, venous and lymphatic vessels were also seen in the medulla of prenatal buffalo ovaries. This supports the earlier findings of Goswami (1985) in buffalo and Priedkalns (1987) in bovine.

The ovarian cortex and medulla was found to be extensively innervated. Majority of the nerve fibres was found to be terminating on the perivascular area (Copenhaver *et al.* 1978, Leson and Leeson 1976). The density of the innervation varied according to species. The cut ovaries have been said to contain many adrenergic nerve fibres while that of man and monkey contained moderate to few (Jacobowitz and Wallach 1967). Fine nerve terminals were observed in the theca cells layer reaching almost up to basement membrane. Whereas, Owman and Sjoberg (1966) found nerve terminals near the follicle in rabbit. Nielson *et al.* (1970) and Fink and Schofilled (1971) correlated the inhibitory action of the nerve plexus on the maturation of follicle and production of steroid.

Rete ovarii were frequently observed in the medulla as well as cortex of prenatal buffalo ovary (Fig.8). They were lined by simple columnar epithelium. Such structures have been reported earlier in cow (Archibald *et al.* 1971), buffalo (Prasad *et al.* 1979, Goswami 1985) and in wide variety of domestic animals (Trautman and Fiebiger 1957, Banks 1981, Priedkalans 1987). In addition coiled branching tubules lined with simple or stratified epithelium appeared as cord of cells. These were the remnants of the embryonic mesonephric tubules. These structures do have neither connection with germinal epithelium nor indication of neofollculogenesis. Such structures have been reported in Egyptian buffalo (Fadle *et al.* 1974) and considered them to be structure associated with neofolliculogenesis.

Interstitial gland cells were observed in the ovary of prenatal age group as reported earlier by Guraya (1977). The interstitial cells were observed in deeper part of medulla. wedged between blood vessels as reported in cattle and buffalo (Guraya 1968, 1985). These epitheloid cells are probably responsible for secretion of steroid hormones, more precisely the androgen (Agarwall and Laloria 1978). Hilar cells were not observed in these animals. The mast cells were observed both in cortex and medulla particularly in the vicinity of blood vessels. Such cells have been demonstrated in cow (Nakamura *et al.* 1987). Studies have revealed that mast cells release histamine (Krishna *et al.* 1989). They appeared degranulated after release of histamine. The degranulation was related by neuronal inputs because neurons have been shown to have close morphological relationship with the mast cells. The histamine regulating the capillary permeability of the ovarian blood supply increases ovarian contractability and subsequent ovulation. No corpus luteum was found.

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Effect of hypermagnesaemia on plasma and salivary magnesium, calcium, phosphate, sodium and potassium concentrations in sheep

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ABSTRACT

The present study was undertaken to have a better understanding about the mineral and electrolyte alterations in the plasma and saliva associated with magnesium infusion. Five experiments were conducted in 3 trained sheep, each with a large rumen cannula. The total collection of saliva was done by slipping a salivary collector at the base of the oesophagus. After 60 min of stabilization, a loading dose of 15 ml magnesium sulphate (25%, w/v) was given intravenously and this solution subsequently infused for 120 min @ 12 ml/hr with the help of an infusion pump. Hypophosphataemia and to some extent hypocalcaemia were observed which may be because of decrease in the parathyroid hormone and increased level of calcitonin in the circulation. In the saliva, the salivary Na: K ratio showed a significant increase following magnesium sulphate infusion. The result of present study has important implications in the treatment of hypomagnesaemia.

Key words: Aldosterone, Calcium, Hypermagnesaemia, Magnesium, Parathormone, Phosphorus

Ruminants are susceptible to a disturbance accompanied by low plasma magnesium known as grass tetany, hypomagnesaemic tetany, grass staggers, lactation tetany, wheat pasture poisoning, and winter tetany (Fontenot *et al.* 1989). This metabolic deficiency of magnesium may be due to a low level of magnesium in the feed or an impairment in utilization of dietary magnesium. The objective of this study was to determine the effect of intravenous magnesium sulphate on the above mentioned electrolytes in the blood and saliva of sheep.

MATERIALS AND METHODS

Adult sheep (N=3) weighing 60-70 kg were prepared with large rumen fistulas. At least 6 weeks before the experiments the fistulas were established under halothane oxygen anaesthesia following induction by an intravenous injection of thiopentone. The fistula was closed with a removable rubber cannula. They were given daily 1.0 kg of feed and straw for feeding. No extra supplementation with common salt was provided.

The fistulated sheep were trained for collection of saliva with the help of a salivary collector and isolated rumen wash technique was used as described by Martens and Rayssigiuer (1980). This involved restraining the conscious sheep by a loose neck yoke and removing the rumen cannula and rumen contents. The salivary collector was slipped at the base of the oesophagus through the rumen cannula. The oesophageal cuff was connected to a nasogastric tube, which was anchored at the nasal end once the cuff was situated in the appropriate place (80-100 mm from the distal end of the oesophagus). Air was allowed to bleed into the lower esophagus to prevent it from collapsing and occluding the outflow of saliva. The rumen contents were returned to the rumen. After the completion of the experiment, the salivary collector was removed and the sheep were fed, and they consumed food and water readily.

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Experimental protocol

After 60 min of stabilization, a loading dose of 15 ml magnesium sulphate (25%, w/v) was given intravenously and this solution subsequently infused for 2 hr @ 12 ml/hr with the help of an infusion pump. Saliva and blood samples were taken every 30 min during the infusion period and for 2 hr after the cessation of infusion. After taking a saliva sample, the rest of the saliva was immediately returned to the rumen.

Analysis

Magnesium and calcium concentrations were determined by atomic absorption spectrophotometry. Ionized calcium was determined immediately after taking the blood sample under anaerobic conditions using a calcium ion selective. Inorganic phosphorus was estimated by autoanalyser using a calorimetric method. Sodium and potassium concentrations were determined by flame photometry. The pH of the saliva was monitored by digital ion analyzer.

RESULTS AND DISCUSSION

Changes in plasma and saliva sodium, potassium and phosphate started appearing after about half an hour from the start of infusion and began to reverse after about 1 hr from the end of the infusion period (Table 1). Infusion of magnesium sulphate resulted in about 2.5-fold increase in the plasma magnesium concentration. Magnesium and calcium are equally potent inhibitors of PTH secretion (Targovink *et al.* 1971). The hyperphosphataemia observed may be due to the decreased PTH secretion in the circulation as PTH infusion causes hypophosphataemia (Dua 1992). Experimentally induced hypermagnesaemia induced calcitonin (CT) in pigs (Littledike and Arnaud 1971) and lowered plasma calcium concentration. They have also made similar observations in cattle. The decrease in the total calcium and ionized calcium observed in this study can also be attributed to the decreased PTH, increased CT and increased plasma phosphate concentration. This observation has an important implication in the treatment of clinical cases of hypomagnesaemia as hypocalcaemia coexists in most of the cases. It is suggested that in the treatment of hypomagnesaemia a combination of calcium and magensium is always better than magnesium alone. There are chances that while treating hypomagnesaemia we may end up in hypocalcaemia.

Table 1. Effect of infusing magnesium sulphate (25%, w/v; 15 ml loading dose and 12 ml/hr for 2 hr) on saliva and plasmamagnesium, calcium (total, Ca and ionizable Ca^{2+}), sodium, potassium and phosphate concentration (mmol/l) along with salivary pH and salivary flow rate (ml/min) in sheep (N=3, n=5). (mean±SEM).

		Before infusion	During infusion	1 hr after end of infusion
Mg	Saliva	0.26±0.02	0.26±0.017	0.23±0.01
	Plasma	0.89±0.09	2.41±0.17*	1.53±0.10*
Ca	Saliva	0.38±0.26	0.37±0.03	0.34±0.02
	Plasma	2.6±0.04	2.56±0.06	2.52±0.05
Ca^{2+}	Plasma	1.45±0.05	1.35±0.04	1.32±0.02*
Na	Saliva	130±5.56	143±6.5	141±4.6
	Plasma	148±1.9	155±2.6	156±2.2*
K	Saliva	23.5±4.1	12.6±2.1*	8.95±1.24*
	Plasma	4.35±0.2	3.68±0.18	4.48±0.19
PO_4	Saliva	12.6±1.09	23.1±2.84*	17.6±2.8*
	Plasma	1.05±0.05	1.42±0.04*	1.26±0.14*
Salivary Na: K		5.53	11.35	15.75
Salivary pH		8.75±0.02	8.84±0.02*	8.83±0.02*
Salivary flow rate		14.7±2.1	9.82±0.79*	12.02±0.89

*Significantly ($P<0.05$) different from before infusion; N=number of sheep; n=number of experiments.

During the infusion of magnesium sulphate plasma sodium started increasing whereas plasma potassium started decreasing resulting in increase in the plasma Na: K ratio. The exact reason for this is not clear. This increased Na: K ratio has inhibitory effect on the release of aldosterone in the circulation. It may be due to this reason that there was improvement in the salivary Na: K ratio from 5.5 to 15.75. This ratio looked like that of sodium replete i.e. sufficient amount of sodium intake is there.

Keeping in view the above observations, we can think of the mechanism of the tetany caused by hypomagnesaemia. During magnesium deficiency, there is decreased plasma Na: K ratio resulting in stimulation of renin-angiotensin and the aldosterone. Since angiotensin is vasoconstrictive, it may have some role in the tetany by increasing the muscle tension. When magnesium is infused to treat hypomagnesaemia, the inhibiton of the renin angiotensin may have a role in the muscle relaxation. It is worthwhile to investigate the role of renin angiotensin system in the tetany caused by hypomagnesaemia in the ruminants.

Hyperphosphataemia observed following magnesium sulphate infusion indicates against the use of phosphorus in the treatment of hypomagnesaemia. Increase in salivary phosphate concentration observed in this study could have been because of increased load and the delivery rate in the arterial plasma (Wright *et al.* 1984). The increased salivary phosphate concentrations in the saliva may have a role in the increased calcium and magnesium absorption from the forestomach of the ruminants (Dua and Care 1999).

Salivary magnesium concentration was not changed immediately following magnesium sulphate infusion because of the lag period of 6-8 hr (Dua and Care 1998), which rules out the possibility of any immediate role of salivary magnesium in the treatment of the hypomagnesaemic tetany. The decrease in the salivary flow rate observed following magnesium sulphate infusion was probably because of the decreased circulatory PTH (Clark *et al.* 1975).

It is concluded that with the intravenous infusion of magnesium sulphate in the healthy sheep there was hypophosphtaemia and to some extent hypocalcaemia which may be due to decrease in the PTH level in the circulation. The increased salivary Na: K ratio following magnesium sulphate infusion could be because of reduced aldosterone concentration. The results of this experiment have important bearing on the treatment of hypomagnesaemia.

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Preparation of eye balls of rat and mice for histopathological examination in toxicological studies

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ABSTRACT

A study was conducted to standardize the procedure for preparation of eye balls of rats and mice, for histopathological evaluation in toxicological studies, using 10% neutral buffered formalin and Davidson's fluid. Davidson's fluid proved to be a good fixative, but prolonged fixation in the same, hardened the lenses. Formalin fixation hardened the lenses to a greater extent and thus resulted in artefactual changes. Better results were obtained with chloroform, as a clearing agent, when compared to xylene. More than 95% results were attained with shorter duration of fixation in Davidson's fluid, along with use of chloroform as a clearing agent during processing.

Key words: Eye ball, Fixative, Davidson's fluid

Toxicologic pathology is gaining more and more importance day by day, especially because of increasing emphasis on the regulatory aspects. Techniques to prepare various tissue slides for histopathological evaluation have been standardized. However, it is very difficult to get good eye sections with lens intact and without any artefacts. There are about 2 800 substances that have some type of untoward effect on ocular tissue (Schiavo 1990). In such a situation, it is very important that a pathologist gets good sections of eyes, for proper interpretation of the findings. Hence the present study was taken up to standardize a procedure for the preparation of eye balls for histopathological evaluation.

MATERIALS AND METHODS

Eyes from in-house bred Wistar rats and Harlan mice formed the material for the study. Five groups were formed in Table 1.

Eyes were dissected out from the orbit along with the optic nerve. Neutral buffered formalin (10%) and Davidson's fluids were used for fixation. Durations of fixation were as mentioned in Table 1. In group 5, xylene was used instead of chloroform as a clearing agent.

Trimming

After fixation, eyes from Davidson's fluid were transferred to 50% ethanol and left overnight. Eyes were trimmed longitudinally at about the level of the optic nerve. Care was taken to slowly saw through the lens, avoiding any mechanical damage to other parts.

Table 1. Duration of fixation

Groups	Number of eyes	Fixative	Fixation time (hr)
1	60	10% neutral buffered formalin	48
2	60	Davidson's fluid	18
3	60	Davidson's fluid	08
4	60	Davidson's fluid	04
5	60	Davidson's fluid	04

Out of 60 eyes, 30 were from rats and 30 from mice.

Processing

The procedure in Table 2 was followed using auto processor.

All the reagents were maintained at ambient temperature. Paraffin infiltration was carried out using paraffin wax maintained at 58°C, under pressure and vacuum.

Embedding

Routine paraffin embedding was performed. Eyes were blocked with the cut side facing down and the optic nerve was slightly pressed to achieve proper orientation.

Sectioning and staining

Sections (5μ) were cut and stained routinely with haematoxylin and eosin.

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Table 2. Procedure followed using auto processor

Reagent	Time (hr)
Ethanol 70%	00:30
Ethanol 80%	00:20
Ethanol 95%	00:20
Ethanol 95%	00:20
Absolute alcohol	00:20
Absolute alcohol	00:20
Chloroform	00:20
Chloroform	00:20
Chloroform	00:20
Paraffin wax	00:30
Paraffin wax	00:30
paraffin wax	00:30

RESULTS AND DISCUSSION

In the eyes from group 1, where 10% neutral buffered formalin was used for fixation, the lens could not be sectioned intact, along with the other structures. Formalin has been reported to be a potentially good fixative for cornea and lens by Duncan (1981). However, in this study, formalin fixation of the eyes hardened the lenses and made the cutting of sections in this group very difficult. Artifactual retinal detachment has been reported by Saunders and Rubin (1975) in formalin fixed eye balls. In the present study also, artifactual changes were observed in retina, cornea and sclera. These changes were probably because of mechanical damage, caused by the hardened lenses, during sectioning. Davidson's fluid has been described as a good fixative for all ocular structures, especially lens and retina (Render and Chemielewski 1992). Likewise, in the present study also, it proved to be a very good fixative for the ocular structures. However, the duration of fixation appeared to be a decisive factor. Results were not satisfactory in groups 2 and 3,where longer durations of fixation were employed. Such long fixation periods resulted in hardening of lenses and hardly any sections could be taken without any artefactual changes. Eye sections were judged by the presence of all structures without any artefactual changes. In group 5, where xylene was used instead of chloroform as a clearing agent, 70% results were obtained. Use of xylene resulted in brittleness and hardening of lenses, because of which artefactual changes were present in eye sections. In group 4, sectioning was much easier, there were minimal artefactual changes and more than 95% results were achieved. This was mainly due to shorter duration of fixation and use of chloroform for clearing. From the present study it was concluded that Davidson's fluid is the fixative of choice for rat and mice eye balls and the tissues should be fixed for a duration of 4 hr, and chloroform must be used as a clearing agent during processing.

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Effect of diclofenac and nimesulide on haematology in dogs

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ABSTRACT

Diclofenac sodium and nimesulide were given @ 3 mg/kg twice daily for 4 days and 2 mg/kg twice daily for 4 days, respectively, orally to dogs (n=4). Among the different haematological parameters assessed, Hb, PCV, TLC and DLC values did not differ significantly when compared with the control group. Although the TEC values in both the diclofenac and nimesulide groups decreased during the course of experiment, the decrease was significant only in the nimesulide group at 96 hr. Hence, the 2 drugs in general did not affect the haematological parameters in dogs.

Key words: Diclofenac, Nimesulide, Haematology

Anti-inflammatory drugs, particularly nonsteroidal anti-inflammatory drugs (NSAIDs) were used to counteract acute and chronic inflammatory conditions. The information on the effects of diclofenac and nimesulide on haematological parameters are scarce (Payen and Shearn 1989).

Hence, the present study was undertaken to elucidate any adverse effects of these drugs on various haematological parameters after administering diclofenac and nimesulide in the dogs.

MATERIALS AND METHODS

Healthy mongrel dogs (12) of about 2 years old with a body weight ranging from 11 to 14 kg were used. They were fed and maintained under standard laboratory conditions. The dogs were kept for 15 days in the laboratory for acclimatization. Diclofenac sodium and nimesulide tablets were used in the present study.

The dogs were divided into 3 groups of 4 dogs comprising equal number of males and females. Group 1 served as control, group 2 was administered diclofenac sodium at 3 mg/kg orally twice daily for 4 days and group 3 was administered nimesulide at 2 mg/kg orally twice daily for 4 days. This dose is also employed in humans for therapeutic purposes. Blood samples were collected from femoral vein before administration of the drugs i.e., at 0 hr and at 6, 12, 24, 48, 72 and 96 hr after the administration of first doses of the drugs using disodium salt of EDTA as the anticoagulant. The samples were stored at –4°C till the parameters were estimated. Following haematological parameters were estimated: haemoglobin (Hb) by Sahli's acid hematin method (Coles 1967), packed-cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC) and differential leukocyte count (DLC) by methods of Benjamin (1985). The results were subjected to Student's 't' test as per Snedecor and Cochran (1976).

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RESULTS AND DISCUSSION

The haematological observations on Hb, TEC, TLC, PCV and DLC have been tabulated (Tables 1-3). The haemoglobin (Hb, g/dl) values in the diclofenac and the nimesulide groups did not differ significantly and ranged from 10.55 ± 0.98 to 11.5 ± 1.19 and from 10.65 ± 0.88 to 11.35 ± 0.57 in the diclofenac and nimesulide groups respectively (Table 1).

The TEC ($\times 10^6$/mm^3) values for the diclofenac group ranged from 4.22 ± 0.33 at 72 hr to 5.49 ± 0.47 at 0 hr. Although the values in the diclofenac group showed a gradual decrease, the decrease was statistically nonsignificant. In the nimesulide group, the values showed a decrease from 5.03 ± 0.46 at 0 hr to 4.57 ± 0.13 at 96 hr. This value was significantly lesser than that of control group (Table 1). The PCV (%) values did not change significantly over the 4 days. They ranged from 40.5 ± 1.55 to 42.5 ± 1.84 and from 39.5 ± 0.95 to 41.5 ± 0.5 in the diclofenac and nimesulide groups respectively (Table 2).

The TLC ($\times 10^3$/mm^3) values for both the diclofenac and nimesulide groups did not differ significantly from the control group. The values ranged from 10.15 ± 0.90 to 14.86 ± 0.82 and from 10.71 ± 0.97 to 13.01 ± 1.79 in diclofenac and

Table 1. Effect of diclofenac and nimesulide on haemoglobin concentration (Hb, g/dl) and total erythrocyte count (TEC,×10^6/mm^3) in dogs (n=4)

Time interval (hr)	Haemoglobin concentration			Total erythrocyte count		
	Control group 1	Diclofenac group 2	Nimesulide group 3	Control group 1	Diclofenac group 2	Nimesulide group 3
0	10.50±0.20	11.50±1.19	11.30±0.90	5.19±0.36	5.49±0.47	5.03±0.46
6	10.35±0.38	10.80±1.08	103.65±0.88	4.91±0.38	5.19±0.59	5.11±0.54
12	10.30±0.29	10.80±1.06	11.05±0.97	4.98±0.80	5.25±0.58	4.82±0.18
24	1.50±0.31	10.75±1.10	12.05±1.03	4.83±0.43	5.19±0.30	4.75±0.20
48	10.65±0.032	11.10±1.22	10.80±0.94	5.06±0.24	5.13±0.39	4.94±0.13
72	10.72±0.33	10.85±1.10	11.20±0.63	4.72±0.06	4.22±0.33	4.82±0.17
96	10.45±0.20	10.55±0.98	11.35±0.57	5.09±0.18	4.62±0.25	4.57±0.13*

Values are given as mean± S.E.M.; *P<0.05, significant.

nimesulide groups, respectively, thus showing a gradual increase (Table 3).

The differential leukocyte counts (DLC, %) have been presented in Table 3. The differential leukocyte counts did not differ significantly with respect to neutrophil, lymphocyte, monocyte or eosinophil counts in both of the treated groups when compared with the control group. The per cent neutrophil counts in the control group varied between 71 ± 0.70 and 74.7 ± 0.47, in the diclofenac group between 68.0 ± 2.58 and 73.2 ± 3.63 and in the nimesulide (oral) group between 71.0 ± 1.29 and 73.7 ± 0.85.

The lymphocyte counts did not alter significantly either in diclofenac or nimesulide group. The values in the diclofenac and nimesulide groups ranged between 26.0 ± 3.48 and 29.5 ± 2.9 8 and 24.5 ± 1.93 and 29.7 ± 1.88, respectively, as compared with the values in the control group which ranged between 24.5 ± 0.50 and 28.0 ± 0.81.

The per cent monocyte values in the control and diclofenac groups varied between 0.50 ± 0.28 and 1.25 ± 0.47 and 0.0 and 2.25 ± 0.25, respectively, and in nimesulide group between 0.5 ± 0.27 and 1.25 ± 0.25. The eosinophil counts in the control, diclofenac and in nimesulide groups varied between 0 ± 0 and 1.0 ± 0.4, 0.25 ± 0.25 and 1.25 ± 0.25 and 0.25 ± 0.25 and 1.25 ± 0.25 respectively.

The Hb, PCV values in the diclofenac and nimesulide groups did not change significantly over the treatment period of 4 days. These results are in accordance with a study where ibuprofen administered to dogs for 7 consecutive days did not produce any significant changes in haemoglobin and PCV values (Raut *et al.* 1995).

The TEC values showed a gradual decrease in both the diclofenac and nimesulide groups over the 4 days treatment period. The values did not change significantly in diclofenac group. However, in nimesulide group at 96 hr TEC decreased significantly (P<0.05).

These findings are similar to earlier findings on other NSAIDs. Aspirin was found to cause iron deficiency anaemia (Miller and Jick 1977) and was also reported to cause haemolytic anaemia (Necheles *et al.* 1970). Phenylbutazone has been reported to cause megaloblatic anaemia (Dukes 1980).

The TLC values showed a gradual increase during the

Table 2. Effect of diclofenac and nimesulide on packed-cell volume (PCV,%) and total leukocyte count (TLC,×10^3/mm^3) in dogs (n=4)

Time interval (hr)	Packed-cell volume			Total erythrocyte count		
	Control group 1	Diclofenac group 2	Nimesulide group 3	Control group 1	Diclofenac group 2	Nimesulide group 3
0	45.0±2.73	41.0±3.85	41.2±0.70	13.20±2.05	10.66±1.46	11.43±0.97
6	44.5±2.95	42.5±1.84	40.7±0.62	12.75±2.16	09.75±1.30	10.71±0.97
12	45.2±2.49	40.5±2.10	41.5±0.50	14.88±2.08	10.15±0.90	11.85±0.56
24	44.0±1.91	40.5±1.55	40.7±0.94	14.02±2.30	12.42±0.99	10.72±0.75
48	44.0±1.87	41.5±2.10	40.2±2.28	12.83±2.17	14.86±0.82	12.30±1.94
72	39.7±2.56	40.7±2.10	39.5±0.95	12.33±2.31	11.80±0.88	12.81±1.67
96	41.7±2.25	41.5±2.25	41.0±1.22	12.89±1.91	12.63±0.65	13.01±1.79

Values are given as mean± S.E.M.; P<0.05

Table 3. Effect of diclofenac and nimesulide on differential leukocyte count (DLC,%) in dogs (n=4)

Time hour	Control (group 1)				Diclofenac (group 2)				Nimesulide (group 3)			
	Neutrophil	Lymphocyte	Monocyte	Eosinophil	Neutrophil	Lymphocyte	Monocyte	Eosinophil	Neutrophil	Lymphocyte	Monocyte	Eosinophil
0	72.5±2.10	25.7±1.88	0.75±0.47	1.00±0.40	70.5±2.62	27.2±1.79	225±0.25	1.00±0.57	71.5±3.56	27.5±3.79	0.75±0.25	0.50±0.28
6	72.0±0.40	26.5±0.28	1.00±0.00	0.50±0.28	73.2±3.63	27.5±0.64	1.50±0.64	0.25±0.25	73.7±3.50	26.2±1.31	0.50±0.28	0.50±0.28
12	71.0±0.70	28.0±0.81	0.75±0.25	0.25±0.25	68.7±2.59	28.7±2.35	2.00±0.81	0.75±0.47	72.0±2.16	29.7±1.88	0.50±0.27	0.25±0.25
24	74.7±0.47	24.5±0.50	0.50±0.28	0.00±0.00	71.0±2.64	27.5±2.39	0.75±0.26	0.75±0.25	71.5±2.72	26.5±2.72	1.25±0.25	0.75±0.25
48	71.5±1.55	26.2±0.50	1.25±0.47	1.00±0.57	72.2±1.70	27.7±1.43	0.00±0.00	0.25±0.25	71.0±1.29	27.5±0.95	1.00±0.40	0.50±0.28
72	71.2±0.85	27.2±0.85	1.00±0.00	0.50±0.28	68.0±2.58	29.5±2.98	1.50±0.28	1.25±0.25	73.7±1.75	24.5±1.93	1.25±0.62	1.25±0.25
96	72.5±0.50	25.7±0.85	1.00±0.40	0.75±0.25	72.7±3.42	26.0±3.48	0.50±0.28	0.50±0.28	73.7±0.85	25.0±1.22	1.00±0.00	0.50±0.28

Values are given as mean ±S.E.M.; P>0.05.

course of treatment but the increase was not significant. Similarly the neutrophil lymphocyte, monocyte and eosinophil counts did not differ significantly in both the treated groups over the 4 days treatment period. Although agranulocytosis has been reported in a few cases of NSAID therapy (Cuthbert 1974) and risks of leukaemia with phenylbutazone has been reported (Leavseley *et al.* 1969). The differential leukocyte counts did not differ significantly with respect to neutrophil, lymphocyte, monocyte or eosinophil counts in both the treated groups when compared with the control group, although there are reports of agranulocytosis in a few cases of NSAID therapy (Cuthbert 1974). Therefore, these 2 drugs in general did not affect the haematological parameters in dogs.

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Histoenzymic distribution of alkaline and acid phosphatases in the ovary of buffalo (*Bubalus bubalis*) with age

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ABSTRACT

The study was conducted on the ovaries of 46 (neonatal 13, prepubertal 3, cyclic 22,and pregnant 8) normal healthy Murrah female buffaloes. The fresh ovarian tissues were collected in the liquid nitrogen for the histoenzymic demonstration of AKPase and ACPase. The tissues were put to cryostat at –20°C to obtain sections at 10 µm on clean glass slides and incubated for phosphatases activity. Histoenzymic study revealed high contents of AKPase and ACPase in primordial, primary secondary and Graafian follicles as compared to atretic follicles. The luteal and interstitial gland cells had high contents of AKPase and ACPase activity.

Key words: ACPase, AKPase, Buffalo, Ovary

The buffalo is known for the poor reproductive performance such as silent heat, low conception rate and long calving interval thus causing heavy economical losses to the farmers. Buffalo ovary is characterized by low number of primordial follicle reserve and high incidence of follicular atresia to the extent of approximate 65% (Guraya 1979, Danell 1987, Deloos *et al.* 1991,Driancourt *et al.* 1991,Bhardwaj and Roy 1999). It has been suggested that low turnover of the Graafian follicle and high frequency of atresia may affect hormone production which, in turn, leads to low reproductive efficiency in buffaloes as compared to that of cattle (Danell 1987, Driancourt *et al.* 1991). However, details of age related histoenzymological studies on ovarian follicles during postnatal development, estrus cycle and pregnancy are scanty and no systematic studies have been reported on buffalo ovary. Hence to understand the follicular development and atresia during postnatal development, knowledge of estrus cycle and pregnancy is prerequisite for the improvement of buffalo production and reproduction. The present study is aimed to elucidate the histoenzymic distribution of AKPase and ACPase in the ovary of buffalo with age.

MATERIALS AND METHODS

The study was conducted on 46 normal healthy Murrah female buffaloes. The details of buffaloes used in studies are given in the Table 1.

Table 1. Particulars of buffaloes used in the study

Sr. No.	Group	Age	Sexual condition	No.of animals	Source
1.	Neonatal	Birth to 1 month	–	13	Ludhiana Dairy complex
2.	Prepubertal	1-3 Years	–	3	Saharanpur abattoir and PAU, Ludhiana Dairy farm
3	Adult	4-10 Years	(a) Cyclic buffalo	22	Saharanpur abattoir and Ludhiana dairy complex
			(b) Pregnant buffalo	8	Saharanpur abattoir and Ludhiana dairy complex

The fresh unfixed ovarian tissues were collected in liquid nitrogen for histoenzymic demonstration of AKPase and ACPase enzymes. The unfixed frozen tissues were put to

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cryostat microtome at –20°C to obtain sections at 10 µm on clean glass slides and were incubated for AKPase and ACPase by Azo dye method (Barka and Anderson 1963).

RESULTS AND DISCUSSION

The results of activities of alkaline phosphatase and acid phosphatase activities in the various components of ovary and follicles in four groups under study have been recorded in the Table 2.

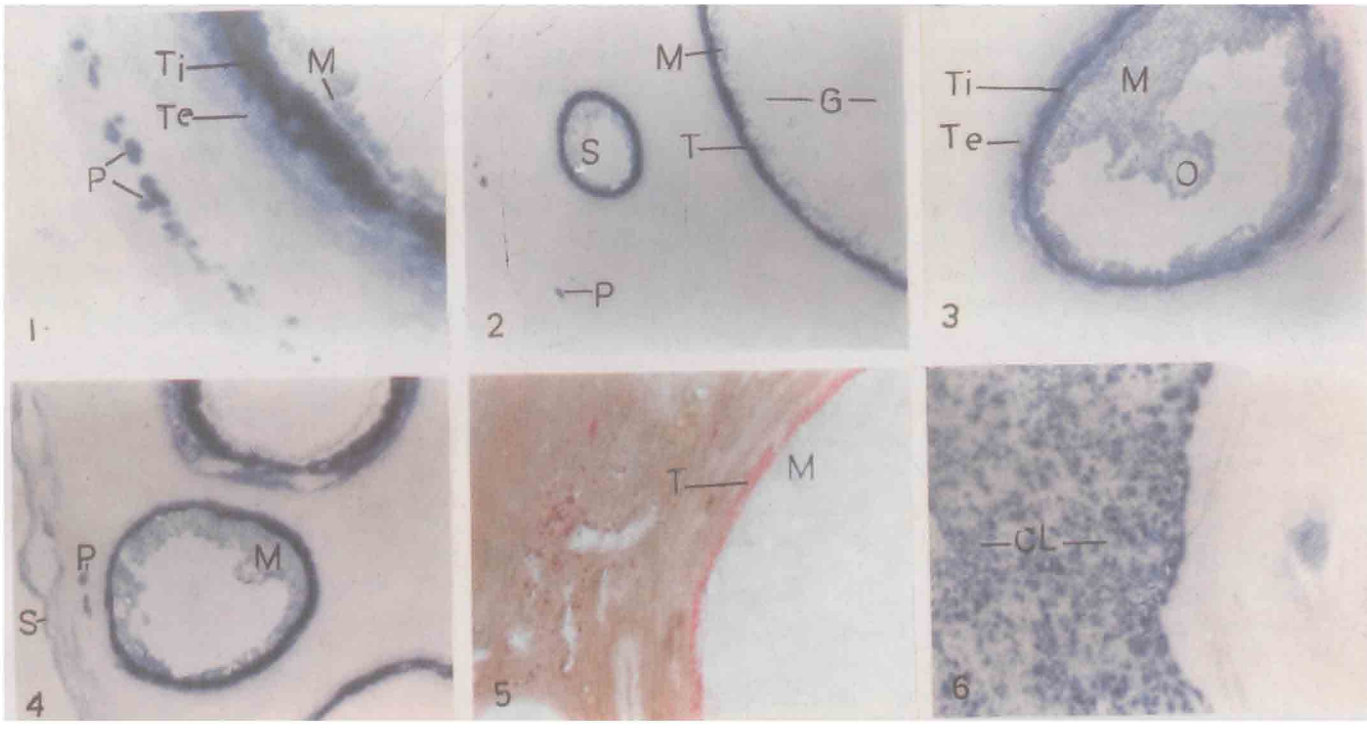
Alkaline phosphatase

The AKPase activity was not appreciable in the surface epithelium, tunica albuginea and ovarian cortex. The primordial and primary follicles showed strong to intense AKPase activity in the all age groups (Figs 1, 2, 3, 4). The activity of AKPase increased in growth and differentiation of follicle and synthesis and transport of substances across the cell walls (Lehniger 1985). The AKPase activity in the theca cells varied from moderate to intense in theca interna all the stages of follicular development in buffalo ovary (Fig. 3). It had decreasing trend during follicular atresia. A high AKPase activity was also reported in theca cells (Nakama 1969). The activity localized in the theca interna indicates the active transport of ions and secretary material across their cellular membrane (Verma and Guraya 1968, Sangha and Guraya 1989).

The AKPase activity in the membrana granulosa was moderate to strong in all types of follicles.However, there was decreasing trend of activity in the atretic follicles (Figs 3, 4). Growing follicles of neonatal animals showed moderate to intense activity in the follicular cells as reported in bovine (Nakama 1969), sheep (Roy and Saigal 1986) and goat (Bhattacharya and Saigal 1990). The AKPase probably plays a role in the metabolism of phosphate esters (Sangha and Guraya 1989). The activity was weak in the zona pellucida and oocyte (Fig.3).

The interstitial gland cells showed moderate to strong activity. The intensity of activity was decreasing with the progression of age.The enzyme is apparently involved in the steroid metabolism and transport process (Brietenecker *et al.* 1978).

The AKPase activity was uniformly strong in the ovarian capillaries in all age groups studied. This supports the earlier



Figs 1-6. **1.** Cryostat section of ovary of: Neonatal buffalo calf showing alkaline phosphatases activity in primordial follicle (P). The wall of graafian follicle had varied activity in membrana granulosa (M). Theca interna (Ti) and theca externa (Te). Azo dye for AKPase. ×35. **2.** Prepubertal calf showing AKPase activity in primordial follicle (P) and secondary follicle (S). The wall of graafian (G) follicle showed varied activity in theca (T) and membrana granulosa cells. Azo dye for AKPase. ×35. **3.** Cyclic buffalo showing activity in oocyte (O), membrana granulosa (M), theca interna (Ti) and Theca externa (Te).Azo dye for AKPase. × 35. **4.** Pregnant buffalo showing activity in surface epithelium (S), primordial follicle (P), membrana granulosa (M) and theca cells of secondary follicle. Azo dye for AKPase. × 35. **5.** Pregnant buffalo showing acid phosphatase activity in membrana granulose (M) and theca cell (T) in large atretic follicle.Azo dye for ACPase. × 35. **6.** Pregnant buffalo showing AKPase activity in luteal cell (CL). Azo dye for AKPase. × 35.

findings of Roy and Saigal (1986) in sheep, required for absorption and transport of ions across the wall of capillaries (Fernley 1971). There was moderate activity of AKPase in the rete ovarii of the ovary of prepubertal onward groups and strong activity in the neonatal. It seems that rete ovarii had some secretary activity in the neonatal groups. However, Goswami (1985) reported weak activity in the rete ovarii of buffalo ovary. Weak to moderate activity for AKPase was observed in the ovarian hilar gland cells of cyclic and pregnant buffaloes. However, Goswami (1985) had reported high concentration of AKPase activity may be related with steroidogenic activity particularly androgen (Harrison and Wier 1977).

The AKPase activity was moderate in corpus luteum spurium and strong to intense in corpus luteum verum (Fig. 6). This supports the earlier findings in cow (Boos *et al.*1988) and buffalo (Singh 1994). The higher AKPase activity of the luteal cells reflected higher secretary and proliferative activity in the luteal cells during formation in cyclic and pregnant buffaloes.

Acid phosphatase

A very mild ACPase activity was observed in the surface epithelium, tunica albugenia and ovarian stroma in ovaries of all the age groups studied. There was weak to moderate activity in the primordial and primary follicles.The activity was weak to moderate in the theca and membrana granulosa cells, mild in zona pellucida and oocyte (Fig.5). This supports the earlier findings of Singh (1993) in the ovarian follicles of buffalo. The distribution of ACPase activity in the theca growing follicles suggests active role of lysosomes in the steroidogenesis (Elfont *et al.* 1977). ACPase is essentially a lysosomal enzyme (Elfont *et al.* 1977).Which increased particularly during atresia of oocyte.

The activity was weak to moderate in the interstitial gland cells. It increased with the progression of age and was maximum in the cyclic group as reported in sheep (Roy and Saigal 1986) and goat (Bhattacharya and Saigal 1990). The activity was possibly associated with steroidogenesis (Takenaka *et al.*1980, Bhattacharya 1980).

ACPase activity in the ovarian blood vessels was negligible. This supports the earlier findings in sheep (Roy and Saigal 1986). The ACPase activity was moderate in the rete ovarii of neonatal group and weak in other groups. Goswami (1985) also observed mild phosphatase activity in the rete ovarii of buffalo ovary. ACPase contents in rete ovarii reflect its participation in hormone secretion. There was weak ACPase activity in the hilar gland cells of buffalo ovary in adult animals. The activity indicated their possible role in steroidogeneses (Elfont *et al.* 1977, Narimoto *et al.* 1985). There was intense ACPase activity in the luteal cells both in the cyclic and pregnant buffalo ovaries, supports the earlier findings of Singh (1994) in buffalo. A strong ACPase activity in luteal cells is related with synthesis and secretion of progesterone (Boos *et al.* 1984, Gasse *et al.* 1984).

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Neutralizing antibodies to peste-des-petits ruminants virus in small ruminants in Andhra Pradesh—A serological survey

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Peste-des-petits ruminants (PPR), an infectious viral disease of sheep and goats is characterized by pyrexia, necrotic stomatitis, diarrhoea and pneumonia. Sheep and goats are at present the only known natural host of the disease. Although cattle and pigs develop serum neutralizing antibodies following experimental PPR virus infection, they are not susceptible to clinical disease (Gibbs *et al.* 1979). The epidemeology of PPR virus is not fully understood (Appel *et al.* 1981) and detailed account of the epidemeology of PPR virus was given by Taylor (1984) who reported that PPR virus circulate in a belt lying across immediately after South of Sahara and Arabian peninsula. During the period 1989-1994, several outbreaks of PPR in sheep were recognized in the Southern states of peninsular India. In each instance, the isolate was confirmed PPR virus by differential nucleic acid hybridization and virus neutralization tests (Shaila *et al.* 1989, 1990). PPR outbreak occurred in sheep in February 1992 in Andhra Pradesh (Subba Rao and Shaila unpublished). Serological investigations were recognized as a faster means of assessing the prevalence of viral infections. The sero-survey spread over different geographical areas would help in understanding the distribution and epidemeology of the disease which would help to contain the malady. So far, no systematic survey has been undertaken in this country to know the extent of the spread of the disease in sheep and goat population.

In view of the paucity of information on the prevalence of PPR virus in India, it was envisaged to carry out a serological survey in small ruminants in Andhra Pradesh as this state had a recent outbreak.

Serum samples (672) were collected from small ruminants from organized farms (155), villages and abattoirs (517) of different districts of Andhra Pradesh. Of these samples 556 were from sheep and 116 from goats. Ninety of these serum samples were collected prior to PPR outbreak in the state. Of these 90 samples, 66 were from sheep and 24 from goats. The sheep belonged to Nellore, Zodpae and Nellore synthetic (Nellore Dorset) breeds.

PPR virus used in this study was an Indian isolate, PPR Ind TN 87/1 which had undergone 14 passages in vero cells and the rinderpest virus (RPV) was the attenuated derivative of the Kebete 'O' strain (RBOK) which had received 24 passages in vero cells. The hyperimmune serum to PPR virus (Ind TN 87/1 strain) and RP virus (RBOK strain) was raised in 2 Indian goats.

Serum samples (672) were analyzed simultaneously using microneutralization test for the presence of neutralizing antibody to PPR virus and RP virus (Rossiter *et al.* 1985). All sera were initially tested for neutralizing antibody to PPR virus at a dilution of 1: 10. All the positive serum samples in the screening test for either virus (PPR or RP) were titrated for neutralizing antibody. The cells were observed daily till complete monolayer formed in cell controls, whereas, virus controls showed complete CPE.

The serum dilutions in which the wells contained 50% and above monolayers were considered to contain neutralizing antibody. The 50% neutralizing end points were calculated using Reed and Muench method (1938). The titres were expressed as VN50 in $\log_{10}$ units.

The sheep serum samples collected, 256 were from Rayalaseema, 116 from Teleangana and 184 from Coastal regions (Table 1). PPR virus neutralizing antibody was observed in 11 (4.30%) and 9 (7.76%) of sheep serum samples of Rayalaseema and Teleangana regions respectively. None of the sheep serum samples from coastal region were positive to PPR virus. None of the 116 goat serum samples from any region, neutralized PPR virus.

An assessment was also made of the cross - reacting antibodies to PPR virus and RP virus in sheep serum samples (556). Goat serum samples (116) were not taken into consideration because none of the goat samples showed

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Table 1. The prevalence of neutralizing antibody to PPR virus in sheep and goat in different regions of Andhra Pradesh

Species	Neutralizing antibody to PPR virus in						Total	
	Rayalseema	Per cent	Telangana	Per cent	Coastal	Per cent	Positive	Per cent
Sheep	11/256	4.30	9/116	7.76	0/184	0.0	20/556	3.6
Goat	0/44	0.0	0/19	0.0	0/53	0.0	0/116	0.0
Total	11/300*	3.67	9/135*	6.67	0/237*	0.0	20/672*	2.98

*Total positive/total samples tested.

neutralizing antibody to PPR virus. Of the 163 sheep serum samples which were positive in screening test to PPR virus and RP virus, 5 samples were positive to PPR virus alone (3.07%) and the rest 15 (9.20%) showed higher titers to PPR virus (1.6 to 2.28 $\log_{10}$ VN_{50}) and lower titers of RP virus (1.20 to 1.5 $\log_{10}$ VN_{50}). None of the goat serum samples was positive to PPR virus antibodies. Of the 556 sheep serum samples, 43 (25.72%) neutralized RP virus with titers ranging from 2.1 to 2.68 $\log^{10}$ VN_{50}. Of these, 143 sheep serum samples which were positive to RP virus 122 (74.85%) samples neutralized RP virus alone while the remaining 21 (12.88%) cross neutralized PPR virus with lower antibody titers (1.4 to 2.0 $\log^{10}$ VN_{50}).

In this study, the serological survey was carried using differential micro neutralization test with PPR virus and RP virus (Taylor 1979, Rossiter *et al.* 1985). Of the 672 serum samples examined from small ruminants in Andhra Pradesh, PPR virus neutralizing antibody was not detected in goats. On the other hand 20 (3.60%) out of 556 samples from sheep were positive to PPR virus. The overall prevalence of PPR virus in small ruminants in Andhra Pradesh was 2.98%. However no comparable results are available from India on the prevalence of PPR as this is the first study in this country. On the contrary, PPR virus is quite prevalent and confined to goats in Africa and the sheep seems to be very resistant (Lefevre and Diallo 1990). Taylor *et al.* (1990) did not observe PPR among sheep in the Middle east, although they seem to be infected with the virus.

The results of the analysis of the cross - reacting neutralizing antibody to PPR virus and / or RP virus in sheep showed that 5 (3.07%) of the samples neutralized PPR virus alone and 112 (74.85%) neutralized RP virus alone. In addition, 15 (9.20%) showed higher titers to PPR virus and low titers to RP virus, whereas 21 (12.88%) showed lower titers to PPR virus as compared to RPV. Out of the 556 sheep serum samples 122 samples were positive to RP virus alone (21.94%). In addition, 21 samples (3.78%) showed cross - reactivity with high RP virus and lower PPR virus antibody titers. However, this type of cross - reactivity with PPR virus were observed only in the outbreak area in and around Palamaner and in Nalgonda and Nizamabad districts wherein there seems to be an outbreak of PPR in neighbouring Maharastra state (Shaila personal communication). For example, the whole of coastal area wherein, not even a single sample was positive to PPR virus none of the RPV virus positive serum samples from coastal area reacted to PPR virus even in low titers.

In conclusion, the present investigation demonstrated that PPR virus was active in the state in Rayalaseema and Teleangana areas. These results also points to the fact that the endemic of RP in small ruminants has to be viewed carefully while interpreting the serological data on PPR. Further, serological data should be obtained on a larger scale both in the state and country to obtain more meaningful information. Attempts should also be made to isolate and identify PPR virus and to assess the geographical distribution and the economic importance of this disease in India.

SUMMARY

These studies were carried out using differential neutralization tests with PPRV and RPV. An assessment was also made of the cross reacting antibodies to PPRV and RPV in small ruminants. Serum samples (672) were collected from various districts of Andhra Pradesh. Of these 556 were from sheep and 116 from goat. They were analyzed, simultaneously for the presence of neutralizing antibody to PPRV and RPV. Of 556 sheep serum samples, 20 (3.60%) reacted to PPRV. Of these 20 positive cases, 5 (3.07%) samples were positive to PPRV alone and the rest 15 (9.20%) showed higher titres of PPRV and lower titres of RPV. None of the goat samples was positive to PPRV antibodies.

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Humoral immune response in rabbits against killed *Mycoplasma* vaccine

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Key words: Agglutinating antibodies, Precipitating antibodies, Rabbits

The present study deals with the humoral immunity in rabbits vaccinated with *Mycoplasma mycoides* subsp. *mycoides* LC (MmmLC) killed vaccine.

For preparation of vaccine an actively growing culture of MmmLC was inoculated into large quantity of MBHS-L medium (Carmichael *et al.* 1972) and incubated at 37°C for 2-3 days for large growth. It was centrifuged at 10 000 rpm for 20 min and washed thrice with PBS pH 7.2. The cells were then sonicated at 10 μ for 10 cycles of 2 min each duration in MSE-soniprep 150. The protein concentration was estimated according to Lowry *et al.* (1951). A single dose of vaccine for rabbit was prepared by mixing 0.5 mg of the above sonicated cell with 0.5 mg of sterile saponin in 0.5 ml PBS. The vaccine was tested for sterility in Robertson's cooked meat medium, blood agar and sabauraud's dextrose agar and incubated at 37°C for 2-3 days. The vaccine was inoculated in 8 rabbits with 0.5 ml dose through S/C route at the back of each rabbit. A booster inoculation was also done with the same dose and route after 28th day PI. The sera samples were collected from each rabbit on day 0, 3, 7, 14, 21, 28 and week 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 PI. The serological tests, viz. slide agglutination test (SAT) and latex agglutination test (LAT) were performed (Srivastava *et al.* 1991). The double immunodiffusion (DID) was conducted according to Lemcke (1964) as adopted by Srivastava (1982).

No antibodies were detected on day 0 (preimmunized) sera of rabbits, indicating that rabbits were free from antibodies to MmmLC. The agglutinating antibodies appeared from seventh day onwards and the maximum response was recorded between 6-8 week PI in both LAT and SAT. The peak response of 3.5 mean titre was obtained at eighth week PI in SAT, while 3.75 mean titre was obtained at sixth week PI in LAT (Fig. 1). The antibodies then declined and disappeared after 22 week PI.

The antibody response in vaccinated rabbits was detected as early as on seventh day PI by all the 3 tests. Although there was not much significant difference in detecting

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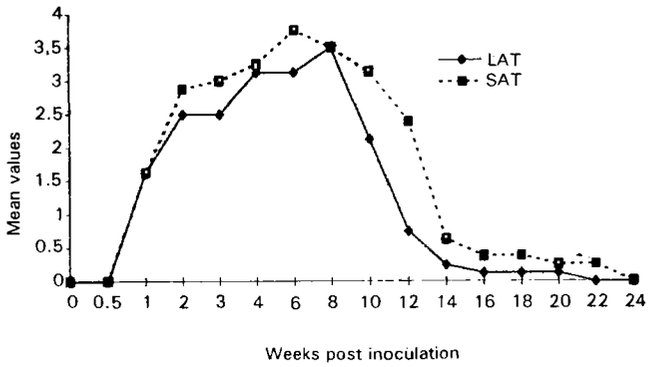


Fig 1. Slide agglutination and latex agglutination values in vaccinated rabbits.

antibodies by LAT and SAT, the former elicited a better response continuously from 1st to 22nd week (Fig.1). Rana and Srivastava (1996) monitored immune response in rabbits vaccinated with *M. mycoides* subsp. *capri* saponin vaccine and observed antibodies by LAT from 3rd day to 14th week PI, however, in our study the antibodies were detectable till 22nd week PI. This is obvious as we have given a booster at 28th day PI. They also observed the LAT to be more sensitive, economical and easy to perform. Srivastava *et al.* (1991) also observed the agglutinins on day 7 PI in goats given *M. capri* vaccine. They did not observe any difference in the sensitivity of LAT and SAT. Singh and Srivastava (1993) observed that the agglutinins are produced earlier (second week) in the rabbits inoculated with the protein of *M. capri* containing saponin than those containing other adjuvants. They also observed maximum response up to 12th week. This delayed and shorter duration of response they observed may be due to the use of different culture vaccine.

The DID has been considered as one of the important serological tests to detect the antibody response in immunized animals. The precipitating antibodies appeared at 7 day PI and reaches its maximum at eighth week and then disappears after 14th week PI. Similar to our result Purohit *et al.* (1982) also observed precipitating antibodies from 7th day to 112 days in sera of rabbits inoculated with *M. capri* culture.

However, Singh and Srivastava (1993) observed the precipitating antibodies as early as 2nd week to 12th week PI in rabbits vaccinated with protein fraction of *M. capri* with saponin, and Rana and Srivastava (1996) could detect precipitating antibodies from 3rd week to 10th week in rabbit sera given *M. capri* saponin vaccine. This difference in the delay in response may be due to the use of different species by the later workers.

From the study it is revealed that the LAT and SAT has advantage over DID in detecting antibodies. However, LAT and SAT appears to be equally sensitive in detecting antibodies, but the former has shown an edge over the later.

SUMMARY

The antibodies response in the sera of rabbits immunized with saponin killed vaccine of *Mycoplasma mycoides* subsp. *mycoides* (LC) was monitored by slide agglutination test (SAT), latex agglutination test (LAT) and by double immunodiffusion (DID). The agglutinating antibodies appeared at 7th day to 20th week post infection (PI), while the precipitating antibodies appeared at 7th day to 12th week PI. The peak response of 3.5 was obtained at eighth week PI in SAT, while 3.75 mean titre was obtained at sixth week PI in LAT.

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Passive protection in mice using *Mycoplasma mycoides* subsp. *mycoides* LC vaccine

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Key words: Mice, *Mycoplasma mycoides* subsp. *mycoides* LC vaccine, Passive protection test

There are no reports available on the use of PMP test on *Mycoplasma mycoides* subsp. *mycoides* LC (Mmm LC) saponin adjuvanted vaccine. Therefore, the present study was carried out to monitor the mouse protective antibodies against the above vaccine.

The vaccine was prepared from MmmLC culture which was grown in mycoplasma tissue culture medium (Carmichael *et al.* 1972) at 37°C for 2-3 days. The growth was centrifuged at 10 000 rpm for 20 min and the pellets were washed and suspended in PBS pH 7.2. The cells were then sonicated at 10 μ for 10 jerks of 2 min each. The protein concentration was estimated as per Lowry *et al.* (1951). A 2.5 mg of sonicated cell protein with 3 mg of saponin in 1.0 ml quantity was used as single dose of vaccine. The sterility of the vaccine was tested in Robertson's cooked meat medium, blood agar and Sabouraud's dextrose agar and was found sterile.

A group of 16 healthy goats were inoculated with 1.0 ml of the above vaccine through S/C route and after 28 day booster was given to all the animals. The sera samples were collected from all the goats on 0 day and at weekly interval up to 4 week and then at fortnightly up to 6 months postinoculation. The sera of each week from all the goats were pooled and used for the test.

The PMP test was conducted as per Dyson and Smith (1975). Five mice were used for inoculation of different pooled sera along with equal number of control. A 0.25 ml of pooled sera was inoculated into each mouse through S/C route. The mice were challenged after 4 hr with 7.3×10^9 cfu of virulent culture of MmmLC through I/P route along with control. The mice were observed for mycoplasmaemia by collecting tail blood in solid and liquid media at 24, 48 and 72 hr of challenge. The absence of mycoplasmaemia was taken as the criterion for protection.

No mouse protective antibodies (MPA) were observed on 0 day goat sera. A 20% protection was observed on 7 day which gradually increased to 80% in 6 week and later on declined to 20% on 14 week (Table 1).

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Table 1. Passive protection (%) in mice with vaccinated goat sera

Group	Week post-immunization											
	0	1	2	3	4	6	8	10	12	14	16	18
Vaccinated	0	20	60	60	60	80	40	60	20	20	0	0
Control	0	0	0	0	0	0	0	0	0	0	0	0

The results were agreeable with Rana *et al.* (1999). However, in contrast Thaker (1987) using whole cell *M. capri* vaccine and Singh and Srivastava (1993) using different fractionated vaccines of *M. capri* in incomplete Freund's adjuvant could not find any protection on day 7 and the maximum response was also observed between 50 and 60%. This indicates that perhaps saponin plays better response in eliciting the MPA. Dyson and Smith (1975) also observed the MPA as early as at 4 weeks in the sera of cattle vaccinated with the 3 attenuated vaccines of *M. mycoides* subsp. *mycoides*, however, the antibodies were seen up to 6 months and 15 days.

SUMMARY

The mouse protective antibodies against the *M. mycoides* LC vaccine were monitored. The mouse protective antibodies were found by day 7 and these increased till 6 week but decreased after that.

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Evaluation of dot-ELISA in the detection of *Taenia solium* cysticercosis in pigs

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Key words: Cysticercosis, Dot- ELISA, *Taenia solium* cysticercosis, *Cysticercus cellulosae*

Detection of *Taenia solium* cysiticercosis in pigs is usually done on postmortem examination. The detection of infection in live animals requires serodiagnosis either by demonstration of circulating antigen, antibody or their complexes. A more simple, rapid and sensitive test, viz. dot-ELISA was evaluated, keeping in view its possible field applicability.

Collection of serum samples

Serum samples (206) were collected from pigs slaughtered at the Bangalore Animal food corporation slaughterhouse which inclued 26 samples from naturally infected animals confirmed on postmortem examination. Serum samples (30) were collected from pigs of organized farms situated in and around Bangalore.

Preparation of antigen

Whole cysticercal antigen (WCA): The antigen was prepared with cysticerci obtained from organs and muscles of naturally infected pigs. The cysticerci were separated in the laboratory and thoroughly washed in phosphate buffered saline (pH 7.2) containing antibiotics (kanamycin sulphate 40mg, nalidixic acid, 40 mg and ampicillin 40mg) and a protease inhibitor 0.01 mM phenyl methyl sulphonyl fluoride. The cysticerci were disrupted in glass tissue homogenizer at 4°C and the homogenate was sonicated 4-times at 20 Kz, lmA for 60 sec, on an ice cold bath. The homogenate was estimated (Bradford 1976). The antigen was stored in aliquots at –20°C till further use.

(b) Antigen-B: Antigen-B (partially purified antigen) was prepared as per the methodology of Bertha *et al.* (1982). The WCA without vesicular fluid was dialysed against 0.5 M acetic acid (pH 2.3) for 12 hr at 4°C. The dialysed material was centrifuged at 14 000 g at 4°C for 1 hr. After estimation of protein content (Bradford 1976) it was adjusted to 0.5mg/ml which was further precipitated with 0.86 N sodium chloride and resuspended in 0.5 M acetic acid.

Procedure for dot-ELISA

The test was conducted with 1× 1cm size nitro-cellulose membrane (NC) with 0.45 μm porocity. 2μl of either WCA or antigen-B in PBS (protein content 10-20μg) was dotted on to the NC-strips. After 5-10 min the blocking was done by immersing the strips in 4% skimmed milk powder and 1% bovine serum albumin (BSA) for 20 min at 37°C. After washing in PBS-tween 20 (0.01 M PBS, pH 7.2 with 0.05% tween 20) the strips were incubated with 1:400 dilution of porcine serum diluted in PBST with 1% BSA at 37°C for 20 min. After proper washing anti-pig IgG-HRP conjugate was added at a predetermined dilution (1 : 3000) which was standardized by chequer board titration. The strips were incubated for 30 min at 37°C. After final washing the substrate solution (3, 3 diamino benzidene tetrahydrochloride-10mg, HIMEDIA, tris buffer-20ml, pH 7.4 and 20μl of 30% v/v hydrogen peroxide) was added and substrate solution was kept for 1 min. Positive reaction could be visualized as clear brown dot at the site of antigen-antibody reaction. The reaction was stopped by distilled water. The sensitivity and specificity of the test was calculated as followed by Konapur *et al.* (1999).

Out of 26 positive serum samples 25 and 22 samples showed positive reaction with antigen-B and WCA respectively. The results of dot- ELISA during the present investigation indicated 96.15 and 100% and 84.61 and 100% specificity by antigen-B and WCA respectively. Out of 180 unknown samples 41.11% (N=74) and 31.11% (N=56) were positive by dot-ELISA when the samples were tested by antigen-B and WCA respectively. All known serum samples (N=30) from farm reared pigs exhibited no positive reaction at the site of antigen-antibody reaction by both WCA and antigen-B.

Dot-ELISA was evaluated in the detection of *Cysticercus*

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cellulosae antigens in CSF of neurocysticerosis patients using whole cyst antigen and 59% sensitivity and 100% specificity were observed by Giron *et al.* (1987). Guo (1989) observed 90.79% sensitivity in pigs with cysticercosis while Li (1995) confirmed 100% cases of cysticercosis by dot-ELISA and reported that test was less expensive and reagent conservative. Draelants *et al.* (1995) found sensitivity and specificity of 87.5 and 93.15% in diagnosis of *Taenia saginta* cysticercosis in cattle. Vaz *et al.* (1996) reported 95.1 and 90.65% sensitivity and specificity in detection of antibodies in CSF using antigen extracts of mambrane and scolex of *Cysticercus cellulosae.*

During the present investigation the sensitivity per cent with antigen B and WCA was 96.15 and 84.61% whereas specificity was 100%. This particular finding on specificity corroborates with the finding of Giron *et al.* (1987) and Li (1995) and partially agreed with the finding of Vaz *et al.* (1996). The sensitivity percentage of present findings with antigen B was fully comparable with the observations of Li (1995), Vaz *et al.* (1996) and partially with Guo (1989), whereas sensitivity percentage of WCA was in agreement with Draelants *et al.* (1995) and partially with Guo (1989).

The sensitivity percentage of dot-ELISA had varied in the present investigation when 2 different antigens were used but the variation was statistically insignificant ($P \leq 0.05$). The variation of sensitivity percentage may be attributed towards the use of antigen B which is more parasite specific being partially purified. Some of the pigs which were considered negative on routine meat inspection but found positive by dot-ELISA could have been having light infection or the cysticerci may have been located in deep regions or there might have been previous exposure to infection. Similar observations on nondetection of *Taenia solium* cysticercosis by meat inspecton but with serological tests such as indirect haemagglutination test and enzyme linked immunosorbent assay were reported by Herbert and Oberg (1974) and Zoli *et al.* (1987) respectively.

SUMMARY

Dot-enzyme linked immuno assay (Dot-ELISA) was evaluated for the detection of *Taenia solium* cysticercosis in pigs using whole cysticercal (WCA) and partially purified antigen (antigen B). Dot-ELISA conducted with antigen B and WCA showed positive reaction in 25 and 19 and 74 and 56 pigs positive on meat inspection and negative on meat inspection respectively. None of the serum samples from farm reared pigs were positive. The sensitivity and specificity of the test was 96.15 and 100% and 84.61 and 100% with antigen B and WCA respectively. This test was simple, easy to perform and can be used for the routine diagnosis of *Taenia solium* cysticercosis in pigs.

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Osteometry of the scapula in tiger

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Key words: Osteometry, Scapula, Tiger

There appears to be meagre information as regards to wild animals which create an important role in the environmental balance. However, Sission (1975) in standard textbook has mentioned salient features of the scapula in dog and cat. The present study was undertaken to put on record the osteometrical parameters of the scapule in tiger as the shoulder has a great range of movement on the chest wall.

The osteometrical study was conducted on the four scapulae of tiger. The bone was 24.52 cm long and 15.25 cm wide with 1:06 scapular index. The scapular spine increased gradually in height dorsoventrally and measured 3.72 cm and 23.87 cm with greatest height and length respectively. The supraspinous and infraspinous fossae showed mean maximum width 7.27 cm and 7.45 cm respectively (Table 1). The scapular spine edge was thick and rough dorsally and at the ventral part it was thin and bent caudally (Fig.1). The short and blunt acromion was opposite the rim of the glenoid cavity. The distance between acromion and lateral aspect of the rim of the glenoid cavity was 3.20 cm. The cranial border was thin and strongly convex and measured 25.50 cm long. The caudal border was shorter (20.12 cm), straight and thick. The dorsal border measured 14.75 cm in length and was thick and convex and beared a band of cartilage. The cranial angle was rounded and the caudal angle was almost at right angle. The glenoid cavity was continuous cranially upon the ventral face of the supraglenoid tubercle, which beared 1.45 cm long coracoid process medially. The circumference and depth of the rim of the glenoid cavity was measured 16.12 cm and 1.50 cm respectively. The mean greatest vertical and transverse diameter of the glenoid cavity were recorded 6.85

Table 1. Osteometry of the scapula in tiger

Parameter	Range (cm)	Mean (cm)
Scapula spine		
Length (greatest)	23.0-25.0	23.87
Height (maximum)	3.0-4.50	3.72
Length of scapula (maximum)	24.0-25.0	24.52
Width of scapula (maximum)	14.5-16.0	15.25
Width of supraspinous fossa (maximum)	7.0-7.6	7.27
Width of infraspinous fossa (maximum)	7.0-7.8	7.45
Length of caudal border	19.5-21.0	20.12
Length of cranial border	23.0-28.0	25.50
Length of dorsal border	14.0-15.8	14.75
Glenoid cavity		
Vertical diameter	6.5-7.2	6.85
Transverse diameter depth	3.5-3.8	3.65
Circumference	15.0-17.0	16.12
Length of coracoid process	1.40-1.50	1.45

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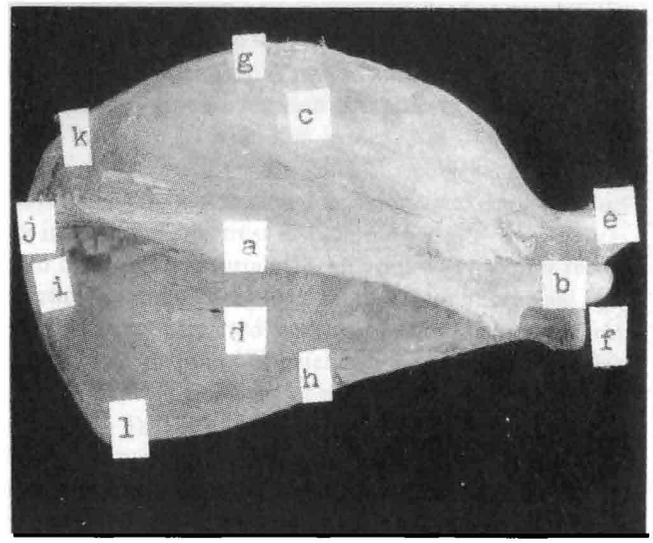


Fig. 1. Right scapula of tiger; lateral view, showing -a, spine; b, acromion; c, supraspinous fossa;d, infraspinous fossa; e, supra-glenoid tubercle;f, glenoid cavity; g, cranial border; h, caudal border; i, dorsal border; j, cartilage; k, cranial angle; and l, caudal angle.

and 3.65 cm respectively.

SUMMARY

Osteometrical parameters of the scapula in tiger are reported here. Length, height and width of scapula spine; vertical and transverse diameter of glenoid cavity; circumference of glenoid cavity; and length of coracoid process were measured. Length of cranial and dorsal borders were also measured.

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Histological studies on cranial cruciate ligament reconstruction in dogs with whole thickness skin, patellar ligament grafts*

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Key words: Dogs, Histology, Cranial cruciate ligament reconstruction, Skin and patellar ligament grafts

Ruptured cranial cruciate ligament is one of the common orthopaedic injuries in dogs. If untreated it leads to degenerative diseases in stifle joint. Recently, successful results have been reported in dogs using an autogenous medial (Lam 1968, Abe *et al.* 1993) or lateral (Shires *et al.* 1984) third of patellar ligament. Bennet and May (1991) and Mcpherson and Allan (1993) recommended combined use of patellar ligament and fascia lata. The present study was undertaken to assess the adaptability of autogenous medial or lateral patellar ligament graft with fascia lata to replace ruptured cranial cruciate ligament in dogs. These procedures were compared with whole thickness skin grafts. Success of these procedures was evaluated basing on functional limb usage and gross and histological changes in the joint and graft tissue.

Mongrels (40) of either sex weighing 15 to 30 kg and without any stifle joint abnormalities were used in the present study.

Following premedication with atropine and trifulupromazine hydrochloride, anaesthesia was induced by intravenous infusion of 2.5% thiopentone sodium @ 25 mg/kg body weight. The animals were intubated and anaesthesia was maintained with ether oxygen mixture using Boyle's anaesthetic apparatus. A lateral parapatellar incision was used for rupture and reconstruction of ruptured cranial cruciate ligament with whole thickness skin or lateral third of patellar ligament and fascia lata. The medial parapatellar incision site was used for reconstruction with medial third of patellar ligament and fascia lata.

Following lateral parapatellar incision, the joint was entered at the level of the patellar ligament, about 1-2 mm lateral to it. The patella was luxated medially in lateral parapatellar approach and laterally in medial parapatellar approach, with the limb in extended position. Strict attention was paid to haemostasis. A part of infra patellar fat pad was excised for better visualization of cranial cruciate ligament, which was severed with limb in flexed position. Displaced patella was reduced on the femoral trochlear groove. The arthrotomy wound was closed in routine manner using No.1 silk in horizontal mattress fashion.

In control group (group 1), no graft was used for reconstruction of ruptured ligament. The ruptured cruciate ligament was reconstructed with whole thickness skin graft (group 2), medial third of patellar ligament with fascia lata (group 3) and lateral third of patellar ligament with fascia lata (group 4). The continuity of the grafts was maintained distally. A tunnel was drilled through the distal end of the lateral femoral condyle. The graft was pulled taut and the free end of the graft was reflected over and anchored to lateral femoro-fabellar fascia and joint capsule using No. '0' silk. The arthrotomy incision was closed in routine manner.

Post-operatively, all the dogs were allowed to use the limb without immobilization. The wounds were dressed daily and cutaneous sutures were removed on 10th post operative day. Two dogs from each group were euthanized at regular intervals of 7, 15, 30, 45, and 60th post-operative day. The joints were observed for the condition of the ligament. The joints were collected and fixed in 10% formal saline. The tissues were processed following procedure of Lillie and Fullmer (1976). Paraffin sections (5μ) were prepared and stained with haematoxylin and eosin (H&E) and van Giesons. The sections were examined microscopically for changes in the remnants of the ligament, graft materials and femoral tunnels.

Microscopic examination of normal cranial cruciate ligament exhibited dense collagen bundle which are arranged parallel to each other. The nuclei of fibrocytes were elongated. In control group, ruptured cranial cruciate ligament showed inflammatory reaction by 7 post-operative day, particularly around the blood vessels. There was hyaline degeneration at 45th day, which was in an advanced stage by 60th day.

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Fig. 1. Section of lateral third of patellar ligment with fascia lata. 45th day.

There was degeneration of hair follicles, epidermis and cutaneous glands in animals which were reconstructed with whole thickness skin graft by 15th day. This showed that the skin is loosing its normal architecture and trying to assume the function of the ligament. New collagen fibres formation was noticed by 30th day. The graft anchored to the bone by 45th day which was firm by 60th day.

In the group grafted with medial third of patellar ligament and fascia lata, it was surrounded by fibrin and red blood cells by seventh day. Granulation tissue invading the graft was observed by 15th day. The graft was highly vascular and new collagen fibres formed by 30th day. There was no difference between graft and newly formed collagenous tissue by 45th day. There were signs of fibres anchoring to the bone. The graft firmly adhered to the bone by 60th day.

In dogs, reconstructed with lateral third of patellar ligament and fascia lata, the graft was surrounded by fibrin and red blood cells at 7th and 15th day. Formation of granulation tissue was evident by 30th day. At 45th day, there was formation of new collagen fibres (Fig.1). There was no difference between the graft and newly formed collagen tissue at 60th day. Vascularization of medial third of patellar ligament with fascia lata graft was more and appeared early when compared to the other two grafts. Vaughan (1963) observed greater vascularization of the graft when passed through a tunnel in bone. New collagen formed early in full thickness skin grafts. However, all the 3 grafts exhibited good amount of collagen by 60th day. This activity might be responsible for converting the graft into ligamentous structure.

The results of microscopic studies suggested the superiority of medial third of patellar ligament with lata for the repair of ruptured cranial cruciate ligament in dogs. Whole thickness skin is the second choice followed by lateral third of patellar ligament with fascia lata.

SUMMARY

Cranial cruciate ligament was ruptured in 40 mongrel dogs of either sex. In 30 dogs, the ruptured ligament was reconstructed with skin, and patellar ligament along with fascialata. The stifles were evaluated up to 60th post-operative day. Control animals showed lameness and anterior drawer symptoms throughout the observation period. Among reconstructed groups, animals with joints stabilized by medial third of patellar ligament along with fascialata showed least degree of lameness and earlier normal functional limb usage when compared to other treated groups. Further, these animals showed early revascularization and collagen formation in grafts.

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Influence of GnRH and eCG on oestrus response and progesterone profile in norgestomet primed postpartum anoestrus cows*

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Key words: Anoestrus, eCG, GnRH, Oestrus induction, Postpartum cow, Progesterone

Progestational compounds are used to induce oestrus and breeding according to oestrus ensured good fertility in anoestrus cattle (Umed Singh *et al.* 1998). After oestrus induction varied oestrus responses are due to dose of progestogen (Moseley *et al.* 1979), inclusion of various hormones along with progestogen (Narasimha Rao and Suryaprakasam 1991), pheramonal effects (Medrano *et al.* 1996) and method of oestrus detection (Cavalieri and Fitzpatrick 1995), were observed. Changes in progesterone profiles in norgestomet synchronized cyclic cows are well established, whereas, in postpartum anoestrus cows during different phases of oestrus induction treatment have not been reported in detail. Hence, the present investigation was carried out to study the pattern of oestrus behaviour at induced oestrus and the progesterone changes before, during and after oestrus induction treatment in postpartum anoestrus cows.

Apparently healthy postpartum anoestrus cows (24) at the Livestock Research Station, Kattupakkam, TNUVAS were randomly divided into 3 (NOR, NOR+GnRH and NOR+eCG) equal (n=8) groups. All the animals were treated with 3 mg norgestomet ear implant and at the time of implant insertion (day 0) 2 ml crestar injection (3 mg norgestomet and 5 mg oestradiol valerate) was administered intramuscularly. The implant was removed on day 9 of the experiment. In the NOR group (control) no drug was administered at implant removal. In NOR+GnRH and NOR+eCG groups 10 µg GnRH analogue and 500 IU equine chorionic gonadotrophin (eCG), respectively, were administered intramuscularly at the time of implant removal.

The animals were kept under constant observation during the treatment. After implant removal, they were observed for oestrus signs at 6 AM, 12 noon, 6 PM and 10 PM daily each for 30 min. Rectal examination was also done at 12 hr interval to confirm oestrus. The oestrus response was defined as the percentage of treated animals that showed oestrus. Onset of oestrus was calculated from the time of implant removal to the first standing to be mounted by other animals. Time between first and last mounting is calculated was duration of oestrus. Intensity of oestrus was scored based on the score card comprising behavioural changes, physiological changes and gynaecological observations. The experimental cows were inseminated at 8 to 12 hr after the onset of oestrus. Blood samples were collected from jugular vein on days -10 (10 days before implant insertion), 0 (implant insertion), 1, 3, 5, 7, 9 (implant removal), 10, 11 (oestrus) and 12. Blood was also collected on day 10 and 21 after AI. Serum was separated and stored at –20°C until assayed. Progesterone was estimated using solid-phase radio immunoassay (RIA) technique with the help of progesterone kit. The data were analyzed using ANOVA (Snedecor and Cochran 1994).

During the implant period, 58.33% of the animals showed varying degrees of oestrus signs like vulval oedema, mucus discharge and uterine tonicity. Similar observation was also made by Escobedo *et al.* (1989) and Favero *et al.* (1995). Favero *et al.* (1995) reported that only 1% of the postpartum cows exhibited oestrus during implant period. The mucus discharge had atypical fern pattern as in the dioestrual and gestational i.e. progesterone phase (Roberts 1971). This may be due to administration of exogenous oestradiol at the time of implant insertion. After implant removal all the animals (100%) exhibited oestrus within 72 hr. Narasimha Rao (1991) observed that 58.4% of the anoestrus cattle treated with progestogen and GnRH showed oestrus response within 2 to 3 days after progestogen withdrawal. Balasubramanian (1989) and Luthra *et al.* (1994) reported 100% oestrus response within 72 hr after implant removal in norgestomet along with eCG treated postpartum anoestrus cows. The effectiveness of norgestomet for inducing behavioural oestrus in cows is due to the combined effect of progestogen priming on the brain and the direct effect of both exogenously administered oestradiol and the high endogenous oestradiol on the hypothalamus (Cavelieri and Fitzpatrick 1995).

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Table 1. Onset, duration and intensity of induced oestrus following oestrus induction in postpartum anoestrus cows

Parameters	NOR	NOR+GnRH	NOR+eCG
Onset of oestrus (mean±SE hr)	39.5±3.66[a]	39.25±8.14[a]	16.38±2.54[b]
Duration of oestrus (mean±SE hr)	23.75±4.20[x]	15.25±2.00[x]	47.13±5.84[y]
Intensity of oestrus			
No. of animals (%)			
Intense	1 (12.5)	2 (25.0)	4 (50.0)
Intermediate	5 (62.5)	1 (12.5)	4 (50.0)
Weak	2 (25.0)	5 (62.5)	0 (00.0)

Group means bearing superscripts a, b differ significantly ($P<0.05$); group means bearing superscripts x, y differ significantly ($P<0.01$).

Onset of oestrus

Significantly earlier onset of oestrus was observed in NOR+eCG group than other 2 groups (Table 1). Similar observation was also reported by Luthra *et al.* (1994) and Umed Singh *et al.* (1998). Mean onset of oestrus in NOR group is in accordance with the observation of Balasubramanian (1989). Beal *et al.* (1984) and Gauthier *et al.* (1984) reported 67 and 72 to 96 hr, respectively, as onset of oestrus after implant removal. In this study, earlier onset of oestrus may be due to reduced dose rate of norgestomet (Moseley *et al.* 1979), early accelerated oestradiol production by exogenous gonadotrophin (Dutta *et al.* 1992) and/or frequent observation of oestrus.

Duration of oestrus

Duration of oestrus was significantly longer in NOR+eCG group than that in NOR and NOR+GnRH groups. This is due to longer half life of eCG resulting in higher concentration of oestradiol in the circulation for a longer duration (Schams *et al.* 1978).

Intensity of oestrus

Intense manifestation of oestrus was observed in eCG treated group. The effect of eCG on multiple follicular maturation resulting in higher endogenous oestradiol production might be responsible for the improved response (Narasimha Rao and Suryaprakasm 1991). The animals injected with GnRH at implant removal showed moderate to poor intensity of oestrus. This might be due to ovulation before follicle could produce enough estradiol to affect behavioural response (Mauer and Rippel 1979). Further, intense manifestation of oestrus is not a common feature in GnRH analogue treated animals (Patabiraman *et al.* 1986). Varying intensity of oestrus was observed in norgestomet treated group. Barnes *et al.* (1981) observed similar response and opined that these might be due to variable ability of individual animal to recover from the influence of this potent suppressor of pituitary function. Improvement in oestrous response, early onset and longer duration of oestrus were observed when a group of animals enter oestrus simultaneously compared with single female (Cavalieri and Fitzpatrick 1995, Medrano *et al.* 1996). This study was carried out in a group of females at a time and this would have also enhanced oestrus response. Serum progesterone levels at 10 days before implant insertion and on the day of implant insertion fluctuated below 1 ng/ml

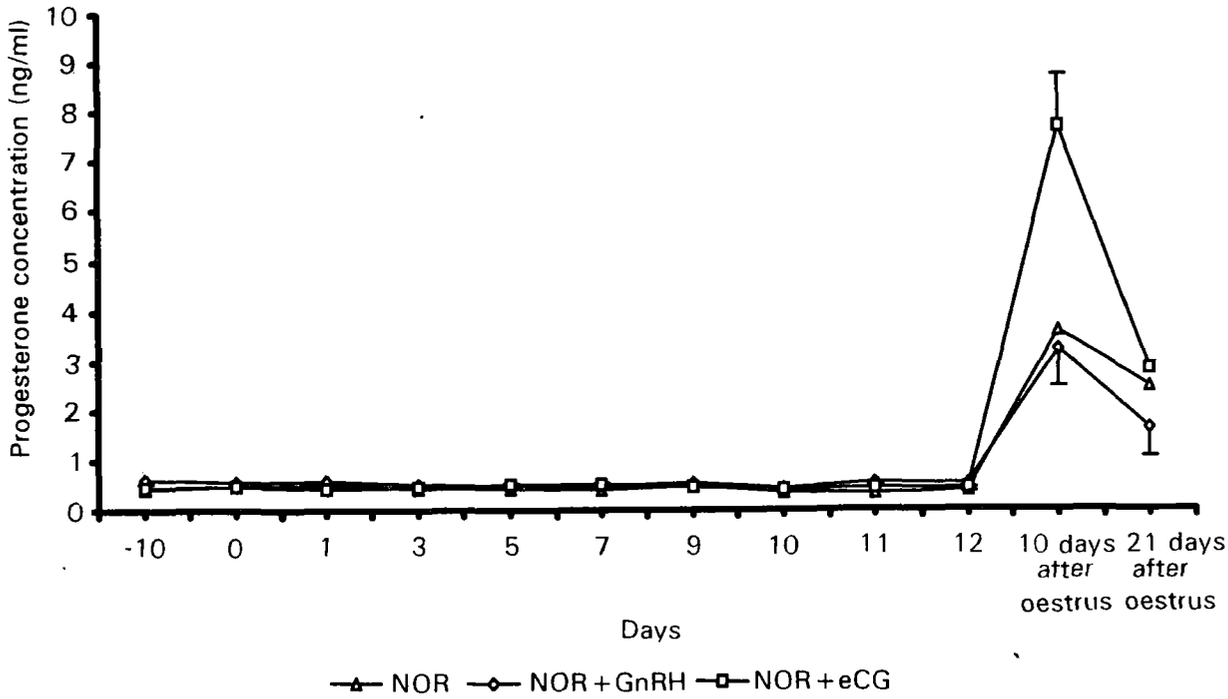


Fig 1. Changes in serum progesterone concentration at various phases of oestrus induction treatment.

(range 0.3 - 0.92 ng/ml), confirmed the true acyclicity of the animals (Dutta *et al.* 1992 Rajasundaram 1994). Lack of changes in serum progesterone concentration during norgestomet treatment observed in this study is because of inability of the RIA to detect norgestomet in the samples because norgestomet does not cross react with progesterone of RIA (Barnes *et al.* 1981).

Progesterone concentrations (ng/ml) on the day of oestrus were significantly higher ($P<0.01$) in NOR+GnRH group than NOR+eCG and NOR groups. This rise may be because of predominant LH activity of GnRH (Wetteman *et al.* 1982) and luteinization of the follicle, if the follicle is not in an appropriate stage of development (Narasimha Rao 1991) as evidenced by low concentration of oestrogen on the day of oestrus in NOR+GnRH group Selvaraju and Rajsundaram 2000.

The mean serum progesterone concentrations on day 10 post-oestrus (7.67±1.92, 3.59±1.29 and 3.21±0.76 ng/ml) in NOR+eCG, NOR and NOR+GnRH had no significant difference between groups. One animal in NOR+eCG group with highest progesterone concentration of 19.11 ng/ml, had double ovulation and was confirmed by rectal examination as suggested by Mikel Jenson *et al.* (1982). Two animals in NOR+GnRH group that had lower progesterone concentration (< 1 ng/ml) on day 10 post-oestrus registered > 1 ng/ml on day 21 post oestrus due to presence of corpus luteum and was confirmed by rectal examination. Whereas 2 animals in NOR group reverted back to anoestrus had basal progesterone concentration (< 1 ng/ml) on days 10 and 21 after oestrus.

The serum progesterone levels were significantly ($P<0.01$) lower in fertile (0.36±0.03 ng/ml) than in non-fertile (0.54±0.04 ng/ml) oestrus. Higher progesterone concentration at the time of oestrus was reported to affect gametes transport, fertilization process and subsequent embryo passage to the uterus (DeSilva *et al.* 1981). Higher progesterone concentrations at oestrus may block LH release and adversely affect oocyte maturation and ovulation (Duchens *et al.* 1994). These cause damage of the oocyte to such an extent that even inseminating close to the time of ovulation may not ensure conception (Duchens *et al.* 1996). It is concluded that eCG than GnRH at implant removal had more significant effect on oestrus response, whereas, GnRH at implant removal had significant effect on P_4 levels at oestrus. The progesterone profile is useful to study ovarian activity, response to hormone treatment and to predict fertility status of the animal.

SUMMARY

Postpartum anoestrus cows (24) were equally divided into 3 groups. The eCG treatment results in earlier onset, longer duration and more intensity of oestrus than GnRH. Estimation of progesterone during different phases of oestrus induction treatment is helpful to study the response to treatment and fertility status of the animal.

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Histoenzymic variations in endometrial phosphatases in buffaloes (*Bubalus bubalis*)

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Key words: Buffalo, Endometrium, Phosphatases, Oestrous cycle

Endometrium is the target organ of ovarian steroids (Hafaz 1964) and metabolise endometrial carbohydrates for nutrition of blastocyst under the influence of oestrogen and progesterone (Hughes *et al.* 1963). Literature lacks information on cyclic histoenzmic function of buffalo edometrium and its significance in maintenance of pre-implantation biochemical milieu, which could be useful in utilization of edometrial biopsies in prediction of fertility. The present investigation was undertaken to predict the fertility of cows on the basis of uterine biochemical environment during late oestrus.

Uterine tissue sampling and histoenzymic demonstration of phosphatases

Genitalia were collected from 20 healthy cycling buffaloes immediately after exsanguination at the Government Abattoir, New Delhi. Those were classified into 2 groups of 10 animals in each group representing follicular and luteal phases of oestrous cycle on the basis of ovarian graafian follicle and corpus luteum, respectively. Genitalia were placed on ice tray and uterine tissues were collected from the uterine horn epsilateral to the functional ovary and were frozen in liquid nitrogen (-196°C) and frozen sections of 8-10μ were cut with cryostat and stained with standard methods for histoenzymic demonstration of ACP and AKP activities (Barka and Anderson 1963) and G-6-P and ATP activities (Chayen *et al.* 1969).

Acid phosphatase (ACP)

In buffaloes, supranuclear region of surface and glandular epithelia evidenced strong ACP reaction during luteal phase and weak reaction during follicular phase of oestrous cycle (Fig.1). Distinctly stronger ACP activity of surface and glandular epithelia of buffalo endometrium during luteal phase could be surmised to its significance in glycogen secretion for nourishment of pre-implanting blastocyst under the influence of progesterone (Murdoch 1970).

Alkaline phosphatase (AKP)

Variations in endometrial AKP activity were distinct during cyclic phases in buffaloes. Recorded strong and weak AKP activities in endometrial surface and glandular epithelia during luteal and follicular phase, respectively, were recorded (Figs 2,3) in buffaloes agrees with reported cyclic pattern in cows (Larson *et al.* 1970). Strong endometrial AKP activity during luteal phase and weak activity during follicular phase could be implicated to progesterone and oestrogen effects, respectively, since progesterone increased endometrial AKP activity while oestradiol decreased AKP activity in ovariectomized cows (Larson *et al.*1970). Increased AKP activity of buffalo endometrium during luteal phase appears to be involved in anaerobic glycolysis process of endometrial carbohydrate metabolism required for survival of implanting blastocyst, since AKP also promotes glucose transfer process of endometrial carbohydrate metabolism (Christie 1966).

Glucose-6-phosphate (G-6-P)

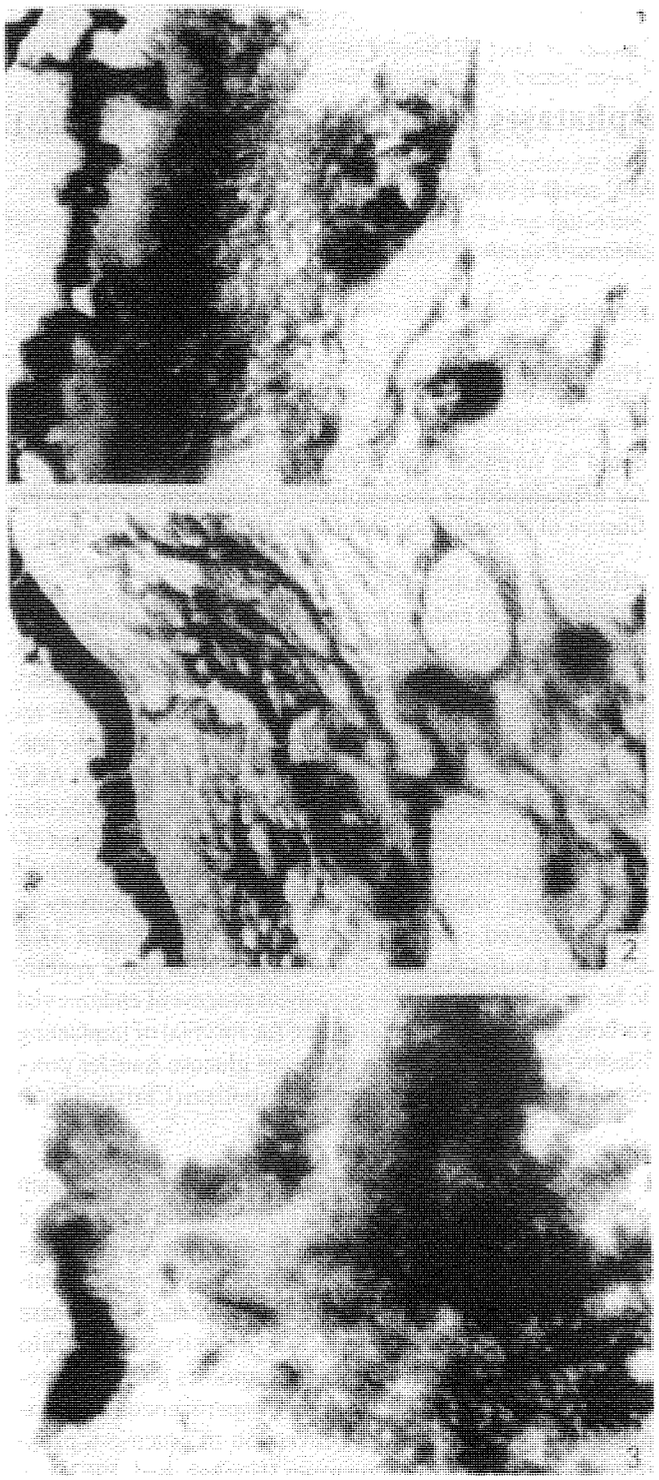
Strong G-6-P activity was noticed in supranuclear region of surface epithelium of buffalo endometrium and in large blood vessels during follicular and luteal phases of oestrous cycle (Fig.4). Maximum G-6-P activity was reported in human endometrium during ovulation period and luteal phase (Wynn 1977). Constant G-6-P activity of buffalo endometrium during cyclic phases promotes glucose secretion in uterine lumen for nutrition of spermatozoa and implanting blastocyst through conversion of glucose-6 phosphate into glucose in endometrium (Hughes *et al.* 1963).

Adenosine triphosphate (ATP)

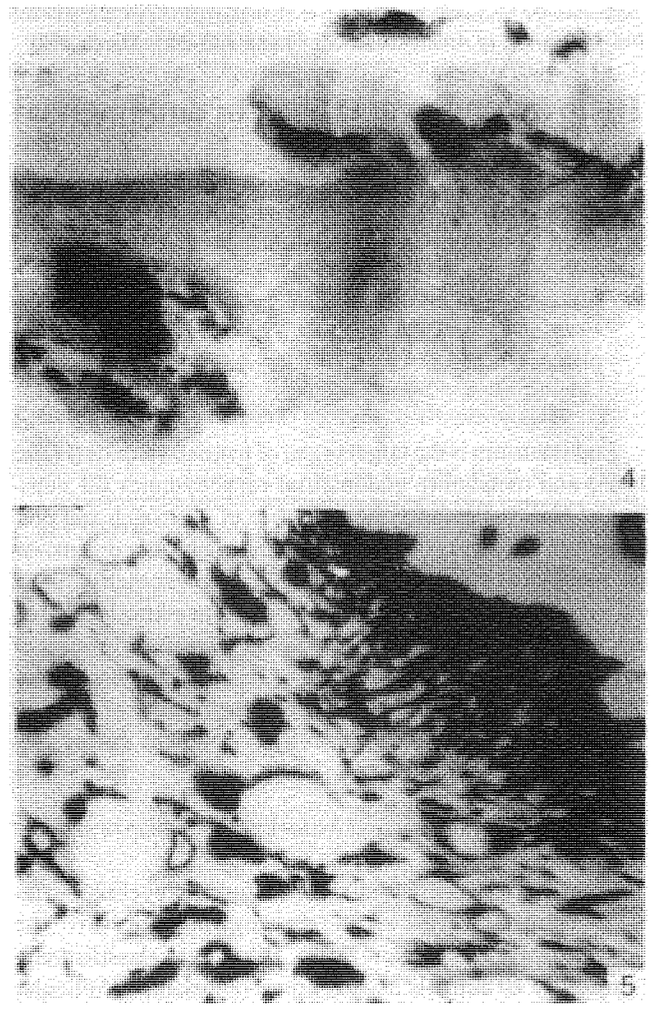
Strong ATP activities in surface and glandular epithelia during follicular phase (Fig.5) was observed in buffaloes. The significantly ($P<0.01$) higher biochemical content of endometrial glycogen during follicular phase than that in luteal phase in cycling buffaloes (Bugalia and Sharma 1990) further substantiated occurrence of endometrial glycogenesis and

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Figs 1-3. **1.** Strong ACP activity in supranuclear region of surface epithelium and superficial gland of buffalo endometrium during luteal phase of oestrous cycle. Azo dye method. ×32. **2.** Surface epithelium and superficial gland of buffalo endometrium showing intense AKP reaction during luteal phase of oestrous cycle. AZO dye method ×32. **3.** Buffalo endometrium showing weak to moderate AKP activity in surface epithelium and connective tissue during follicular phase of oestrous cycle. Azo dye method. ×128.



Figs 4-5. **4.** Strong G-6-P activity in supranuclear region of surface epithelium in buffalo endometrium during luteal phase of oestrous cycle. Wachstein and Moisol method. ×50. **5.** Buffalo endometrium showing strong ATP activity in surface epithelium and blood vessels and intense apical ATP reaction in superficial glands during follicular phase of oestrous cycle. Calcium activated method (Myosin type). ×16.

anaerobic glycolysis during follicular and luteal phases, respectively. Phosphatases in bovine endometrium regulate glycogenesis and glycolysis processes of carbohydrate metabolism for nourishment of blastocyst till implantation.

SUMMARY

Uterine tissues were collected from 20 cycling buffaloes during follicular and luteal phases for histoenzymic demonstration of acid phosphatase (ACP), alkaline phosphatase (AKP), glucose-6-phosphatase (G-6-P) and adenosine triphosphatase (ATP) in endometrium. Distinct cyclic variations were recorded in activities of ACP, AKP and ATP in buffalo endometrium. Strong ACP and AKP activities were demonstrated in surface and glandular epithelia of

endometrium during luteal phase and weak activities during follicular phase and moderate during luteal phase in buffaloes. However, strong G-6-P activity was observed in surface epithelium of buffalo endometrium during both the cyclic phases without distinct cyclic variations. Significance of phosphatases in carbohydrate metabolism of endometrium and pre-implantation uterine milieu have been reported.

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Concentrations of histamine and 5-hydroxytryptamine in certain reproductive tissues of buffaloes *(Bubalus bubalis)*

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Key words: Buffalo, Histamine, 5-HT, Reproductive tissues, Serotonin

Neuroendocrine system plays an important role in regulating the rhythmicity of sexual behaviour and the reproductive functions. Several investigators have revealed a close relationship between autacoids and certain reproductive hormones in regulation of ovarian functions. The oestrogen-induced hyperaemia and increase in microcirculation is mimicked by histamine (Piacsek and Huth 1971). Histamine also causes vasodilatation, increase in the capillary permeability and blood flow to the ovaries (Krishan *et al.* 1986) and thus may also regulate the permeability of ovarian follicles to LH (Halterman and Murdoch 1986). Like histamine, 5-HT plays an important role in the ovulation process (Claussell and Soliman 1978). Present study was, therefore, aimed to determine the concentrations of histamine and 5-HT in the ovary and uterus of both the non-pregnant and pregnant buffaloes *(Bubalus bubalis)* and placenta of pregnant animals to unravel the possible physiological/clinico-pathological significance of these autacoids in female reproduction.

The ovaries and uteri from pregnant and nonpregnant buffaloes of non-descript breed and of varying ages, were collected from the local abattoir of Mathura. From the nonpregnant animals, ovaries and uterine tissues from midcornual region were collected and transported to laboratory in chilled normal saline solution. Whereas, from the pregnant animals, the ovaries, uterine tissues (intercotyledonary area of gravid horn from the site of maximum attachment) and placenta (maternal cotyledons, foetal cotyledons and intercotyledonary area) were collected separately as described above.

Histamine and 5-HT were extracted (Parrat and West 1957). Histamine and 5-HT concentrations in the above tissues were determined by bioassay method using guineapig ileum and rat fundus, respectively, and the results expressed as µg/g tissue (Parry 1970).

The data were subjected to statistical analysis by using student 't' test (Snedecor and Cochran 1967).

The mean concentrations of histamine and 5-HT in the ovaries of non-pregnant buffaloes revealed that the ovarian histamine was significantly ($P<0.05$) higher in pregnant animals as compared to nonpregnant buffaloes (Table 1). Certain hormones are known to augment the blood supply to their target organs. LH-induced ovarian hyperaemia in rat and its effect on the microcirculation was similar to that of histamine (Schayer 1962). And, it was later suggested that histamine regulates the ovarian microcirculation and mediates the action of LH on ovarian function (Hunter and Leatherman 1968).

Histamine plays an important role in progesterone synthesis (Krishna and Terranova 1985, Schmidt *et al.* 1987) and the mechanism by which histamine is released to stimulate ovarian steroidogenesis may involve, in part, the mast cell-degranulation induced by LH. *In vitro* perfusion of preovulatory follicles of rats with histamine stimulated progesterone secretion through the activation of H_2 histaminergic receptors (Schmidt *et al.* 1987).

The 5-HT plays a role in the regulation of reproductive processes and its presence in the ovaries was reported in rats (Claussel and Soliman 1978), buffalo (Srivastava 1984, Wani 1985), goat (Raviprakash 1986) and cow (Battista *et al.* 1987). Exogenous 5-HT prolonged the estrous cycle in rats (Vaughan *et al.* 1969) and ewes (Domanski *et al.* 1975). The 5-HT is also reported to involve in the control of prolactin secretion in sheep and this action is exerted at the level of hypothalamus and not directly at the posterior pituitary (Thomas *et al.* 1988). Further, 5-HT appears to have an inhibitory role on ovulation in rats (Wilson and Mc Donald 1974) and demonstrated to stimulate the production of progesterone by dispersed bovine luteal cells *in vitro* (Battista and Condon 1980).

As in the ovaries, uterine histamine concentration in gravid uterus was also significantly ($P<0.05$) higher than in the nongravid uterus. However, there was no significant difference in the uterine 5-HT concentrations between nonpregnant

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Table 1. Histamine and 5-HT concentrations (μg/g) in ovary, uterus and placenta of buffaloes (n=5-11)

Tissue	Histamine		5-HT	
	Nonpregnant	Pregnant	Nonpregnant	Pregnant
Ovary	0.21±0.9[a]	4.58±1.53[b]	0.87±0.23	0.59±0.15
	(0.00-0.50)	(1.91-10.17)	(0.19-1.73)	(0.44-0.74)
Uterus	1.52±0.46[a]	5.15±0.79[b]	0.71±0.12	0.49±0.11
	(0.00-2.69)	(3.23-8.76)	(0.25-1.15)	(0.12-0.92)
Placenta				
FC		0.59±0.26		0.25±0.06[a]
		(0.00-2.56)		(0.01-0.52)
FICA		0.61±0.19[a]		0.48±0.06[b]
		(0.00-1.21)		(0.19-0.75)
MC		5.29±0.66[b]		0.21±0.02[a]
		(2.52-7.54)		(0.14-0.32)

Means with different superscripts in a row for the same parameter differ significantly (P<0.05); means with different superscripts within a column for different parameters in placenta differ significantly (P<0.05); data in parentheses indicate range of the data; FC, foetal cotyledons; FICA, foetal intercotyledonary area; MC, maternal cotyledons.

and pregnant animals. Significantly increased level of uterine histamine concentration during pregnancy may play an important role in embryonic growth (Kahlson and Rosengreen 1968). Further, histamine also exerts dilatory effect on microcirculation of the uterus (Szego 1965) thus resulting in increased permeability of the tissues to nutrients, and promotes the metabolic activity and growth of the foetus (Singh *et al.* 1974). The role of 5-HT on the uterus is still to be elucidated and needs further studies.

The histamine concentration in maternal cotyledons was significantly (P<0.01) higher than the foetal cotyledons and foetal intercotyledonary area (Table 1). The 5-HT concentration was significantly (P<0.05) higher in fetal intercotyledonary area than foetal cotyledons and maternal cotyledons. The higher concentration of histamine in the maternal cotyledons may also play a role in facilitating the microcirculation which in turn promotes the foetal growth as discussed earlier.

These observations suggest that significant elevation of histamine in the uterus, ovaries and the internal cotyledons of pregnant buffaloes compared to that of nonpregnant animals may be playing an important role by increasing the blood flow to the ovaries and uterus for the maintenance of pregnancy.

SUMMARY

Histamine and 5-hydroxytryptamine (5-HT) concentrations were estimated in the ovaries, uterus and placenta of non-pregnant and pregnant buffaloes. Histamine and 5-HT were extracted by standard techniques and bioassayed on guineapig ileum and rat fundus for histamine and 5-HT respectively. Histamine concentration was significantly higher in ovaries and uteri of pregnant animals compared to non-pregnant. In the placenta, it was higher in maternal cotyledons than that in foetal cotyledons or the foetal intercotyledonary area. Whereas, 5-HT concentration was markedly higher in the ovaries and the uterus of non-pregnant buffaloes compared to pregnant animals. The placental 5-HT was significantly higher in the foetal intercotyledonary area than the foetal or maternal cotyledons.

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Production and characterization of a sensitive antiserum against progesterone

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Key words: Antiserum, Antiserum Production, Characterization, Progesterone

In both popular procedures, radio-immunoassay (RIA) or enzyme-immunoassay (EIA), a major limiting factor for practical application of this technology is the high cost of importation of progesterone specific antiserum. Therefore the present study was conducted to develop an indigenous progesterone antiserum for practical exploitation of progesterone analysis in body fluids of bovines.

Procedure

Animals: Soviet Chinchilla rabbits (4,male) were taken for production of antiserum against progesterone-11α-OH-hemisuccinate-BSA and they were housed in the small animal house of the institute.

Antigen preparation and immunization: progesterone-α-OH-hemisuccinate-BSA 3 mg was dissolved in phosphate buffer saline ($NaPO_4$ 0.04M, NaCl 0.15M; 1mg/ml). After complete dissolution the above solution was emulsified with an equal volume of Freund's Complete Adjuvant.

Rabbits were administered 300µl (150µg antigen) of the immunogen emulsified in Freund's complete adjuvant intradermally at 30-40 sites on the flanks. Six booster immunisations with the same amount were administered in similar fashion at intervals of 1 to 1.5 months. Blood samples (2ml) were collected in clean test-tubes 2 days after booster administration and at weekly intervals, from the immunized rabbits. The blood samples were allowed to clot at room temperature for 30 min and serum was separated by centrifugation.

Serial dilutions of antiserum from 1:100 to 1:8000 were made to estimate the titre by direct radioimmunoassay method (Kamboj and Prakash 1993). Apart from titre determination the antiserum was evaluated for sensitivity and cross-reactivity with various related steroids. Biological validation of the antiserum was conducted by estimating progesterone in blood plasma samples obtained by jugular venipuncture of 1 pregnant and 1 cycling buffalo on alternate days for 1 month and comparing the results with the concentration obtained using a previously characterized antiserum (received from Dr H H D Meyer, Institut fuer Physiologie, Weihenstephan, Germany).

Evaluation

Respondents: Out of 4 rabbits 50% rabbits responded by producing antiserum showing a titer of 1:4000 in RIA. The antiserum response was seen after 3 booster immunizations but there was no appreciable improvement with subsequent immunizations.

Antisera sensitivity: The sensitivity of the antiserum produced by the 2 rabbits (rb-1, rb-6) was evaluated by observing the standard curves in a progesterone RIA (Kamboj and Prakash 1993). The comparisons of the two standard curves (Fig.1) showed 50% binding sensitivity of the assay using rb-6 antiserum which was 2-3-fold better than that obtained using rb-1 antiserum (70 pg vs 200 pg/tube respectively). The sensitivity of the antiserum rb-6 was 8 pg/tube progesterone which corresponded to 0.4 ng/ml plasma when standards prepared in charcoal-treated hormon free plasma were used. Hence, for further characterization, only antiserum from rb-1 was selected.

Specificity: The cross reactivity of progesterone antiserum

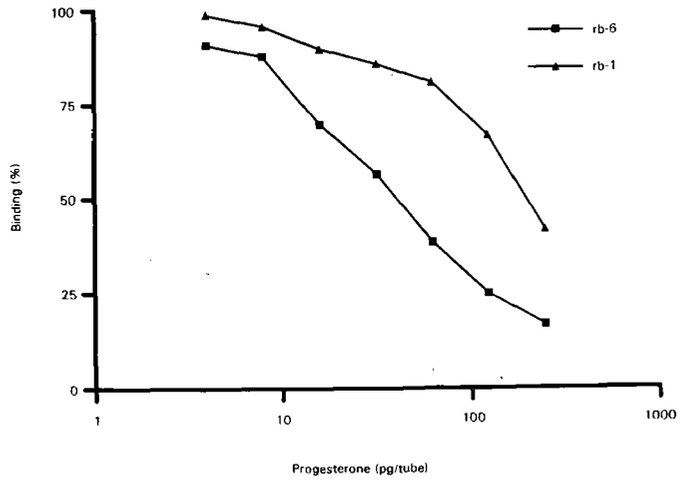


Fig. 1. Comparison of standard curve of progesterone using different antisera.

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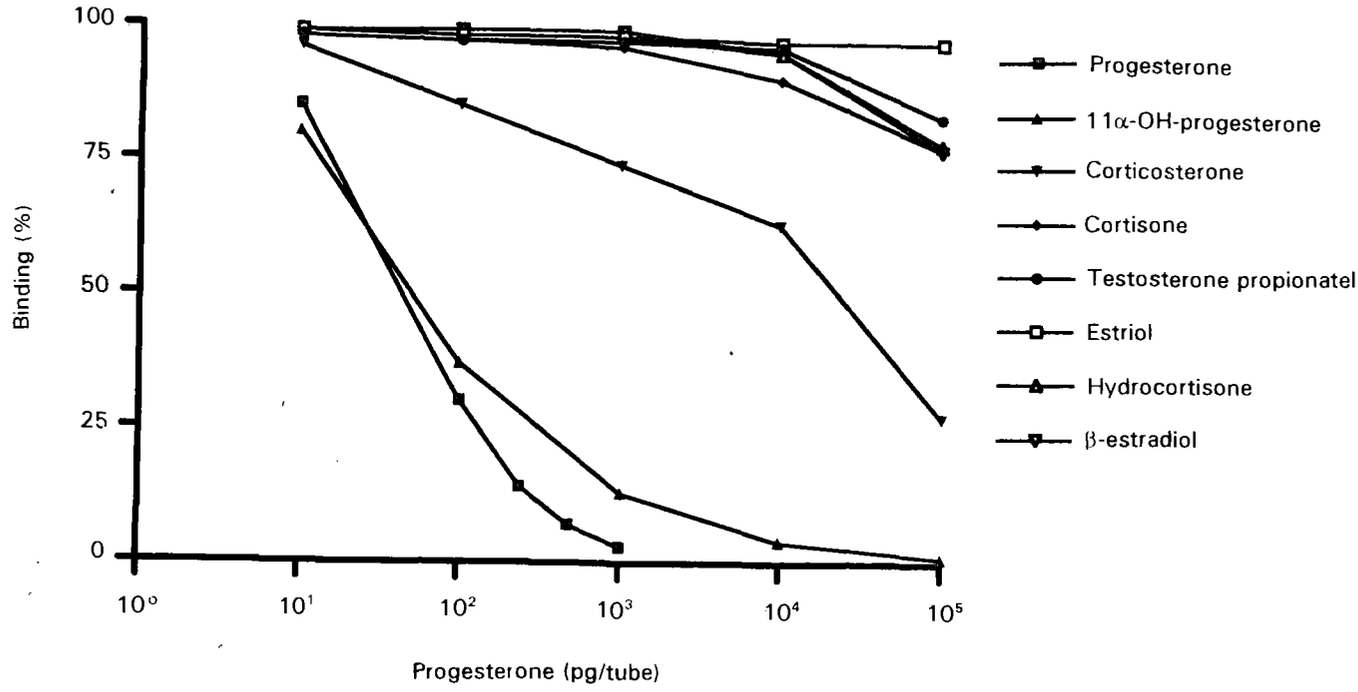


Fig. 2 Cross reactivity of progesterone antiserum against 8 steroids.

against 8 steroids, viz. progesterone, 11α-OH-progesterone, corticosterone, hydrocortisone, β-estradiol, cortisone, estriol and testosterone propionate, is presented in Fig. 2. The antiserum cross-reacted 90% with 11α-hydroxyprogesterone and <0.20% with corticosterone. Cross-reactions with all other steroids tested was <0.001.

Biological validation of progesterone antiserum: The progesterone concentrations obtained from blood samples collected from 1 pregnant and 1 cycling buffalo by using the raised antiserum (rb-6) and a previously characterized antiserum, is presented in Fig.3. The progesterone values obtained using the 2 antisera were highly correlated-0.81 and 0.90-for samples obtained from pregnant and cycling buffaloes respectively.

The results obtained clearly indicate the successful indigenous production of progesterone antiserum. The sensitivity obtained (8pg/tube) was sufficiently high to estimate the progesterone concentrations in blood plasma. The calibration curve ranges of 8 pg/tube to 250 pg/tube corresponded to 0.4 ng/ml to 12.5 ng/ml and covered the whole range of possible physiologic variations in progesterone concentrations. The high sensitivity obtained permits the application of a direct

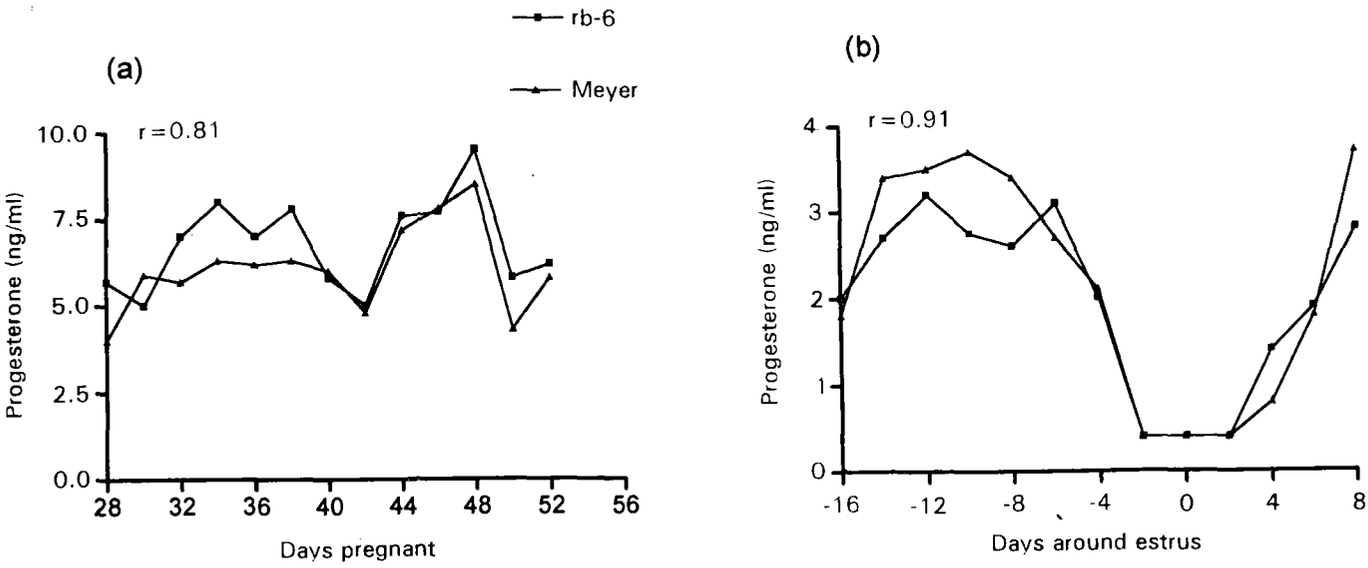


Fig. 3. Comparison of plasma progesterone concentrations using different antisera.

progesterone RIA routinely used in the lab (Kamboj and Prakash 1993) and was demonstrated by using the antiserum for progesterone estimations from blood samples of buffaloes during (a) pregnancy when concentrations are expected to be high, and (b) during cyclicty when concentrations are expected to rise and fall depending on the stage of estrous cycle (Fig.3).

The cross reactivities of the antiserum against various related steroids showed that the antiserum is highly specific for progesterone determination in RIA. The high correlation between progesterone values obtained by estimating the same samples using the indigenously produced antiserum and a previously characterized antiserum also confirms the high specificity of the antiserum. The observation that antiserum produced by different rabbits being dissimilar in characteristics (Fig.1) showed variability among animals pertaining to immunological response to the same antigen.

The indigenous progesterone antiserum production makes the hormone assay less expersive and obviates the requirement of its importation from commercial sources thereby resulting in foreign exchange saving to the country.

SUMMARY

For raising antiserum against progesterone-11a-hydroxyhemisuccinate-BSA, 4 male Chinchilla rabbits were taken. The sensitivity of the antiserum raised from 1 of these 2 rabbits was 8 pg/tube when tested in a progesterone radioimmunoassay. The antiserum was specific for binding to progesterone and exhibited very low cross-reactivity to other steroids tested. The progesterone concentrations obtained using the 2 antisera were highly correlated and similar, testifying further the high specificity of the anti-progesterone serum produced.

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Comparative study on the performance of liquid and deep frozen semen in artificial insemination in the southern Bangladesh

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Key words: Conception rate, Frozen semen, Liquid semen

In Bangladesh, large-scale use of artificial insemination (AI) was introduced in 1975-76 by establishing District AI centers. From the beginning, AI was done by liquid semen. Deep frozen semen was introduced in 1986 and used along with liquid semen. The existing AI program of Bangladesh covers only 15% of the cattle population and conception rate (CR) is much lower in AI than that of natural services (Bhuiyan 1997). The present study was carried out in the southern part of Bangladesh and a little work has been done on AI in this region. Therefore the present work was undertaken with an aim to compare the performance of liquid and deep frozen semen in AI program. In addition, Cruz *et al.*(1995) observed that CR varies with season. In this study it was planned to investigate the effect of season on CR.

The experiment was conducted at a selective area in Khulna, Bangladesh. AI was carried during August 1996 to December 1998. Both liquid and deep frozen semen were used for AI. Liquid semen was produced from 4 crossbred bulls of Khulna District AI centre. The collected fresh semen was diluted with egg yolk citrate buffer solution. Diluted liquid semen consisted of 20 to 25 million spermatozoa per dose (1 ml) in which sperm motility was 65 to 70%. In this experiment, 61 cows and heifers were inseminated with liquid semen. On the other hand, the average motility of deep frozen semen was 60% and number of motile spermatozoa per dose (0.25ml) was 30 million. A total of 123 cows and heifers were inseminated with deep frozen semen. Insemination was done by recto-vaginal method between 8 to 24 hr after onset of estrous. Out of 184 inseminated animals, 62 were pure local (indigenous zebu), 64 were local × Friesian and 58 were local × Sahiwal crossbreds. Year round AI programme was divided into 3 seasons, summer (March to June), monsoon (July to September) and winter (October to February) considering average ambient temperature, humidity and rainfall. Data were analyzed by statistical package 'MINITAB'. General Linear Model was used to analyze the effects of semen type (liquid or deep frozen) and season of AI on conception rate.

Significant difference ($P<0.05$) was found in conception rate (CR) between liquid and deep frozen semen. The higher and lower CR was found in liquid semen (65.57 ± 6.13%) and deep frozen semen (49.59 ± 4.53%), respectively, and the overall mean CR was 54.89 ± 3.68%. The findings were consistent with those of Shamsuddin *et al.* (1987). They found CR of 53.25% and 48.65% with liquid semen and deep frozen semen respectively. However, Howlader *et al.* (1997) had a different observation. They found higher CR with deep frozen semen (64.92%) than with liquid semen (58.83%). These differences might be due to variation in the fertility of donor bulls, improper maintenance of cold chain of liquid and deep frozen semen, faulty thawing techniques of deep frozen semen, variation in the concentration and motility of spermatozoa in semen, etc. CR with deep frozen semen has been reported by some workers as 62.51% by Das *et al.* (1990-93), 51.87% by Qureshi (1979), 48.5% by Prakash and Saini (1987), and 40.18% by Nair (1975).

The effect of season of AI on CR was found insignificant ($P>0.05$). The highest CR was found in monsoon (59.66 ± 4.52%) followed by summer (51.52 ± 8.83%) and winter (40.62 ± 8.82%). Possible reason is that the availability of naturally grown grasses is abundant in monsoon and scarcity of grasses in winter. Cruz *et al.* (1995) found significant effect of season of AI on CR. They found average CR in *Bos taurus* × *Bos indicus* as 61.7%, 55.9% and 54.9% in December-March, April-July and August-November respectively. Finally, it might be concluded here that the CR with liquid semen was higher than deep frozen semen. Proper preservation and handling of deep frozen semen as well as appropriate thawing techniques would enable to increase the efficiency of deep frozen semen.

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SUMMARY

The performance of liquid and deep frozen semen was

studied in AI programme. The effect of season on conception rate was AI programme. The effect of season on conception rate was also studied. A significant difference was found in CR between liquid and deep frozen semen. The effect of season on AI and CR was found insignificant.

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Bacterial load in buck semen of different Indian breeds

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Key words: Bacterial load, Goat, Semen

The main source of bacteria present in the semen of healthy animal is prepuce and its contents are mixed with the semen during ejaculation (Hare 1985). Other sources of contamination include inflammatory foci in the genital tract, the skin of animals, semen collection and processing equipment, animal handlers and laboratory personnel and semen extenders (Kendrick *et al.* 1975, Riek *et al.* 1980). The reports on bacterial load of buck semen is scanty (Naidu *et al.* 1991, Sharma and Deka 1986), however, lot of information is available on bovine semen (Meyer *et al.* 1980, Ibrahim 1983). Present study was undertaken to know the possible bacterial load in the semen of different breeds of goat and their distribution in different seasons and age groups.

Semen was collected from 6 different breeds of goats, viz. Barbari, Jamunapari, Marwari, Kutchi, Sirohi and Jakhrana. On the basis of age, bucks were classified into groups named as group 1- young (below 2 years), group 2- adult (2-6 years) and group 3- old (above 6 years). All animals were apparently healthy. These animals were maintained under semi-intensive system of management at Physiology, Reproduction and Shelter Management Division, CIRG, Makhdoom.

Semen samples from 6 breeds of goat were collected at weekly intervals throughout the year. Year was divided on the basis of mean daily temperature and microenvironment into cool (temperature range 5.28°C to 21.29°C, December to February), moderate (temperature range 13.17°C to 28.33°C, March,October and November), hot dry (temperature range 26.22°C to 46.0°C, April to June), and hot humid (temperature range 21.50°C to 38.40°C, July to September) period.

Semen samples (337) for different breeds of goats were taken for determination of bacterial load. Semen sample (0.1-0.2 ml) immediately after collection was kept in sterilized vial and there were processed for determination of bacterial load at 3 different dilution rates (10^2, 10^3 and 10^4) by single plate count method (SPC). Briefly, 1 ml semen was introduced into 3 petri-dishes for each dilution after which 15-20 ml blood agar medium is added and thoroughly mixed by rotating the plate. When the medium solidified, the organisms are trapped in the gel. It is supposed that 1 organism gives rise to 1 colony, hence, a colony count was performed on the plate revealed the viable microbial population of the inoculum. Plates were kept at 37°C for 24 hr in inverted position. Colonies were counted by illuminating them from below. Number of bacteria was calculated as per the formula:

$$\left.\begin{matrix}\text{Number of bacteria}\\ \text{per ml of semen}\end{matrix}\right\} = \text{Number of colonies counted on plate} \times \text{dilution rate of sample}$$

Least-squares analysis of variance (Snedecor and Cocharn 1980) was used to workout the significance of the results.The isolates were identified as per Buchanan and Gibbson (1974).

In present study 337 semen samples of different breeds of goats were processed for bacterial load. Bacterial load averaged 24.75±1.0, 16.16±0.8 and 9.36±0.6 CFU/ml at 10^2, 10^3 and 10^4 dilution rates respectively. Breed was found to have significant effect on bacterial load and among the breeds Jamunapari bucks had the highest bacterial load irrespective of season of study followed by Kutchi,Jakhrana, Barbari, Sirohi and Marwari at 10^2 dilution rate (Table 1).

Age has no significant effect on bacterial load as all age groups revealed more or less same counts. However, season (period) has significant effect on bacterial load. Bacterial load was highest in cool period and lowest in hot humid period and hot dry period at the 10^2 dilution rate (Table 1). The higher value of bacterial load during cool period may be due to the dampness in the soil where the bucks were housed and the close contact of the preputial sheath with soil.

Least-square analysis of variance revealed significant effect ($P< 0.01$) of breed, period and interaction between breed and period on the bacterial load, while age has no significant effect on the trait. No significant difference was observed among Barbari, Jakhrana and Kutchi. Bacterial load decreased due to washing of preputial sheath prior to semen collection.

In a study in 6 bucks, bacterial load of 80.9, 34.5 and 13.2 at 10^2, 10^3, 10^4 dilution rates was reported in prewash semen samples (Naidu *et al.* 1991). This figure is much higher

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Table 1. Least-squares mean±SE for bacterial load (CFU/ml) in buck semen

Source	n	Dilution rate		
		10^2	10^3	10^4
Overall mean	337	24.75±1.0	16.16±0.8	9.39±0.6
Breed				
Jamunapari	17	37.48±2.9	25.78±2.3	17.47±1.7
Barbari	42	24.93±2.3	14.97±1.8	7.88±1.4
Jakhrana	8	25.25±3.7	16.67±2.8	9.74±2.2
Marwari	109	15.63±1.5	8.37±1.1	3.99±0.9
Kutchi	69	26.41±1.0	19.18±1.3	11.60±0.9
Sirohi	92	19.79±1.5	11.18±1.2	5.64±0.9
Age group				
1	70	25.24±1.7	16.68±1.3	9.26±1.0
2	229	24.82±1.2	16.07±0.9	9.42±0.7
3	38	24.19±2.0	15.73±1.6	9.47±1.2
Period				
Cool	34	39.84±2.1	29.00±1.6	19.79±1.3
Moderate	94	24.19±1.6	13.79±1.3	6.34±0.9
Hot dry	55	18.07±1.9	11.43±1.5	6.30±1.2
Hot humid	154	16.91±1.7	10.31±1.4	5.11±1.1

than the present finding. The mean percentage of reduction in the number of colonies consequent to preputial washings was 45, 55, and 94, respectively, in the said dilutions (Naidu *et al.* 1991). The average bacteria in neat semen without addition of antibiotics was 13 000 bacteria per ml of buffalo semen (Verma *et al.* 1996).

The most common contaminants in the semen were *E.coli, Bacillus* sp., *Pseudomonas* sp., *Staphylococcus* sp. The reduction of bacterial count in post wash samples suggested that the main source of bacteria is the prepuce. Though these organisms do not have definite pathogenic significance, but there increasing load may affect the motility and viability of sperms in the semen.

Till date no information is available on regulatory threshold limit of bacterial load in buck semen. Hence the work is needed in the said area as semen with higher bacterial load impairs the reproductive functions of male and female.

Therefore, present study emphasized that there is a need to establish a limit on the number of nonpathogenic organisms that can be present in buck semen to be used for artificial insemination.

SUMMARY

The semen of different Indian goat breeds, viz. Barbari, Jamunapari, Marwari, Kutchi, Sirohi and Jakhrana was studied to find out the effect of cool (December to February), moderate (March, October and November), hot dry (April to June) and hot humid (July to September) and age on bacterial load in buck semen. Semen of Jamunapari buks showed highest bacterial load (37.48 CFU/ml) irrespective of seasons. Age has no significant effect on bacterial load. However, periods (seasons) had significant effect on bacterial load. Most of the bacterial contaminants were of non-pathogenic nature.

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Milk yield, its composition and performance of Malpura ewes under protocol of grazing and supplementation

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ABSTRACT

The reported study was conducted to assess potential milk yield of Malpura ewes under protocol of grazing with concentrate supplementation @ 1.5% of body weight. The bio mass yield of *Cenchrus ciliaris* dominated pasture was 18.2 q/ha and the mouth grab sample of the vegetation contained 8.3% CP while the concentrate mixture offered to the ewes had 15.7% CP. Milk yield of the ewes was 525 ml on the date of lambing and peak yield was recorded on 1st, 2nd an 3rd week of lactation (548 ml) and the yield gradually declined by 12th week of lactation. Based on lactation records of 3 months, the daily milk yield of the ewes was 496 g/day. The average milk fat content of the ewes in total lactation length of 90 days was 6.1%: the milk fat content increased with decrease in milk yield (r = - 0.62). On an average the milk contained 3.4% lactose, 5.1% protein, 9.5% SNF and 15.8% TS. The ash content was higher in 1st and 2nd week (3.3%) than other periods (0.9%). The ewes showed linear gain in body weight after termination of pregnancy in lactation phase of the study. It is concluded that average milk yield of Malpura ewes was 496 g/day in the lactation period of 3 months. Further the lactating ewes showed linear gain in body weight under protocol of grazing with concentrate supplementation @ 1.5% body weight which was adequate for their requirements.

Key words: Ewe, Milk composition, Milk yield

Under field condition, in western part of the country, the ewes have potential yield of 300-400 ml/day, of which about 150-200 ml is stripped and rest is allowed to be suckled by the lambs (Riyazuddin *et al.* 1998). Organized feeding management on research farm under protocol of grazing with supplementation indicated that, Bharat Merino ewes have daily milk yield of 643 g (Singh and Singh 1987). Such information for Malpura breed is however lacking in literature.

The reported study was therefore conducted at the research farm to assess potential milk yield of Malpura ewes under protocol of grazing with supplementation.

MATERIALS AND METHODS

Malpura ewes (12), 3- to 4-year-old, in advance stage of pregnancy were adopted for the study. All the ewes were in second parity. Prior to adoption in the experiment, the ewes were maintained on grazing for 8 hr on *Cenchrus ciliaris* dominated pasture under supervision of a grazer followed by free choice conserved fodder in hay racks, in the animal shed. Before initiation of the study all the ewes were dewormed, vaccinated against common contagious diseases, and during the course of the study given veterinary aid as and when required. The experimental flock had free access to clean drinking water twice daily, in the morning before taking them out for grazing and in the afternoon, on their return from grazing area. The ewes after grazing were housed during night in a side open asbestos roofed animal shed.

The adopted pregnant ewes in addition to free grazing individually received concentrate supplementation @ 1.25% of their body weight. The concentrate (barley 62%, groundnut-cake 15%, wheat bran 20%, mineral mixture 2% and salt 1%) offer for each ewe was revised in weekly intervals based on their body weight changes. The chemical composition of the concentrate mixture and mouth grab sample of vegetation consumed by the animals are presented in Table 1.The ewes after termination of pregnancy were followed up in lactation phase till 3 months post lambing . The concentrate allowance was increased to 1.5% of ewe's body weight to meet the added requirement of lactation phase. The composition of the concentrate mixture, grazing protocol and feeding management were, however, similar to those of pregnancy phase. After termination of pregnancy, the ewes were initially confined in a pen for 7 days followed by grazing and supplementation as in pregnancy phase. Milk yield of all the ewes was recorded in weekly intervals by lamb suckling method along with estimation of milk composition. The

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Table 1. Chemical composition of mouth grab samples and concentrate mixture (% DM)

Attributes	Mouth grab sample of vegetation	Concentrate mixture
Crude protein (CP)	8.31	15.70
Neutral detergent fibre (NDF)	70.59	48.37
Acid detergent fibre (ADE)	54.26	18.91
Cellulose	37.69	14.17
Lignin	6.12	4.14
GE (kcal/g DM)	4.13	4.51

experiment was continued for 90 days.

Biomass yield and chemical analysis

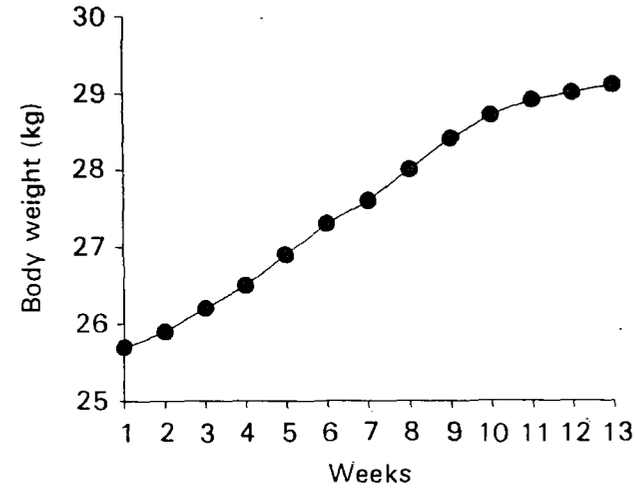
Biomass yield of the pasture (Tadmore *et al.* 1975) and per cent distribution of plant species in grazing (Holechek et al. 1986) were assessed. Milk yield of the ewes was estimated by lamb suckling method. Fat, lactose, milk protein, SNF, total solids and ash content of the milk were estimated (AOAC 1984). Fibre fractions of mouth grab sample was estimated as per Goering and Van Soest (1969).

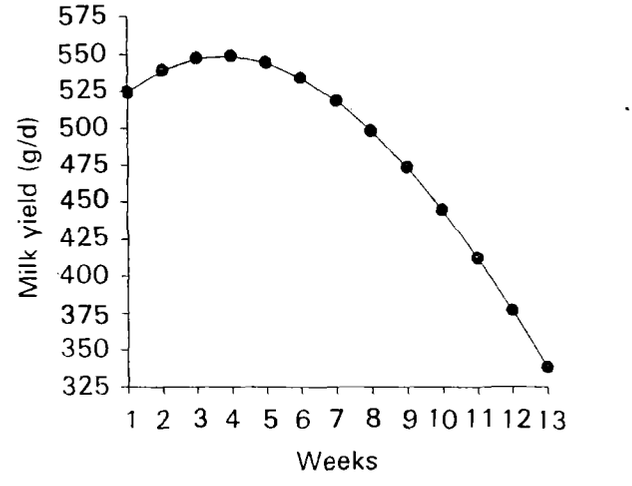
Statistical analysis

The generated data were subjected to analysis of variance (Snedecor and Cochran 1967) and significant group differences were compared by Duncan's multiple range test (Duncan 1955). Body weight changes and milk yield of individual ewe during lactation phase was charted by fitting polynomial curve and the pooled constants are presented graphically.

RESULTS AND DISCUSSION

The biomass yield of the *Cenchrus* dominated pasture was





Figs 1-2. **1.** Body weight changes of ewes during lactation phase. **2.** Milk yield of the ewes during the experiment.

Table 2. Milk composition of the ewes on weekly basis

Weeks	Fat%	Lactose%	Protein%	SNF%	TS%	Ash%
1	3.15±0.19	2.66±0.40	4.76±0.37	13.74±1.31	16.89±1.25	3.15±0.19
2	3.47±0.18	2.62±0.28	4.58±0.17	12.40±0.78	16.86±0.88	3.47±0.18
3	4.28±0.34	3.04±0.29	5.43±0.09	11.36±0.81	15.63±0.93	0.69±0.04
4	5.57±0.49	3.79±0.40	5.33±0.36	10.15±0.45	15.17±0.83	0.72±0.05
5	5.58±0.64	2.41±0.59	5.75±0.16	8.26±0.41	13.85±0.41	1.23±0.10
6	6.95±0.76	2.33±0.60	5.67±0.11	7.49±0.36	14.28±0.72	1.05±0.15
7	6.73±0.50	3.29±0.31	5.45±0.32	9.16±0.55	15.89±1.00	1.03±0.02
8	7.18±0.60	4.57±0.24	4.81±0.37	9.40±0.96	16.59±1.22	0.92±0.14
9	7.23±0.53	5.29±0.29	3.61±0.18	8.62±0.26	15.85±0.55	0.87±0.13
10	7.52±0.42	4.29±0.39	5.15±0.36	10.54±0.18	17.97±0.55	0.84±0.02
11	7.77±0.34	3.12±0.53	5.58±0.31	7.20±1.09	14.99±1.16	0.85±0.07
12	7.62±0.29	3.31±0.39	5.29±0.31	7.49±1.02	15.10±1.04	0.84±0.06
Average	6.08±0.48	3.39±0.26	5.12±0.17	9.50±0.59	15.80±0.34	1.3±0.27

18.2 q/ha. The pasture was dominated by *Cenchrus ciliaris* (73%), *Sorghum helepanse* (4.5%), *Achyranthes aspera* (4.0%), *Zizyphus nummularia* (3.8%), *Commelina forskalei* (1.5%), *Vigna sinensis* (1.1%), *Vermonia cineraria* (0.4%) and others seasonal shrubs/forbs (11.7%). Shinde *et al.* (1996) reported similar composition of pasture vegetation. The mouth grab sample of the vegetation contained 8.3% CP and the concentrate mixture offered to the ewes had 15.7% CP (Table 1).

The pattern of milk yield of the Malpura ewes from lambing till weaning of the lamb, is presented in Fig.1. Milk yield of the ewes was 525 ml on the day of lambing and peak yield was recorded on 1st , 2nd and 3rd week (548 ml) of lactation (Fig. 2). Thereafter the yield gradually declined till 12th week of lactation. Based on lactation records of 3 months it was estimated that on an average the daily milk yield of the ewes was 496 g/day. The daily milk yield Malpura was lower than Bharat Merino ewes maintained under organized feeding management (Singh and Singh 1987) while, the yield was higher than the mixed breed of ewes maintained by farmers under free grazing on natural range land with top feed supplementation (Riyazuddin *et al.* 1998).

The average milk fat of the ewes in total lactation length of 90 days was 6.1%. which is in agreement with 6.8% average milk fat reported for sheep from Middle Eastern region (Bhattacharya and Harb 1973). The milk fat content was lower ($P<0.01$) in 1st and 2nd week (3.3%) and gradually increased with progression of lactation length reaching 7.6% by the end of the study (Table 2). The daily milk yield and fat% were inversely related ($r = -0.62$) which is a consistent finding in other studies (Patel and Dave 1982). The lactose content of the milk also varied significantly ($P<0.01$) over the lactation length with overall average of 3.39 g% while no definite trend could be established over the experimental period. Similarly the total protein content also varied ($P<0.01$) over the weeks with overall average value of 5.1 g%. The SNF was, however, significantly ($P<0.01$) higher in the 1st week of lactation (13.7 g%) and gradually decreased to 7.5 g% by 12 week. The variation in SNF values over period was as per the convention. The total solid content of the milk remained similar over the 12 weeks period and ranged from 13.8 to 16.9%. The ash content was higher ($P<0.01$) in 1st and 2nd week of lactation (3.3%) than other periods (0.9%). Mba *et al.* (1975) also observed that percentage of fat, protein and lactose decreased with advancing of lactation.

Body weight changes of the experimental ewes are presented in Fig.1. The ewes showed gain in body weight after termination of their pregnancy: the body weight was 25.7 kg on the day of lambing which increased to 29.1 kg by the end of the study. Gain in body weight during the lactation phase indicated that the nutrient intake of the ewes under the experimental protocol of grazing with supplementation @ 1.5% of body weight was more than adequate for their requirement.

It is concluded that average milk yield of Malpura ewes was 496 g/day in the lactation period of 3 months and that the milk yield and its composition over the lactation period followed the conventional trend. Further the lactating ewes showed linear gain in body weight under protocol of grazing with concentrate supplementation @ 1.5% body weight which was adequate in meeting their requirements.

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In situ protein degradability of certain feedstuffs in the rumen of cattle

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ABSTRACT

In situ crude protein (CP) disappearance of soybean extraction, sunflower extraction, rapeseed extraction, maize gluten meal-60 (60% CP), maize gluten meal-40 (40% CP), copra-cake extraction, safflower-cake, broken rice, jowar grain, ragi grain, rice polish and maize bran in the rumen at different incubation periods were determined by nylon bag technique using 3 adult fistulated steers. The residual CP values were transformed to natural logarithms and then subjected to linear regression to arrive at degradation constants. The effective degradability was calculated for an assumed out flow rate of 5%/hr. The effective CP degradability of soybean extraction, sunflower extraction, rapeseed extraction, maize gluten meal-60, maize gluten meal-40, copra-cake extraction, safflower-cake, broken rice, jowar grain, ragi grain, rice polish and maize bran was 54, 54, 69, 21, 37, 33, 63, 32, 15, 32, 47 and 37% respectively. Maize gluten meal-60, maize gluten meal-40, soybean extraction and copra-cake extractions provided high amounts of UDP (490, 252, 210 and 150g) per kg dry matter as compared to other protein supplements. The energy supplements (ragi grain, jowar grain, broken rice, maize bran and rice polish), although in small quantities, contribute more undegradable protein than rumen degradable protein at an outflow rate of 5%/hr.

Key words: Energy supplements, Protein supplements, Protein degradability, RDP, UDP

The metabolizable protein systems (ARC 1980, NRC 1985) proposed as an improvement to the digestible crude protein (DCP) system for protein evaluation of feeds and for expression of protein requirement of ruminants, require the description of dietary crude protein in terms of rumen degradable protein (RDP) and undegradable protein (UDP). This not only applies to the protein supplements but also to the cereals and cereal by-products which make up a considerable proportion of ration for ruminants. The protein degradability values varies considerably due to the source of raw materials and the processing methodology. Therefore, it is essential to have more data on protein degradability of feedstuffs to have a comprehensive knowledge. The present study was, therefore, undertaken to assess the protein degradability of some commonly used protein sources, cereals, millets and cereal by-products.

MATERIALS AND METHODS

Adult crossbred steers (3) of about 350 kg body weight fitted with large rumen canula were used to estimate the degradability of feedstuffs. Animals were fed concentrate mixture (maize 30%, groundnut-cake 25%, wheat bran 42%, mineral mixture 2% and salt 1%), green grass (para grass) and ragi straw to meet the nutrient requirement for maintenance (ICAR 1985).

Protein sources such as soybean extractions, sunflower extractions, rapeseed extractions, maize gluten-meal 60 (60%CP), maize gluten-meal 40 (40%CP), copra-cake extractions, safflower-cake and energy sources such as broken rice, jowar grain and ragi grain and by-products like rice polish and maize bran were used for the study. The feed samples were ground to a particle size of 2-3mm. However, soybean extractions, rice polish, maize bran etc., were smaller in particle size and used as such. The samples were placed in nylon bags (pore size 40mm) of size 100 mm × 170 mm and suspended in the rumen of fistulated animals for 3, 6, 9, 12 and 24 hr.

Nylon bags were introduced into the rumen in descending order (i.e. 24 hr was introduced first), so that all bags were removed and washed at the same time. Control samples (0 hr) were washed without incubation in the rumen in a washing machine and dried to a constant weight at 60°C as was also practiced for nylon bags removed after specific incubation in the rumen. Each sample was tested in duplicate for 2 consecutive days in the rumen of 3 animals. The dry matter (DM) and crude protein (CP) disappearing from samples at different incubation periods were estimated (Mehrez and Orskov 1977). The residual CP values were transformed to natural logarithms and then subjected to linear regression

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Table 1. Per cent of DM and protein disappeared from feedstuffs incubated in the rumen for different periods of time (Average of 12 observations)

Feedstuff		Period of incubation (hr)				
		3	6	9	12	24
Soybean extraction	DM	21.85±2.23	31.62±1.95	44.24±1.47	57.24±1.70	79.26±2.53
	Protein	8.30±1.65	23.19±1.44	42.57±2.51	54.22±2.15	66.73±2.16
Sunflower extraction	DM	15.17±2.88	21.00±3.29	33.11±3.73	37.01±3.72	47.38±2.09
	Protein	25.45±2.58	35.60±5.41	45.42±4.51	46.92±2.97	65.45±2.06
Rapeseed extraction	DM	56.50±0.94	60.61±1.02	63.99±1.66	64.86±0.80	66.60±0.71
	Protein	50.50±5.38	52.61±4.06	56.58±4.56	62.10±6.89	68.90±1.06
Maize gluten-meal	DM	14.35±0.98	18.30±1.08	21.79±1.18	28.32±1.40	43.84±1.95
(60%CP)	Protein	3.15±1.04	5.21±1.07	8.88±1.16	15.06±1.29	30.75±1.93
Maize gluten-meal	DM	9.89±0.49	17.02±0.61	27.61±1.78	31.36±1.07	57.67±1.70
(40%CP)	Protein	11.25±0.86	20.95±1.08	28.66±1.96	28.71±2.02	44.10±3.06
Copra-cake extraction	DM	16.65±1.02	21.72±0.91	23.33±2.21	24.06±1.56	35.55±1.21
	Protein	22.13±1.96	23.85±1.05	24.11±1.90	30.00±2.99	42.54±2.59
Safflower-cake	DM	8.28±0.72	14.56±0.53	17.99±0.55	21.38±0.29	25.59±0.46
	Protein	47.07±2.49	55.06±1.82	56.44±1.91	57.59±1.12	71.85±1.18
Broken rice	DM	3.06±0.48	9.58±1.72	14.50±1.69	28.37±2.05	83.31±3.40
	Protein	3.20±0.85	10.18±1.51	12.68±1.32	21.64±4.75	55.90±2.66
Jowar grain	DM	7.72±0.64	15.46±1.61	26.35±1.27	32.29±2.19	75.26±1.36
	Protein	1.00±0.23	3.19±0.87	7.33±1.24	9.45±0.79	59.63±2.97
Ragi grain	DM	15.53±4.83	30.18±1.77	45.01±1.67	57.55±1.94	74.82±1.36
	Protein	4.13±1.28	8.59±2.11	13.69±1.79	26.69±2.99	42.26±0.15
Rice polish	DM	12.48±1.58	17.87±2.01	27.26±2.5	35.60±1.94	48.55±1.54
	Protein	14.17±2.33	20.42±1.44	32.97±1.68	42.29±1.74	56.66±1.39
Maize bran	DM	13.95±0.94	23.97±1.7	32.83±6.20	39.99±1.75	59.98±8.98
	Protein	2.86±0.86	11.65±1.73	21.77±1.33	28.91±1.67	55.92±2.69

(Snedecor and Cochran 1968). The degradation constant was calculated at 3 to 12 hr period. The effective degradability was calculated for an assumed outflow rate of 5% / hr (Miller 1980).

RESULTS AND DISCUSSION

The percentage of DM and CP disappearing from feedstuffs at various hours of rumen incubation is given in Table 1. In protein supplements and cereal by-products the trend of disappearance was similar for both dry matter and protein except of safflower-cake. The DM disappearance of safflower-cake was much lower (26 %) than that of protein disappearance (72 %) during 24 hr of incubation in the rumen. The lower rate of DM disappearance may be attributed to higher fibre (30 %) in safflower-cake. Sampath and Sivaraman (1985) also observed a poor correlation between protein and DM disappearance in high fibrous feeds. In energy supplements, the disappearance of DM and protein was very slow up to 12 hr of rumen incubation after which there was a

Table 2. Crude protein, degradability, RDP and UDP content of feedstuffs at an outflow rate of 5% /hr

Feedstuff	CP (%)	Degradability (%)	RDP g/kg DM	UDP g/kg DM
Soybean extraction	46	54	250	210
Rapeseed extraction	37	69	260	110
Sunflower extraction	30	54	160	140
Maize gluten-meal (60%CP)	62	21	130	490
Maize gluten-meal (40%CP)	40	37	148	252
Copra-cake extractions	23	33	80	150
Safflower-cake	23	63	150	80
Broken rice	11	32	35	75
Ragi grain	10	32	32	68
Jowar grain	14	15	21	119
Rice polish	13	47	61	69
Maize bran	16	37	59	101

steep rise. This slow rate of degradation up to 12 hr in grains may be attributed to the hard coat in these materials, which makes them more resistant to microbial attack.

Among the protein supplements studied, the rapeseed extraction and safflower extraction were highly susceptible to rumen microbial degradation with ruminal protein degradability values of more than 60% at an outflow rate of 5% / hr, while the maize gluten-meal-60 was least degraded with 21% degradability at the same outflow rate (Table 2). Other protein supplements such as soybean extraction, sunflower extraction, copra-cake extraction and maize gluten-meal-40 had the degradability values in the range of 30 to 60%. Thus the maize gluten-meal provided the highest amount of UDP/kg DM, while the rapeseed extraction provided the highest amount of RDP/kg DM. High degradability values of 70 and 73% for rapeseed-meal and 77% for safflower-cake were reported by Sinha and Singhal (1997), Wadhwa *et al.* (1993), and Anjaneya Prasad (1997), respectively. Low degradability value of 22% for maize gluten-meal and degradability value of 38 and 54% for soybean extractions were reported by Chaturvedi and Walli (1995), Garg (1997) and Anjaneya Prasad (1997), respectively. It is difficult to fix absolute protein degradability values for feedstuffs. Several factors introduce considerable variations into what is supposed to be standard for comparison. It is realistic therefore, to determine the relative protein degradability values for different feedstuffs and to rank them relative to one another. The protein degradability values for rapeseed extractions, safflower extraction, maize gluten-meal-60, soybean extraction and sunflower extractions obtained in this investigation are in the range of earlier reported values. However, in copra-cake extraction, the 33% ruminal protein degradability in this study is slightly higher than the value of 21% reported by Garg (1997) and 20% reported by Kalbande and Thomas (1999). This variation may be attributed to the temperature and time to which the copra-cake is subjected during oil extraction process. These processing factors affect the susceptibility of feedstuff for ruminal degradation. Same is also the case with maize gluten-meal sample with low protein (40%) which had degradability of 37% as compared to 21% for the maize gluten with 60% CP.

Information is limited about the ruminal protein degradability values of energy supplements and cereal by-products such as broken rice, ragi grain, jowar grain, rice polish and maize bran. Although, the contribution of these feed ingredients in terms of crude protein is lower as compared to protein supplements, they make up a considerable proportion of concentrate mixture in the diet of ruminants in southern parts of India. Their protein content vary from 10 to 16%. The effective ruminal protein degradability of broken rice and ragi grain were 32%, while that of jowar grain was 15% at ruminal outflow rate of 5%/ hr. The slow rate of degradation of these grains in the rumen up to 12 hr period because of their hard coat is reflected in the lower effective crude protein degradability. The lower degradability of these grains in the rumen is of considerable importance in feeding of lactating animals as some of these grains (e.g., broken rice and ragi) are rich in lysine and methionine (Gopalan *et al.* 1986) which are considered as limiting amino acids for milk production.The effective crude protein degradability of rice polish and maize bran is 47 and 37 %, respectively. These by-products form the important ingredients of the concentrate mixture of lactating animals because of their bulky nature. They contain higher amounts of crude protein (13 to 16%) and thus they contribute more UDP as compared to grains.

It can be concluded from these results that maize gluten-meal-60, maize gluten-meal-40, soybean extraction and copra extraction are good sources of bypass protein and the energy supplements and cereal by-products (ragi grain, jowar grain, broken rice, maize bran and rice polish), although in small quantities, contribute more UDP than that of RDP at an outflow rate of 5%/hr.

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Effect of replacing different levels of skimmed milk powder with fish-meal on nutrient utilization

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ABSTRACT

Piglets (136), each weaned fractionally at 5 kg weight, after 3 weeks of age, belonging to Landrace, Tamworth and their crosses with *desi* were taken for the present study. The control group (T_1) was fed standard pre-starter diet (20% CP and 3500 Kcal DE/kg of diet) containing 8% skimmed milk powder (SMP). Experimental groups T_2 and T_3 were fed diets in which 25 and 50% of SMP was replaced by fish-meal, respectively, on isoproteinous and isocaloric basis. The average body weight at eighth week of age in T_1, T_2 and T_3 diets, did not differ significantly. There was no significant difference among diets with respect to nutrient utilization. The feed conversion efficiency in T_1, T_2 and T_3 groups were 2.17: 1, 2.25: 1 and 2.32: 1, respectively. The cost of feed/kg gain in body weight were significantly higher in T_1 (Rs 13.36) than that in T_2 (Rs 12.50) and T_3 (Rs 12.38), the difference between later 2 diets was nonsignificant. It was thus concluded that 50% of the SMP can economically be replaced by fish-meal without affecting the palatability and nutritive values of the pre-starter diets besides growth rate of piglets.

Key words: Cheaper diets, Nutrient utilization, Pigs

The present study was planned to see the effect of replacing SMP with fish-meal to cut down the cost of feed per unit gain without affecting the growth performance of piglets.

MATERIALS AND METHODS

Piglets (136) belonging to Landrace, Tamworth and their crosses with *desi*, were weaned fractionally reaching 5 kg body weight after 3 weeks of age. The control group (T_1) was standard pre-starter diet (20% CP and 3500 Kcal DE/kg diet) containing 8% SMP. In the other 2 diets SMP was replaced by fish-meal to the extent of 25% (T_2) and 50% (T_3). The animals were maintained on isonitrogenous and isocaloric diet (Table 1). After 8 weeks of feeding, 2 piglets from each litter were selected randomly and subjected to metabolic trial. An acclimatization period of 2 days was, before 4 days of collection period (King *et al.* 1960) allowed for adjusting the piglet in the metabolic cage. Feeds and faeces samples were analysed for all the proximate principles, and urine for nitrogen content (AOA 1980). Calcium in feed, faeces and urine was determined (Talpatra *et al.* 1940). Phosphorus in feed, faeces and urine was estimated calorimetrically (Ward and Johnstone 1962). Ballistic bomb callorometer was used for the estimation of energy of feed and faeces. The data were analysed statistically (Snedecor and Cochran 1968).

Table 1. Per cent chemical composition of diets in different treatments

Particulars	T_1	T_2	T_3
CP	20.02	20.12	20.08
EE	10.04	10.13	10.09
CF	4.26	4.11	4.18
NFE	56.85	57.07	57.21
Total ash	8.83	8.57	8.44
OM	91.17	91.43	91.56
Ca	1.46	1.68	1.75
P	0.58	0.65	0.72
Lysine (calculated)	1.00	1.00	1.00
Meth + cyst (calculated)	0.67	0.66	0.66
GE (kcal/kg)	4089	4076	4066
DE (kcal/kg)	3522	3495	3468

RESULTS AND DISCUSSION

Per cent chemical composition of T_1, T_2 and T_3 diets in terms of CP, EE, CF, NFE, total ash, DM, Ca, P and energy contents were almost same (Table 1). Diets had nonsignificant influence on DM intake of piglets. AS CP contents and their

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Table 2. Average growth rate and economics of feeding in different treatment groups

Particulars	Dietary groups		
	T_1	T_2	T_3
Birth weight (kg)	1.02±0.02 (86)	1.11±0.14 (25)	1.06±0.03 (25)^{ns}
Wt. at 8th week (kg)	7.36±0.12 (77)	7.17±0.40 (24)	7.34±0.18 (24)^{ns}
Daily weight gain (g)	113.06±2.02(77)	105.48±7.18(24)	111.09±3.13(24)^{ns}
Total feed consumed/litter (kg)	26.73±3.83(11)	29.50±10.12(3)	29.65±4.71(3)
Total weight gain/litter (kg)	12.45±1.83(11)	13.32±4.86(3)	12.85±2.18(3)
Feed conversion ratio	2.174:1±0.03(11)	2.25:1±0.01(3)	2.32:1±0.07(3)^{ns}
Economics of feeding (Rs/kg gain)	13.36^a	12.50^b	12.38^b

Figures in parentheses indicate number of observations. NS-Nonsignificant in the row. Means having different superscripts in the row differ significantly.

digestibilities in all the 3 diets are almost same, so, CP and DCP intake did not differ significantly (Table 3). However, significantly higher DE intake was recorded in T_1 than T_2. This might be because of slightly increased DM intake besides slightly more DE content in the diets of this group, however, it did not differ significantly from T_3.

Digestibility coefficient and nutritive value

Digestibility of DM, CP, CF, NFE, OM and GE and nutritive values in terms of DCP and DE of 3 diets did not differ significantly among themselves (Table 3) indicating that all the diets were digested with similar degree in piglets. The result is in quite agreement with the findings of Newport

Table 3. Nutrient utilization of different diets in piglets

Particulars	T_1	T_2	T_3
Nutrient digestibility (%)			
DM	83.81±0.37	83.64±0.46	83.29±0.41^{ns}
CP	82.18±0.49	81.20±1.38	80.92±1.54^{ns}
EE	92.51±0.26	93.12±0.71	92.18±0.24^{ns}
CF	54.14±0.60	54.29±1.44	53.43±1.24^{ns}
NFE	89.28±0.36	87.71±0.73	88.83±0.60^{ns}
OM	86.26±0.32	84.94±0.69	86.81±0.88^{ns}
GE	86.14±0.86	85.74±1.89	85.29±1.98^{ns}
Nutritive value			
DCP (%)	16.45±0.10	16.34±0.28	16.25±0.31^{ns}
DE (Dcal/kg)	3522±35	3495±77	3468±80
Nutrient intake per 100 kg body weight			
DM (kg)	4.98±0.17	4.60±0.38	4.76±0.24^{ns}
CP (kg)	0.98±0.03^{ns}	0.93±0.08	0.98±0.05^{ns}
DCP (kg)	0.80±0.03	0.75±0.06	0.77±0.04^{ns}
DE (Mcal)	19.55±0.68^a	16.08±1.32^b	16.51±0.84^{ab}
Balance (g/day/piglet)			
Nitrogen			
Out go faeces	2.67±0.22	3.74±0.47	2.47±0.35
Out go urine	2.34±0.19	2.60±0.31	2.09±0.08
Retention	9.79±0.99	10.00±0.53	9.47±1.39^{ns}
Calcium			
Out go faeces	2.25±0.18	4.30±0.70	3.08±0.65
Out go urine	0.43±0.14	0.33±0.01	0.32±0.06
Retention	3.96±0.37	3.90±0.39	4.12±0.20^{ns}
Phosphorus			
Out go faeces	0.59±0.05	0.99±0.20	0.88±0.29
Out go urine	0.04±0.01	0.07±0.02	0.08±0.03
Retention	2.01±0.18	2.24±0.11	2.19±0.10^{ns}

N-Nonsignificant, means bearing different superscripts differed significantly.

and Keal (1983) who observed that protein source had no effect on apparent digestibility of nitrogen.

Piglets fed T_1, T_2 and T_3 diets were positive N, Ca and P balance which did not differ significantly among themselves (Table 3) indicating that utilization of nutritent of these diets are almost similar. Newport and Keal (1983) also observed that protein source has no effect on apparent digestibility of nitrogen and its retention in piglets.

Growth rate

Birth weight, weight at eighth week of age and weight gained by piglets of T_1, T_2 and T_3 groups, did not differ significantly (Table 2) indicating that all the 3 diets are equally efficient so far as their performance is concerned. Nonsignificant difference between ration T_2 in which SMP was completely replaced by fish-meal and ration T_1 in which 4% SMP was mixed in ration was also reported by Devi and Singh (1998) and Devi *et al.* (1999). The present finding is in quite agreement with that of Newport and Keal (1983). DeBoer *et al.* (1988) also reported that piglets growth performance was not adversely affected by mixture of different sources of protein or solely on animal protein diets.

Feed conversion efficiency (FCE)

The quantity of various pre-starter diets (T_1, T_2 and T_3) required to produce 1 kg gain in body weight were 2.17, 2.25 and 2.32 kg, respectively, which did not differ significantly indicating that all the 3 diets were equally efficient in producing unit gain in body weight. The results are quite similar to those reported by Devi and Singh (1998a). The FCE of these weaner diets are quite normal and comparable with those of Wong (1985). However, Postavalau and Chura (1987) reported lower FCE varying from 3.56 to 3.87 kg/kg gain. The variation may be due to differences in diet composition and breeds used.

Economics of production

The cost of T_1 feed for per kg body weight gain was significantly higher (Rs 13.36) than that of T_2 (Rs 12.50) and T_3 (Rs 12.38), the differences between latter 2 was nonsignificant. The cost incurred on preparation of feed T_2 and T_3 was 6.44 and 7.34% less, respectively, in comparison to T_1 feed. Significantly lower cost of feed for unit gain in body weight was also reported by Devi and Singh (1998a). The present findings are also in good agreement with the report of Newport and Keal (1983), who reported that FCE was not affected whether SMP was used as the sole source of protein or mixture of soybean and fish-meal.

Incidence of diarrhoea and mortality

Incidence of diarrhoea and mortality in piglets maintained on T_1, T_2 and T_3 diets did not differ significantly either from birth to 3 weeks or from 4 to 8 weeks of age indicating that there was no effect of replacing varying levels of SMP by fish-meal in pre-starter diets. Devi and Singh (1998) also reported nonsignificant difference in mortality rate of piglets kept on T_1 (with SMP diet) and T_2 (without SMP diet).

It could be concluded from the studies that replacing 25 and 50% of SMP with fish-meal on isonitrogenous basis in the diet of early weaned piglets did not affect the nutrient intake and digestibility. 50% replacement of SMP with fish-meal worked out to be economical in terms of feed conversion efficiency.

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The combined methionine and cystine requirement of growing cockerels in the tropics

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ABSTRACT

Methionine + cystine (met + cys) requirements of cockerels aged between 8 and 16 weeks of age was studied by the nutrient-response relationship. Experimental diets were prepared by supplementing common basal diets with graded levels of met such that dietary met ranged between 0.21 and 69% of the diet. The birds were fed and watered *ad lib.* for 56 days (8-16 weeks). Dietary supplementation increased weight gain, feed conversion efficiency and protein efficiency ratio (PER) with optimal levels obtained between 0.29% (met .55% met + cys) and 0.37% met (0.63% met + cys). The dressing percentage was significantly affected by dietary treatments but optimal level (0.29%) was lower than where highest weight gain and feed efficiency were obtained.

Key words: Cockerels, Cystine, Methionine

The commercial cockerel chickens in the tropics has until recently been the forgotten member of the poultry fraternity with regard to nutritional research, apart from the few reports available (Okosun and Tewe 1987, Okosun 1987) there is lack of definite information on their specific requirements especially for amino acids which had obviously hindered progress in cockerel rearing. Therefore, additional information still need to be established if cockerel production is to become an attractive component of the tropical poultry industry.

MATERIALS AND METHODS

Barred Harco cockerels (240) at 8 weeks of age were weighed and divided into 16 groups of 15 birds each. Two such groups were randomly assigned to each dietary treatment.

Experimental diets (8) were formulated by adding graded levels of DL-met to the standard basal diet in 0.08% step-wise increments such that dietary met ranged between 0.21 and 0.69% of the diet (Table 1). A standard fish-meal control diet was also formulated and the total sulphur amino acids (Met + Cys) of test ingredients were 0.34% for maize, 0.38% (maize offals), 1.16% for groundnut-cake and fish-meal (2.4%).

The mixing of diets were as recommended by Larrabee (1976). All the rations were isoproteinous (16% Cp) and isocaloric (2250 Kcal ME/kg). The cockerels were managed in deep litter pens in an open sided tropical type house. Continuous light was provided, feed and water were supplied *ad lib.*

Weekly body weight, feed consumption and mortality were recorded. Nitrogen retention (Okosun 1987) and carcass measurements (Oluyemi and Roberts 1979) were estimated in the 16th week. At the 16th week, chickens from each replicate diet were bled and the pooled blood were kept and serum separated (Okosun 1987). The analyses of serum total protein and uric acid were as stated by Gelson and Ackerman (1975). Proximate composition of ingredients by AOAC (1980) procedure and amino acids (column chromatography). Data were subjected to analyses of variance and the significance of differences were assessed by applying Duncan's multiple range test (Steel and Torrie 1980).

RESULTS AND DISCUSSION

The birds fed on the rations containing 0.23% methionine and above had significantly ($P < 0.05$) better weight gain, feed per gain ratio than those of 0.21% met diet (Table 3). Apart from diet 2 (0.21% met) where significant reduction in body weight was observed, birds on other treatments grew fairly well. Optimal weight was obtained by growing the finishing cockerels on diets containing up to 0.37% met (0.63% Met + Cys). The observed lowered growth depression at the lower dietary met level may be because of inadequacy (Hewilt and Lewis 1969).

Feed consumption value revealed that the lowest intake was recorded at the 0.61% met diet which was only signifi-

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Table 1. Percentage composition of diets

Ingredients	Diets							
	1	2	3	4	5	6	7	8
Yellow maize	27.91	29.17	29.17	29.17	29.17	29.17	29.17	29.17
Groundnut-cake	10.28	14.28	14.18	14.18	14.18	14.18	14.18	14.18
Maize-offals	55.81	52.74	52.66	52.58	52.50	52.42	52.34	52.26
Fish-meal	2.50	-	-	-	-	-	-	-
Bone-meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Oyster shell	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Premix*	0.25	0.25	2.25	0.25	2.25	0.25	0.25	0.25
Salt (NaCl)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	
DL-methionine	-	-	0.08	0.16	0.24	0.32	0.40	0.48
L-lysine	-	0.41	0.41	0.41	0.41	0.41	0.41	0.41
Calculated composition:								
Crude protein (%)	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00
ME (Kcal/kg diet)	22.50	22.50	22.50	22.50	22.50	22.50	22.50	22.50
Methionine (%)	0.23	0.21	0.29	0.37	0.45	0.53	0.61	0.69
Met+cys (%)	0.49	0.34	0.55	0.63	0.71	0.79	0.87	0.90
Lysine (%)	0.56	0.90	0.90	0.90	0.90	0.90	0.90	0.90

The vitamin-mineral premix supplied the following per kg of diet: vitamin A, 500 IU, vitamin D_3, 1000 IU, vitamin E, 8mg, vitamin K_3, 0.4mg, vitamin B_2, 1.2mg, vitamin B_3, 1mg, vitamin B_{12}, 0.004mg, niacin, 3mg, vitamin C, 4mg, cholin, 112mg, manganese, 24mg, iron, 8mg, copper, 16mg, zine, 18mg, iodine, 0.5mg, selenium, 0.048mg, and BHT antioxidant.

cantly ($P<0.05$) different from those of cockerels fed the lowest level (0.21%) of dietary met. Also, apart from diet 6, feed consumption appeared to be higher at lower supplemental level below optimal diets. This higher feed intake recorded at lower dietary met is in agreement with what was observed in other chicken (Carew and Hill 1961) that birds over consume feed in met deficiency. Protein efficiency ratio of experimental birds ranged between 1.02 in diet 2 (0.21% dietary met), and 1.78 in 0.61% met (diet 7). There was an improvement between diets 2 and 7 except for the lower but nonsignificant decrease at the 0.53% dietary met (diet 6). None of the synthetic amino acid supplemented groups was significantly affected by dietary treatments.

The met +cys level where the best growth and feed conversion efficiency was obtained is similar to previous report (Izquierdo *et al.* 1988) for young chicks. However, the estimated met + cys (0.63%) level as found optimum for the cockerels is higher than that of the counterpart replacement pullets which may be due to their faster growth rate (Hannaganan *et al.* 1988) or higher energy in those reports.

The highest total protein and lowest uric acid concentrations of serum were recorded in birds maintained on diets containing met between 0.37 and 0.61%.

Dressing percentage revealed that the values obtained for the cockerels fed with 0.29 and 0.69% met diets were significantly ($P<0.05$) higher than those of the other dietary

Table 2. Performance characteristics of cockerel finishers fed graded level dietary met

Parameters	Diets (met. level-% diet)							
	*1(0.23)	2(0.21)	3(0.29)	4(0.37)	5(0.45)	6(0.53)	7(0.61)	8(0.69)
Weight gain (g/bird/day)	17.64±022	12.73±2.90	16.74±0.22	18.87±0.34	16.85±0.58	16.54±1.96	17.00±0.86	17.18±0.37
Daily feed consumption (g/bird)	77.35±0.77	77.03±3.68	83.49±1.98	70.39±0.96	69.94±3.67	78.25±2.93	67.46±0.12	74.30±2.22
Feed/gain ratio (g feed/g gain)	4.39±0.42	6.06±0.42	4.99±0.27	3.73±0.20	4.20±0.05	4.70±0.57	4.12±0.24	4.39±0.26
Protein efficiency ratio (g gain/g protein intake)	1.43±0.01	1.03±0.08	1.26±0.02	1.68±0.06	1.51±0.03	1.36±0.19	1.78±0.08	1.45±0.07
Mortality (%)	-	3.0	-	-	-	-	-	-

*Control fish-meal diet: Means on the same row with different superscripts are significantly different ($P<0.05$).

Table 3. Nitrogen retention serum metabolites and dressing percentage of finisher cockerels fed graded levels of dietary methionine

	Diets (Met. Level-% diet)							
Parameters	1(0.23)	2(0.21)	3(0.29)	4(0.37)	5(0.45)	6(0.53)	7(0.61)	8(0.69)
Daily nitrogen retention (%)	2.26±0.17	2.09±0.18	2.07±0.07	2.06±0.42	2.08±0.08	1.88±0.09	2.05±0.21	2.13±0.20
Nitrogen retention (%)	81.92±3.05	81.15±0.13	81.03±5.56	79.33±3.84	78.58±1.46	78.58±1.46	73.92±4.49	74.59±2.17
Serum total protein (mg/dl)	6.18±0.01	8.19±0.09	8.26±0.02	9.97±0.05	8.08±0.03	8.28±0.00	10.55±0.59	7.59±0.02
Serum uric acid (mg/dl)	3.95±0.06	4.84±0.02	3.11±0.02	2.61±0.00	3.04±0.16	3.22±0.02	2.63±0.02	4.80±0.08
Dressing percentage	67.51	68.39	82.14	69.50	68.97	68.50	73.79	83.33

Means on the same row with different superscript are significantly ($P<0.05$) different.

groups. The dietary treatment had no significant effect on flesh-to-bone ratio, comb/size and the spleen. Also, the carcass yield obtained in the various dietary groups was within the range reported by Oluyemi and Roberts (1979) as optimum for the meat chickens. Thus under the conditions of this study, it may be inferred that allowing for safety margins, 0.37% methionine (0.63% met + cys) in diet may be optimum for cockerels aged 9 to 16 weeks.

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Factors affecting early expressed body type traits in Sirohi goats

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Key words: Body type traits, Goat, Sirohi

In meat producing animals like sheep and goats, external body measurements could be a reliable indicator of its future performance with respect to live body weights, if and only if, a correlation has been identified among these traits of interest. Keeping in view, this study has been planned to characterize Sirohi goats on the basis of body height (BH), body length (BL), heart girth (HG), paunch girth (PG) and ear length (ER) at different stages of early growth i.e. birth (BH0, BL0, HG0, PG0 and ER0), 3 months (BH3, BL3, HG3, PG3 and ER3) and 6 months of age (BH6, BL6, HG6, Pg6 and ER6). For the study, 173 Sirohi kids of both sexes born through the mating of 14 purebred Sirohi sires during 1996-97 and 1997-98 were used. These kids were maintained under semi-intensive system of management. Weaning of these kids was done at the age of 3 months. Afterwards, these kids were allowed to browse in the typical arid to semiarid pastures available with the institute for about 8-10 hr daily. Analysis of data was done with Model 2 of the least squares' and maximum likelihood computer program, PC-2 Version (Harvey 1990).

Overall least-squares means for body type traits indicated linearity with age (Table 1). The heritability estimates (h^2) of all the body type traits were low (Table 1), except HG0, ER3 and ER6, which ranged from moderate to high with high standard errors. Such large sampling errors reduce the reliability of the estimates and lead to hazy inferences.

The effect of sire was nonsignificant on almost all the traits, except ER3, which showed that it is highly significant. At the same time, the regression of dam weight on these postnatal body measurement traits was linear and highly significant in nature (Table 1), on almost all the traits, except BH0, which expressed significant effect of this factor. Therefore, it could be concluded that a minimum optimum weight of dam is essential before permitting it for the first service/ insemination, just to achieve better performance with respect to weights/ measurements at later stages of life.

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The effect of sex was highly significant on almost all the traits, except BL3 (nonsignificant). It was observed that males performed better than females at all stages of measurements (Table 1). Here, it is essential to mention that it is a well established fact that males are always heavier than females at all stages of growth in Sirohi goats (Tomar *et al.* 1997, Tomar *et al.* 1998 and Tomar *et al.* 1999).

Similarly, the effect of year of kidding was highly significant on most of the traits, except BL6 (significant at $P<=0.05$) and nonsignificant effects on BH0 and PG3 (Table 1). The year 1997-98 was better as compared to the year 1996-97 for expression of most of the traits. It might be due to the fact that during 1996-97 concentrate supplementation withdrawal from the feeding schedule of does also affected the body type/body conformation traits.

In the same fashion, effect of type of birth (single vs. twins) was also observed to be highly significant on almost all the traits indicating that single born kids expressed larger measurements as compared to kids born as twins.

Therefore, it could be concluded on the basis of present findings that animals with higher body measurements at initial phases of growth will perform better with respect to even body weight traits at later stages of growth, but it requires a detailed study of this subject.

SUMMARY

Sirohi goats were characterized on the basis of body height, body length, heart girth, paunch girth and ear length as different stages of early growth. The performance of animals with higher body measurements at initial stage, was better at later stages of growth.

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Table 1. Least-squares means (cm) and heritability estimates for body type traits in Sirohi kids

Pqrticulars	Obs	BH0	BL0	HG0	PG0	ER0	BH3	BL3	HG3	PG3	ER3	BH6	BL6	HG6	PG6	ER6
Overall	173	34.77 ±0.25	31.07 ±0.22	30.86 ±0.27	29.18 ±0.28	12.10 ±0.16	50.02 ±0.51	47.38 ±0.48	48.01 ±0.40	51.51 ±0.51	15.88 ±0.26	57.14 ±0.70	55.02 ±0.67	56.35 ±0.71	61.10 ±0.76	17.37 ±0.32
Sex		**	**	**	**	**	**	Ns	**	**	**	**	**	**	**	**
Male	87	35.17 ±0.28	31.44 ±0.24	31.50 ±0.29	29.97 ±0.31	12.46 ±0.18	50.92 ±0.56	47.57 ±0.55	48.65 ±0.45	52.40 ±0.57	16.16 ±0.27	59.53 ±0.80	56.77 ±0.77	58.04 ±0.81	63.24 ±0.87	18.06 ±0.34
Female	86	34.36 ±0.29	30.70 ±0.26	30.22 ±0.30	28.38 ±0.33	11.73 ±0.19	49.11 ±0.59	47.19 ±0.59	47.36 ±0.48	5062 ±0.60	15.60 ±0.28	54.76 ±0.82	53.27 ±0.82	54.66 ±0.86	58.96 ±0.93	16.68 ±0.35
Year of kidding	Ns	**	**	**	**	**	**	**	Ns	**	*	**	**	**	**	
1996-97	112	34.98 ±0.30	30.18 ±0.27	30.01 ±0.30	28.29 ±0.34	11.69 ±0.19	48.04 ±0.61	45.95 ±0.61	46.51 ±0.50	50.70 ±0.62	15.32 ±0.29	55.18 ±0.88	53.81 ±0.84	54.07 ±0.89	57.88 ±0.96	16.61 ±0.36
1997-98	61	34.56 ±0.34	31.95 ±0.31	31.71 ±0.33	30.06 ±0.39	12.51 ±0.22	51.99 ±0.69	48.82 ±0.70	49.45 ±0.58	52.32 ±0.72	16.43 ±0.31	59.10 ±1.02	56.23 ±0.98	58.64 ±1.03	64.32 ±1.11	18.13 ±0.40
Type of birth		*	**	**	**	*	**	**	**	**	**	**	**	**	**	Ns
Single	155	35.23 ±0.20	32.14 ±0.17	31.72 ±0.23	30.33 ±0.21	12.39 ±0.13	52.46 ±0.40	50.07 ±0.35	50.79 ±0.28	54.29 ±0.38	15.99 ±0.24	60.33 ±0.50	57.80 ±0.48	59.10 ±0.51	64.53 ±0.55	17.74 ±0.27
Twins	18	34.31 ±0.43	29.99 ±0.40	30.00 ±0.40	28.02 ±0.50	11.81 ±0.28	48.58 ±0.88	44.70 ±0.92	45.23 ±0.76	48.73 ±0.93	15.76 ±0.36	53.93 ±1.33	52.24 ±1.28	53.61 ±1.35	57.67 ±1.45	17.00 ±0.49
Regression of dam weight	*	**	**	**	**	**	**	**	**	**	**	**	**	**	**	
		0.006 ±0.002	0.007 ±0.002	0.008 ±0.002	0.010 ±0.003	0.005 ±0.001	0.024 ±0.005	0.031 ±0.006	0.030 ±0.005	0.035 ±0.006	0.006 ±0.001	0.040 ±0.009	0.032 ±0.008	0.030 ±0.009	0.035 ±0.009	0.008 ±0.003
h^2 values		0.069 ±0.162	0.040 ±0.153	0.238 ±0.212	0.041 ±0.153	0.072 ±0.163	0.073 ±0.163	>1.0	>1.0	0.022 ±0.147	0.455 ±0.269	>1.0	>1.0	>1.0	>1.0	0.195 ±0.200

**Significant at P<=0.01;* significant at P<=0.05; NS nonsignificant.

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Comparative efficacy of ELISA with TLC for determination of aflatoxin B_1 in animal feeds

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Key words: Aflatoxin B_1, Animal feeds, ELISA, TLC

Aflatoxins (AFs) are mycotoxins produced by several species of *Aspergillus* of which AFB_1 is most important from the point of view of animal and human health (CAST 1989, Eaton and Groopman 1994). Approximately 25% of the world's food supply is contaminated by mycotoxins annually (CAST 1989). But the detection and control method in our country in not adequate. So, it is important to develop a rapid, sensitive and reproducible technique for quick determination of AFB_1. The available techniques (TLC, GLC, LC, HPLC etc.) are time consuming and very costly. The possible solution to overcome the problem is use of the ELISA which has become very popular in the last few years for the screening of various food/feeds (Chu 1986, Fukal and Kas 1989).

Hyperimmune serum against AFB_1 was developed in rabbit (Gaur *et al.* 1980). Aflatoxin B_1 has been extracted and purified from the feed samples and detected by TLC (AOAC 1990). The toxin (AFB_1) was also detected by ELISA (Ram *et al.* 1986) using AFB_1-HRP conjugate (Pestka *et al.* 1981). Standard curve was prepared using standard AFB_1 and compared with the result of sample tested in both the cases.

The comparative efficacy and applicability of ELISA over TLC in large scale detection of AFB_1 in feed samples was determined based on sensitivity, time requirement, cost, simplicity, application and precisions. With 2 dimensional TLC (silica gel F_{254} aluminium sheets), as low as 1 to 1.5 ng of AFB_1 was detected and the result simulated the findings of Shotwell *et al.* (1981). Patterson and Roberts (1979) detected up to 0.1 ng of AFB_1 in extract prepared from maize and other simple feeds. The detection level could vary depending upon the interferences in a given sample. Confirmation of AFB_1 on TLC plate was important, because the impurities present in the extracts of feed samples may sometimes gave similar fluorescence as observed in AFB_1, causing over estimation or false positivity. It could be overcome by trifluoroacetic acid (TFA) treatment followed by H_2SO_4 spray. The impurities were completely suppressed but not AFB_1, which would only be converted to aflatoxin B_1 hemiacetal (AFB_{2a}) and gave characteristic yellow fluorescence.

Use of an alternative TLC solvent system in conjunction with the CB method cleanup was also studied. Single solvent, such as hexane, ethyl ether, chloroform and combination of solvents, using ethylether, methanol, water, ethyl acetate, formic acid and ammonia in various combinations and proportions were used. But the best result was obtained by using the combination of toluene – ethylacetate - formic acid (6 : 3 : 1, v/v/v) and chloroform-acetone (9 : 1, v/v) and were used throughout the study. Improvement of sample cleanup prior to TLC was made by introducing silica gel column chromatography (Thouvenot and Morfin 1983) with various solvents like, diethyl ether as washing solvent and chloroform-methanol mixture (97 : 3, v/v) as elution solvent.

Enzyme linked immunosorbent assay (ELISA) for AFB_1 detection was sensitive, specific and rapid. The direct competitive ELISA used in this study was based on competition between free AFB_1 in the sample extract and the AFB_1-HRP conjugate for the specific solid phase antibody. The procedure requires water-soluble reagents for mixing sample extract with enzyme conjugate. Water miscible methanol was used to extract AFB_1 from contaminated samples. In the final mixture of sample extract 35% methanol could be included and enzyme conjugated without substantial loss of ELISA performance. Presence of 1% DMF in the extraction solvent and enzyme conjugate dilution buffer (blocking reagent) eliminated the turbidity without diminishing assay activity. Microtiter wells were treated with BSA and glutaraldehyde to reduce interwell variability of the sample replicates (Ram *et al.* 1986).

This ELISA method has the advantage of using a crude solvent extract without further cleanup. On the basis of preliminary spiking trials, the minimum AFB_1 detection level was 50 pg/assay. These results are in agreement with Ram *et al.* (1986) and Chu *et al.* (987). A sample extract of ELISA

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could be prepared within 20 min in our studies. Chu *et al.* (1987) reported nearly similar type of result. But in contrast to our results, Ram *et al.* (1986) extracted the toxin for ELISA within 5 min. By using a 96 wells microtiter plate, as high as 30 feed samples in triplicate could be tested at a time and it took 2.5 hr to complete the assay. The number of samples could further be increased up to 36 in a 96 wells microtiter ELISA plate (Ram *et al.* 1986). The overall time requirement for completion of assay varies from 30 min to 2 hr (Ram *et al.* 1986, Chu *et al.* 1987, Beaver *et al.* 1990, Hongyo *et al.* 1992).

Non-specific reactions are always a big problem in ELISA systems, causing incorrect data. It is a unique phenomenon that about 5-fold dilution with water exerts a major effect on the elimination of nonspecific reaction from the ELISA system. Because of high sensitivity and possible matrix interference effect 1 : 20 or higher dilution of samples was necessary in each assay. Consequently, less sample matrix was introduced into the assay system and subsequently, less interference. This is the major advantage of the current method over the previous protocols (EL-Nakib *et al.* 1981) in eliminating matrix interferences.

The microtiter plate ELISA is gaining wide spread acceptance in clinical laboratories and a number of automated devices facilitating these assays are available. The direct ELISA method for AFB_1 described here has high sensitivity, precisions, simplicity and the advantage of both time saving and cost effective (Table 1). Therefore, if different parts of a large sample are chosen and every sample is assayed by ELISA, an overall accurate level of AFB_1 content of the sample can be obtained within a very short time. The correlation coefficient (r) between the results of TLC and ELISA gave a better (+) r value (0.929) which was statistically highly significant ($P<0.01$) and indicated the accuracy of our ELISA result.

SUMMARY

The comparative efficacy and applicability of ELISA over TLG in large scale detection of aflatoxin in feed samples was determined. The ELISA gave better and accurate results than TLC.

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On farm trials on silage: Quality and palatability of maize stover silage

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Maize and sorghum are the main stable food crops of Bundelkhand region. After harvesting the cobs of maize, crops remain in the field for 3-4 months and fed to the animals as per need of the farmer. Thus large area is not available for rabi sowing. To overcome this problem, earlier attempt was made by Singh *et al.* (1993) to ensile the green stover; where it was observed that the efficiency of nutrient utilization was better form silage than fresh stover. Keeping this in view, the present study was undertaken at farmer's field at Ambabi village (Jhansi) by ensiling maize stover incorporating urea @ 0.25% to popularize this technique of silage making.

After taking the maize cobs 50% of the maize stover was ensiled in a *kaccha* pit (1.5 m × 1.5 m × 1.5 m) lined with plastic sheet and rest was sun-dried. Stover was chaffed by hand chaff cutter. At the time of ensiling, urea was added @ 0.25% on fresh weight basis. Silo was opened after 60 days of ensiling and silage quality was evaluated. Feeding experiment of 7 days duration was conducted to record the intake of silage and dried stover on different livestock available with the farmer.

Dry matter in the original sample was estimated by drying at 100°C for 24 hr which was used for calculation of various constituents in silage. Original samples were analyzed for crude protein (AOAC 1970) and water soluble carbohydrates (Johnson *et al.* 1966). Buffering capacity was estimated by taking suitable aliquot of water extract of the plant and titration with standard H_2SO_4 to the level of pH 4.0. Silage samples were analyzed for pH, CP, NH_3-N (Conway 1957), acetic and butyric acid (Daniel 1970), lactic acid (Barker and Summerson 1941) and alcohol (Snell and Snell 1957). Flieg Index was calculated as per the method of Zimmer (1966).

The fresh maize stover contained dry matter 40.4%, crude protein 6.62%, water soluble carbohydrates 4.80% and buffering capacity 6.20 mE/100 g dry matter. Organoleptic test revealed that the silage emitted good aroma and appeared yellowish green in color. pH of the silage was towards higher side (5.03). Addition of urea during ensiling of maize and sorghum stovers raised pH (Mowat *et al.*1976 and Singh *et al.* 1993). Ammonia nitrogen was 0.41% indicating that added urea was completely converted into ammonia, which confirmed the observation of Thomas *et al.* (1975). Acetic acid (0.75%) and butyric acid (0.08%) were at lower side. The concentration of lactic acid was 3.27%. Singh *et al.* (1993) also found higher lactic acid (3.54%) in sorghum stover silage. But Collenbrander *et al.* (1971) detected a lower level of lactic acid (2.92%) during ensiling of maize stover with 0.5% urea. The higher level of lactic acid in the present investigation reflected the better quality of silage, which was due to conducive environment in silo pit during ensiling. On the basis of Flieg Index (78), silage quality was very good. The maximum dry matter intake (kg/100 kg body weight) was recorded in calf (2.55), followed by buffalo (2.44), cow (1.91) and bullock (1.55), whereas dry matter intake as percentage of body weight from dried maize stover were 1.77 in calf, 1.36 in buffalo, 1.34 in cow and 1.15 in bullock. A similar level of dry matter intake from sorghum stover in growing heifers was observed by Singh *et al.* (1993).

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SUMMARY

The quality and palatability of maize stovers silage was studied. The silage quality was very good as it emitted good aroma. Silage pH was 5.02. Lactic acid concentration was 3.27% and butyric acid was at low level (0.08%). Flieg index was 78. Palatability of maize stover silage was very high as compared to the dried stovers. Dry-matter intake was higher for silage as compared to dried stovers.

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Effect of replacing fish-meal by soybean on growth performance of piglets

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Utilization of soybean grain in pig ration after suitable treatment for the inactivation of various antinutritional factors present in it, was planned in lieu of fish-meal. The coarsely ground soybean grain was subjected to pressure cooking in a vertical type autoclave at 1.05 kg/cm^2 pressure for 10 min (Bajpai and Gupta 1971), dried and mixed in the concentrate mixture to replace fish-meal.

Weaned piglets (27; Tamworth × *desi*) of 3 to 4 months age having about similar weight were selected and divided randomly into 3 equal groups. They were fed as per NRC (1973) feeding standards on isonitrogenous and isocaloric diets of 3 different compositions (Table 1). The fish-meal component of control group (T_1) was totally replaced by heat treated soybean (T_2) and soybean supplemented with lysine and methionine (T_3). Feeding trial continued for 3 months and during it body weights of pigs were recorded at weekly interval. The data were analyzed statistically (Snedecor and Cochran 1968).

Table 1. Per cent ingredient and chemical coposition of various concentrate mixture

Particulars	T_1	T_2	T_3
Ingredient composition			
Maize	50.0	46.5	46.5
Groundnut-cake	20.0	20.0	20.0
Wheat bran	20.0	20.0	20.0
Fish-meal	6.5	-	-
Soybean	-	10.0	10.0
Dicalcium phosphate	1.0	1.0	1.0
Mineral mixture	2.0	2.0	2.0
Common salt	0.5	0.5	0.5
Chemical compostion (% DMB)			
Crude protein (N×6.25)	20.86	20.54	20.74
Crude fibre	4.39	4.34	4.28
Ether extract	4.75	4.86	4.99
Nitrogen free extract	60.99	62.44	62.33
Total ash	9.01	7.82	7.56
Calcium	0.49	0.52	0.47
Phosphours	0.39	0.49	0.52

Lysine and methionine were added @ 10g/100kg of concentrate mixture in T_3.

The initial average body weight of piglets of T_1, T_2 and T_3 groups did not differ significantly between the groups (Table 1). Analysis of variance showed nonsignificant influence of different diets on weekly body weight during the whole experiment of 13 weeks. The overall daily weight in piglets fed three diets showed nonsignificant differences (Table 2). Replacement of total quantity of fish-meal of the control diet (T_1) with heat treated soybean (T_2) and heat treated soybean

Table 2. Average weekly body weight (kg) of piglets in various diets

	Experimental groups		
Week	T_1	T_2	T_3
Initial	13.78±1.28	13.67±1.12	13.55±1.42
1	15.61±1.73	15.55±1.46	15.72±1.85
2	17.05±1.64	17.05±1.64	17.16±2.08
3	19.33±2.09	19.00±2.70	19.05±2.23
4	21.44±2.33	21.94±1.93	21.00±2.44
5	23.50±2.63	23.61±2.17	22.77±2.62
6	25.33±2.82	25.66±2.06	24.33±2.82
7	27.38±2.67	27.16±2.46	26.33±3.41
8	29.77±3.04	30.16±2.54	29.61±3.50
9	31.66±3.80	32.16±2.87	32.08±3.27
10	35.22±3.66	35.50±2.93	34.83±3.38
11	38.00±3.77	38.77±2.66	37.38±3.82
12	41.16±2.087	41.38±2.83	41.20±3.96
13	44.44±4.44	44.50±2.89	43.38±4.01
Total gain (g)	30.67±3.39	30.83±1.88	29.83±2.67
Weekly gain (kg)	2.36±0.26	30.83±0.15	2.30±0.20
Daily gain (g)	337.03±37.26	338.79±20.72	327.80±28.29

Differences among all the experimental groups were nonsignificant. Each value is an average of 9 observations.

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supplemented with lysine and methionine (T_3) had no adverse effect on the growth performance of pigs. The supplementation of lysine-methionine in the diet had no extra advantage which revealed that level of these essential amino acids in other 2 diets were also adequate to maintain the growth requirements. Krider and Carroll (1971) studied in laboratory animals and concluded that methionine or a combination of cystine and methionine is the limiting amino acid of soybean protein. But in this study probably the level of protein recommended by NRC (1973) were sufficient to prevent this limitation of essential amino acids. The present finding is also in agreement with the report of Castell and Cliplef (1990).

The comparable growth performance of fish-meal and soybean containing diet (T_2 and T_3) may be attributed to inactivation of undesirable thermolabile factor such as trypsin inhibitor, haemagglutinine and saponins, because of heat treatment (Krokhina 1983). However, untreated (raw) soybean to growing-finishing pigs resulted in reduction in body weight (Pontif *et al.*1988). Present findings are comparable with the observation of Moshkutelo *et al.* (1986), who noted that pigs given heat-treated or autoclaved soybean and pea grew faster than those given untreated. Cost per kg live weight gain during this study was minimum in T_2 group (Rs 23.49) followed by T_3 (Rs 24.67) and T_1 (Rs 26.28) group (Kumar *et al.* 2000). From the present findings it can be concluded that fish-meal in growing pig ration can be completely replaced by heat-treated crushed soybean without having any adverse effect on growth rate.

SUMMARY

Tamworth × *desi* (27) growing piglets, 3-4 months-old, were utilized for this study. Concentrate mixture, viz. T_1, T_2, and T_3 were formulated to feed the piglets. T_1 having 6.5% fish-meal served as control whereas, fish-meal was completely replaced by pressure cooked crushed soybean on isonitrogenous basis in T_2 and T_3 diets. In T_3 diet, lysine and methionine was also added @ 10g/100 kg of concentrate mixture. The results showed nonsignificant effect of diets on piglet weight at different weeks besides daily weekly body weight gain, except for weight gain during fourth week. Replacement of fish-meal with heat treated soybean had no adverse effect on growth of piglets. Hence, fish-meal can completely be replaced by heat treated soybean in grower ration without affecting the performance of growing piglets.

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Effect of different stages of weaning and pre-starter diets on the performance of crossbred piglets

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Key words: Early weaning, Piglets, Weaner diets

Under the normal management practices of pig raising in India, the piglets are weaned at 8 weeks of age. The mortality rate of piglets and farrowing interval of sows seem to be higher at 8 weeks in comparison to early weaning. Hence, present study was carried out for overall improvement for economical pig production. Growth studies were conducted on 444 piglets of 60 sows belonging to Landrace (LR), Tamworth (T) and their crosses with *desi* (D), maintained at the Pig Breeding Farm of Ranchi Veterinary College (BAU). They were randomly divided into 5 weaning groups, viz. weaned at 3 weeks (W_3), 4 weeks (W_4), 5 weeks (W_5), 6 weeks (W_6) and 8 weeks (W_8) of age.

The piglets of each weaning group were offered 4 types of weaner diets as creep from 2 weeks of age. They were kept on the weaner diets up to 8 weeks of age. After 8 weeks, piglets of all the groups were switched over to similar type of grower diet. Pigs were fed *ad lib*. Ingredient composition of weaner and grower diets along with actual chemical composition were presented in Table 1. Weaning was done by removing the dam from the farrowing pen and leaving the piglets in the same pen to protect from displacement shock. The differences in daily weight gain among different weaning groups were nonsignificant during first 3 weeks (Table 2). Nonsignificant differences among the body weight of piglets of different weaning groups was expected because the piglets of all the weaning groups were reared on similar type of feeding regime (suckling the mother + creep feed) up to 3 weeks of age. The critical review of data with regard to effect of age at weaning on daily weight gain during third to eighth week of age showed highest daily weight gain in the piglets of W_6 group followed by W_5 and W_8 groups, however, they did not differ significantly among themselves. But the piglets belonging to these groups had significantly higher average daily weight gain than those of W_3 and W_4 groups, which did not differ significantly from each other (Table 2). Slow growth immediately after weaning may be because of shock and stress of being separated from their dams. Such growth curve is typical of early weaned piglets. Bhatia *et al.* (1985) also got similar results in Landrace pigs. Retarded growth rate in piglets just after weaning was also reported by Devi and Singh (1998) in 'T and D' piglets. During 9th to 12th weeks of age, although the highest daily weight gain was recorded in piglets weaned at fifth week, but the differences among different

Table 1. Ingredient composition (%) of experimental diets

Ingredient	Weaner				Grower
	T_1	T_2	T_3	T_4	
Maize	38.0	31.0	53.0	51.0	60.0
Barley	15.5	14.0	10.0	8.0	-
Bajra	3.0	14.0	-	6.5	-
Groundnut-cake	13.5	13.0	7.0	6.5	13.0
Wheat bran	-	-	-	-	17.0
Fish-meal	9.0	9.0	9.0	9.0	7.0
Skimmed milk powder	8.0	8.0	8.0	8.0	-
Jaggery	5.0	5.0	5.0	5.0	-
Lard	5.0	3.0	5.0	3.0	-
Dicalcium phosphate	1.0	1.0	1.0	1.0	1.0
Calcium carbonate	0.5	0.5	0.5	0.5	0.5
Sodium chloride	0.4	0.4	0.4	0.4	0.4
Mineral and vit.pre-,mix*	1.1	1.1	1.1	1.1	1.1
Antibiotics mix**					
Chemical completion					
CP (%)	20.05	20.03	18.06	18.06	16.36
DE (Kcal/kg)	3502	3311	3480	3385	3200
Lysine (%)	1.00	1.02	1.00	0.92	-
Meth + cyst (%)	0.67	0.66	0.60	0.60	-

*Mixture fortified with $FeSO_4.7H_2O$ and $ZnSO_4.7H_2O$. Supplied (per kg diet): Ca, 2.7 mg; P, 0.75 mg; Mn, 137.5 mg; Fe, 158 mg; Zn, 103 mg; I, 5 mg; vit A, 25000 IU; vit B_{12}, 10 mg; calcium pantothenate, 20 g; vit B_6, 2 mg; vit B_{12}, 28μg; vit D_3, 3125 IU; vit E, 4 IU; choline chloride, 50 mg.

**Sulphadimidine 100mg/kg diet; furazolidone, 50 mg/kg diet.

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Table 2. Average daily weight gain (g) of growing piglets at different stages of weaning

	Age at weaning					
Age (week)	3rd wk	4th wk	5th wk	6th wk	8th wk	over all
1 wk^{NS}	135.84	141.71	141.74	138.67	136.25	140.02
	±8.32	±8.56	±9.08	±7.65	±05.8	±3.52
	(83)	(87)	(85)	(76)	(93)	(424)
2 wk^{NS}	138.88	131.93	146.89	157.28	115.15	138.40
	±10.00	±10.87	±18.68	±11.51	±14.23	±6.49
	(83)	(79)	(82)	(75)	(88)	(407)
3 wk^{NS}	121.63	114.71	138.92	152.53	106.83	127.49
	±9.65	±10.20	±12.60	±14.59	±14.52	±6.15
	(82)	(74)	(80)	(74)	(84)	(394)
4 wk**	-17.07^{a}	108.28^{a}	115.20^{b}	104.28^{b}	87.85^{b}	77.81
	±11.02	±14.77	±12.62	±7.47	±13.74	±5.21
	(82)	(68)	(78)	(74)	(83)	(385)
5wk**	38.64^{a}	2.21^{b}	117.52^{c}	123.36^{c}	97.88^{c}	77.44
	±13.80	±13.93	±11.34	±13.99	±16.00	±7.12
	(79)	(68)	(78)	(74)	(81)	(380)
6wk**	56.54^{a}	68.16^{a}	55.36^{a}	159.25^{b}	121.68^{b}	92.78
	±12.85	±11.93	±14.91	±20.98	±18.63	±8.23
	(73)	(68)	(77)	(74)	(79)	(371)
7wk^{NS}	98.86	85.07	126.04	88.71	133.99	107.76
	±14.24	±16.48	±13.72	19.38	±21.12	9.25
	(69)	(63)	(77)	(74)	(78)	(361)
8wk^{NS}	116.93	107.00	172.69	163.82	152.68	144.40
	±20.12	±15.42	±19.40	±30.27	±17.58	±11.18
	(68)	(62)	(75)	(73)	(78)	(356)
3-8wk**	68.83^{a}	79.29^{a}	120.96^{b}	132.01^{b}	116.82^{b}	104.91
	±9.39	±12.45	±11.62	±12.06	±11.84	±5.82
	(68)	(62)	(75)	(73)	(78)	(356)
9-12wk^{NS}	79.75	76.83	97.99	94.86	75.89	85.31
	±11.20	±7.27	±9.49	±8.56	±12.22	±4.83
	(68)	(61)	(72)	(72)	(75)	(348)

NS, Nonsignificant; **, P<0.01; mean under the same superscript did not differ significantly; figures in parentheses indicate number of observations.

stages of weaning were nonsignificant (Table 2). Similar results were reported by Bhatia *et al.* (1985). Significantly higher daily weight gain was recorded in piglets maintained on T_1 diet than of T_4 at fifth week of age (Table 3). Although the total daily weight gain by piglets of different dietary groups during third to eighth week of age were statistically nonsignificant, but maximum weight was gained by piglets of T_1 group followed by T_2, T_3 and T_4 groups indicating that diet T_1 (high energy high protein) was the best diet suitable for early weaning. Newport and Keal (1982) also observed that higher levels of both protein and energy improved performance in early weaning piglets. Compbell and Dunkin (1983) also observed increased growth rate of piglets on higher energy intake.

The effect of diets and age of weaning had nonsignificant influence on feed conversion efficiency (Table 4). It indicates that potency of all diets are almost equal to produce

Table 3. Average daily weight gain (g) of growing piglets on different weaner diets

Age	Diets			
(week)	T_1	T_2	T_3	T_4
1^{NS}	138.71±7.70	129.84±9.68	146.20±5.91	141.47±7.15
2^{NS}	142.83±9.48	129.15±16.82	146.96±11.80	133.15±10.23
3^{NS}	124.08±9.42	127.74±12.50	127.25±13.13	128.63±12.38
4^{NS}	83.55±13.35	75.89±18.52	78.61±19.05	80.79±16.18
5*	106.52±18.91^{a}	74.10±14.3^{ab}	69.22±17.30^{b}	53.85±13.67^{b}
6^{NS}	110.32±24.78	94.14±16.25	89.41±14.91	74.96±13.08
7^{NS}	128.40±20.19	115.52±16.86	98.14±11.16	84.07±12.18
8^{NS}	173.39±26.24	149.81±18.66	118.21±15.49	129.07±14.15
3-8^{NS}	119.75±15.01	105.07±12.65	96.80±9.77	91.90±8.40
9-12^{NS}	93.14±9.79	111.89±37.14	84.18±8.89	73.42±7.83

NS, Nonsignificant; *, P<0.05; Means under the same superscript did not differ significantly.

unit growth in piglets. The slightly higher FCE of T_1 and T_2 dietary groups than T_3 and T_4 may be because of higher proportion of protein in T_1 and T_2 than that of T_3 and T_4 groups (20% vs 18%). Although the effect of age at weaning on FCE was nonsignificant, but better efficiency was observed in the piglets weaned at fifth week than those weaned at sixth week followed by third week and fourth week. The results are comparable to Devi and Singh (1998a) who recorded FCE to be 2.80 ± 0.27 and 3.00 ± 0.17 in 2 types of diets weaned at fifth week of age.

The cost of various diets (T_1 to T_4) for 1 kg gain in body weight of piglets was calculated to be Rs 14.49, 14.62, 17.27 and 17.94, respectively (Table 5). As expected, due to better performance of piglets on T_1 and T_2 diets, minimum cost incurred on it for unit body weight gain. T_3 and T_4 diets were proved to be costlier by 19.19 and 23.81%, respectively, when compared to T_1 diet. Thus, T_1 and T_2 diets proved to be the best from economic point of view.

The cost involved for 1 kg gain was calculated to be

Table 4. Average feed conversion efficiency of each litter.

	No. of litter	Total feed consumed	Total weight gain (kg)	Feed efficiency (kg feed/kg gain)
		Stages of weaning		
3rd wk	9	44.87±5.33	17.62±3.16	2.84±0.28
4th wk	10	29.44±3.59	10.57±1.67	3.05±0.33
5th wk	11	36.45±3.50	15.85±1.66	2.34±0.12
6th wk	11	26.89±4.06	12.09±2.55	2.52±0.19
		Type of feeds		
T_1	10	37.62±4.58	16.81±2.70	2.36±0.14
T_2	10	35.75±5.48	15.57±2.65	2.43±0.18
T_3	11	32.30±4.95	12.51±2.35	2.84±0.24
T_4	10	30.59±2.72	11.02±0.32	3.04±0.32

Differences among means were nonsignificant.

Table 5. Average cost (Rs)/kg gain in body weight of pigs fed to different weaning and dietary groups

Weaning groups	Dietary groups				Overall Av. (weaning groups)
	T_1	T_2	T_3	T_4	
W_3	13.89 ±1.89 (3)	16.09 ±2.65 (2)	17.34 ±1.49 (2)	22.88 ±4.43 (2)	17.14^{NS} ±1.58 (9)
W_4	15.02 ±5.58 (2)	14.46 ±2.05 (2)	20.30 ±4.91 (2)	21.21 ±3.94 (2)	18.35 ±1.93 (2)
W_5	15.84 ±2.07 (3)	13.14 ±1.37 (3)	14.82 ±1.03 (3)	12.18 ±0.27 (2)	14.16 ±0.76 (11)
W_6	12.81 ±1.79 (2)	15.22 ±3.05 (3)	16.64 ±2.34 (3)	15.21 ±2.10 (3)	15.17 ±1.13 (11)
Overall average (dietary groups)	14.49 ±0.85 (10)	14.62 ±1.06 (10)	17.27 ±1.46 (11)	17.94^{NS} ±1.91 (10)	

Figures in parentheses indicate number of litters. NS, Nonsignificant.

Rs 17.14, 18.35, 14.16 and 15.17 in piglets of W_3, W_4, W_5 and W_6 weaning groups, respectively (Table 5). Thus, minimum feed cost per kg gain in body weight was of the piglets weaned at 5 weeks of age.

SUMMARY

Growth studies were conducted on 444 piglets belonging to Landrace (LR), Tamworth (T) and their crosses with *desi* (D). The piglets were divided into 5 weaning groups. They were reared on 4 types of weaner diets (pre-starter diets) up to 8 weeks of age and thereafter on standard grower diet up to 12 weeks of age. The differences among first 3 and between latter 2 weaning groups were significant statistically. The effect of high protein high energy (T_1), high protein low energy (T_2), low protein high energy (T_3) and low protein low energy (T_4) had nonsignificant influence on daily weight gain at different ages. Feed efficiency ratios did not differ significantly among different weaning groups. On the basis of overall results it was concluded that the piglets can be weaned at 5 weeks of age and reared on weaner diet having 20% CP and 3500 Kcal DE/kg diet for the maximum profit.

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β-carotene, nitrate and composition of *dub* grass (*Cynodone dactylan*) at monthly intervals

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Key words: Acid detergent fibre, β-carotene, *Cynodone dactylan*, Neutral detergent fibre

Dub (Cynodone dactylan), a perennial non leguminous grass that grows in the field throughout the year, is very much palatable and nutritious under village conditions. The present investigation was undertaken to study the β-carotene and nitrate content in *dub* and chemical composition of *dub* at monthly intervals. The information on β-carotene nitrate and chemical composition of *dub* grass is not available for throughout the year. Carotene is an important component of green fodder and a precursor of vitamin A (Church and Pond 1982). Nitrate causes poisoning in livestock (Naylor and Ralston 1991) and nitrate also hampers the conversion of β-carotene into vitamin A (Hoar *et al.* 1988). Therefore present study was undertaken to determine the β- carotene and nitrate contents of *dub* grass during the whole year.

Dub grass samples were collected monthly from the IVRI, Izatnagar, during July 1995 to June 1996. Every month 6 samples of grasses were collected from various places of field and determined for protein (AOAC 1980), β-carotene (Pathak *et al.* 1996), nitrate (Krishna and Ranjhan 1991) and neutral detergent fibre and acid detergent fibre (Georing and Vansoest 1970). Statistical analysis of data was done according to Snedecor and Cochran (1980).

β-carotene content was highest in August (Table1). During this month grasses were succulent. Minimum β-carotene level was in June when grasses were not in good position, due to lack of rain. Highest NDF was in November and lowest in May. Maximum ADF values were obtained in October and minimum during June. Maximum nitrate was observed during February and minimum during July. Maximum protein percentage was observed during June and minimum during September.

Neutral detergent fibre and acid detergent fibre content increased with maturity and this is on agreement with earlier reports (Sen and Ray 1971, Gampawar and Bhaiswar 1985).

Table 1. Average β-carotene, NDF and ADF nitrate and crude protein contents in dub grass (dry matter basis)

Month	Particulars				
	β-Carotene (ppm)	NDF (%)	ADF (%)	Nitrate (%)	Crude protein (%)
July	390.05±4.87	72.23±2.75	34.06±0.67	0.116±0.0	9.33±0.43
August	396.87±5.78	70.73±2.13	40.17±0.75	0.135±0.0	8.35±0.10
September	376.08±11.15	76.88±1.08	49.34±2.14	0.151±0.01	5.72±0.26
October	308.25±4.11	75.99±1.39	52.74±1.27	0.173±0.02	6.21±0.33
November	253.7±10.28	77.70±1.04	44.64±0.32	0.176±0.02	5.29±0.26
December	277.04±8.09	76.90±1.49	48.27±1.49	0.133±0.01	9.52±0.32
January	228.80±7.90	70.47±0.85	41.72±0.65	0.192±0.03	10.0±0.32
February	281.89±3.80	73.99±0.94	41.45±1.26	0.238±0.01	8.91±0.31
March	202.60±8.19	75.12±0.63	43.30±1.38	0.222±0.02	9.02±0.53
April	199.76±7.09	59.33±3.78	30.91±0.04	0.206±0.00	8.13±0.73
May	181.0±5.50	53.65±3.15	31.04±0.72	0.20±0.00	7.62±0.53
June	150.34±11.64	63.32±1.62	29.62±2.41	0.194±0.00	10.41±0.84

The carotene content decreased as plant matures, this is also similar to the results of other reports (Ranjhan 1993). However, the nitrate content is dependent on environmental condition and other factors (Kapoor *et al.*1973 and Naylor

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and Ralston 1991).

Above results showed that there are wide monthly variation in the composition of grass for nitrate and β-carotene. However, nitrate content in grasses is below the dangerous levels so grass is a very good source of carotene for livestock feeding.

SUMMARY

β-carotene and nitrate contents of *dub* grass during the whole year were decided. The β-carotene showed wide monthly variation but nitrate content was not below critical level throughout the year.

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Soil estimates (pH, N and OM), biomass yield and chemical composition of vegetation of community range land in semi-arid region of Rajasthan

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Key words: Biomass, Chemical composition, Rangeland, Semi-arid, Soil estimates

Rural animal production system in semi-arid regions of Rajasthan is based on pasture grazing on natural community range land. About 80-90% of community grazing land is under poor to very poor conditions due to overstocking and round-the-year grazing without rest to the pasture. The climax vegetation of the public rangelands in the hot region, is never achieved due to intensive and continuous grazing pressure (Karim 1999). The biomass yield of semi-arid community grazing land has been recorded during monsoon, winter and summer and considering the annual biomass yield of natural range land to be 1.3q / ha, the resource is sufficient to maintain at the best 0.3 adult cattle unit (ACU) round-the-year. As against stipulated carrying capacity, the stocking density on such land range from 1.0 to 51.1 ACU in different locations (Shanker and Gupta 1992). The animals under such poor availability of feed are surviving at the existing stocking density and providing production at low level for the sustenance of the farmers is a pertinent question to be answered. The reported study was therefore conducted to assess biomass yield, quality of vegetation and soil estimates, affecting the pasture growth of semi-arid community rangeland. A survey in Malpura, Toda Rai Singh, Deoli and Niwai cluster of villages of Tonk district was conducted after pre-monsoon shower.

Tonk district was divided in to 4 different regions, viz. Malpura, Toda, Deoli and Niwai cluster of villages, on the basis of differences in soil conditions as per the existing soil map. Samples from community range land were collected from 5 to 10 cluster of villages from each region. Thirty-two samples each of vegetation and soil were collected from 5-10 village community grazing land from 4 regions. The approved method of biomass yield estimation could not be applied due to very short height of vegetation and in its place all the above ground vegetation was manually scraped using *khurpy* for yield estimation.

One square metre iron frame was used to estimate biomass yield of unit area and expressed in terms of hectare. After deducting the samples were weighed and representative samples were drawn for laboratory analysis. Simultaneously soil samples were collected with the help of soil Auger at 20 cm depth in the range land. About 1 kg soil sample was taken to laboratory for analysis.

Nitrogen content of forage (washed and unwashed) and soil was estimated as per AOAC (1990). The crude protein content of forage was calculated (CP%=%N × 6.25). Dry matter yield of the range land was estimated by drying the vegetation samples at 60°C to a constant weight and the yield is presented as q/ha. pH of the soil samples was estimated following the procedure of Iswaran (1980).

Data were subjected to analysis of variance and correlation among various soil and forage estimates (Snedecor and Cochran 1968). Significant differences were compared by Duncan's multiple range test (Duncan 1955).

Biomass and dry matter yield of forage was significantly ($P<0.05$) higher in Malpura and Toad Rai Singh compared to

Table 1. Biomass yield (q/ha) of community range land

Region		Fresh forage yield	DM yield
Malpura	Range	5.72-19.55	4.57-12.70
	Mean	10.36[a]	7.55[a]
	SE	1.33	0.76
Toda	Range	5.18-15.57	4.14-14.97
	Mean	13.20[a]	9.50[a]
	SE	3.90	2.38
Deoli	Range	4.25-7.59	2.75-6.07
	Mean	5.08[b]	4.16[b]
	SE	0.69	0.56
Niwai	Range	1.90-8.06	1.52-7.03
	Mean	5.90[b]	5.14[b]
	SE	0.98	0.87
Over all	Mean	8.63	6.59
	SE	1.91	1.20

Values bearing different superscripts in column differ significantly ($P<0.05$).

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Table 2. Crude protein content of forage (%)

Region	Unwashed	Washed
Malpura	5.73[b]	7.42
Toda	6.74[a]	7.04
Deoli	6.30[a]	7.70
Niwai	7.05[a]	7.00
Mean	6.55	7.29

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

Table 3. Average soil estimates

Region	pH	%N	%OM
Malpura	6.82	0.116[b]	3.48[a]
Toda	7.10	0.086[b]	2.43[b]
Deoli	7.14	0.084[b]	1.31[c]
Niwai	7.62	0.973[a]	2.12[b]
Mean	7.17	0.316	2.34

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

Deoli and Niwai regions (Table 1). Overall yield of fresh forage and dry matter was 8.63 and 6.57 q/ha, which is far below than the reported yield of protected range land in a semi-arid location of Rajasthan (Shinde *et al.* 1996, Karim 1998). Since community grazing land are poorly managed, over grazed and have thinner plant population of different pasture species, hence the observed lower biomass yield is obvious. Biomass yield of community rang land ranged from 0.89 to 1.57 q/ha (Shankar and Gupta 1992, Sankhyan *et al.* 1999) in semi-arid region, which is much lower than the biomass yield observed in this study. Vegetation yield varied may be due to the estimation methodology applied in the 2 studies. In this study forage was scraped with *khurpy*, which overestimate the forage yield by adding the inedible portions of the pasture in total biomass yield.

Crude protein content of unwashed forage samples was higher (P<0.05) in Malpura compared to other 3 regions (Table 2), while the yield was similar in washed samples of forage from the 4 regions. Contamination of forage with soil reduced the CP content of unwashed samples, which was eliminated in water washed samples. CP content of forage ranged from 7.00 to 7.70% which would be considered adequate for maintenance of animals at pasture (Mc Dowell 1985).

Average soil estimates, viz. pH, nitrogen and organic matter are presented in Table 3. Soil pH was nearly neutral in Malpura, Toad and Deli regions, whereas, it was slightly basic in Niwai region. OM content of the soil was also different among the 4 regions and ranged from 1.31 to 3.48%. Soil organic matter was lowest (P<0.05) in Deoli compared to other 3 regions. N content ranged from 0.084 to 0.973%. While N content of soil in Niwai region was higher (P<0.05) than the other regions.

It is concluded that after pre-monsoon shower the biomass yield of semi-arid community grazing land range 5.08 to 13.20 q/ha. The biomass yield is sufficient to maintain 2 sheep or 0.4 ACU round-the-year. The CP content of biomass was sufficient to maintain the animals, while the pH and N-content of the soil were the main constraints of community grazing lands for pasture growth.

SUMMARY

Biomass yield, quality of vegetation and soil estimates, affecting the pasture growth of semi-arid community rangeland, were studies. The nutritive value of grazing land was sufficient to meet the requirement of animals.

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Relationship between body measurements and chevon production in Barbari kids*

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Body measurements are indicative of body size and weight and as such the live body weight and carcass weight can be predicted from body measurements (Bhattacharya *et al.* 1984 and Mishra *et al.* 1987). This information is much valuable in field conditions where weighing facilities are not avaibale. Moreover, data on carcass weight or meat yield can be obtained without sacrificing the amimal. Das *et al.* (1990) reported that body weights of Barbari and Jamunapari kids were predicted using body measurements at birth, 3 months and 6 months of age. The present work, however, aimed at establishing relationship of body measurements with body weight, carcass weight and chevon yield in Barbari kids at an average slaughter age of 10 months.

Data on body weight and body measurements from 16 male Barbari kids with an age of 4-6 months and an initial average weight of 10.7 kg, were recorded for 5 months (Sep-Jan 1999). Measurements were taken on the following body parts: Body length (BL, point of shoulder to the pin bone), body height (BH, base of the hoof to the highest point of the wither), heart girth (HG, circumference of the body immediately behind the fore-legs), paunch girth (PG, around the circumference of the umblicus), neck circumference (NC, circumference at the base of the neck) and thigh circumference (TC, around the middle of the thigh). All goats were slaughtered for carcass evaluation. The hot carcass weight was calculated as fasted body weight less the weight of edible offals (liver, kidneys, heart and spleen) and non-edible offals. The chevon yield was taken as the sum of hot carcass weight and edible offals. For prediction of body weight, carcass weight and meat yield with the help of body measurements and for estimation of carcass weight or meat yield on body weight data, the different biometrical models were fitted to the data and analysed by simple and multiple regression analysis (Snedecor and Cochran 1968). The partial regressions of body weight or carcass weight or meat yield on all the above 6 body measurements and on the best 2 body $\ldots + b_i x_i$ where Y is a meat production trait, a, is an intercept, b_i, partial regression coefficients of Y on X_i and X_i is a body measurement. The simple regression of carcass weight or meat yield on final body weight were also calculated by using the model: $Y=a+b_i x_i$ where, Y is the carcass weight or meat yield, a is an intercept, b_i is the regression coefficients of Y on x_i and x_i is final body weight. The coefficient of determination (R^2) and multiple correlation coefficients (R) for each regression equation were determined for the purpose of constructing the best prediction equation.

The average values of final body measurements were 51.64±0.92, 57.72±0.94, 57.21±0.84, 63.24±0.88, 31.25±0.56 and 25.38±0.56 and 25.38±0.66 cm for BL, BH, HG, PG, NC and TC respectively. The respective mean final body weight, hot carcass weight and chevon yield were 17.69±0.77, 7.27±0.41 and 7.77±0.43 kg. The present result is comparable with the report of Singh *et al.* (1979) for body length, height and paunch girth of Barbari she goats. However, the value for heart girth in this study was slightly lower compared with the above reported value which might be because of sex difference. Kushwaha (1996) recored higher mean value for heart girth in female yearling goats than the males. The average values for body height, heart girth and neck circumferences are in agreement with the findings of Kushwaha (1996) for male yearling goats. Kesava Rao *et al.* (1985) reported comparable mean slaughter weight of market goats ranged from 17.2 to 19.6 kg and carcass weight from 7.9 to 9.0 kg in local Barbari goats.

Correlation coefficient values between all body measurements and body measurements with body weight, hot carcass weight and chevon yield were positive and significant (Table 1). Similar significant and positive correlation coefficient was reported between body weight and body measurements such as with heart girth, height at withers and body length in Marwari kids (Wadhwani and Patel 1993). Bose and Basu (1984) also observed strong correlation between

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Table 1. Correlation coefficients among body measurements and with carcass traits

Measurements	BL	NC	BH	HG	TC	PG
NC	0.753**	-	-	-	-	-
BH	0.811**	0.786**	-	-	-	-
HG	0.882**	0.753**	0.902**	-	-	-
TC	0.855**	0.887**	0.776**	0.877**	-	-
PG	0.698**	0.568*	0.746**	0.895**	0.801**	-
FBW	0.827**	0.771**	0.869**	0.950**	0.877**	0.873**
HCW	0.846**	0.850**	0.868**	0.925**	0.925**	0.804**
MY	0.845**	0.844**	0.865**	0.925**	0.923**	0.805**

*P<0.05, **P<0.01; FBW=Final body weight, HCW=Hot carcass weight, MY=Meat/Chevon yield.

Table 2. Simlpe and multiple regression equations for body weight and chevon production traits in Barbari kids

Dependant trait	Prediction equations	R^2 (%)	R
FBW	$Y= - 21.121-0.065X_1+0.149X_2+0.053X_3+ 0.595X_4+0.124X_5+0.129X_6$	91.8	0.958**
FBW	$Y= -32.954+0.774X_4+0.10X_6$	90.5	0.951**
MY	$Y= -13.049-0.082X_1-0.029X_2+0.082X_3+ 0.308X_4+0.40X_5-0.103X_6$	92.3	0.961**
MY	$Y= -15.033+0.257X_4+0.318X_5$	91.0	0.954**
MY	$Y= -1.819+0.541X_7$ (r=0.962)**	-	-
HCW	$Y= -12.563-0.076X_1-0.011X_2+0.081X_3+ 0.278X_4+0.368X_5-0.091X_6$	92.7	0.963**
HCW	$Y= -14.134+0.239X_4+0.305X_5$	91.2	0.955**
HCW	$Y= -1.7399+0.508X_7$ (r=0.961)**	-	-

X_1=BL, X_2=NC, X_3=BH, X_4=HG, X_5=TC, X_6 =PG, X_7=FBW ** (P<0.01)

body measurements (length, height, heart and paunch girth) with slaughter weight and carcass weight in Beetal goats. Similar parameters have positive correlations with body weight in Barbari female goats (Singh *et al.* 1979).

A prediction equation (Table 2) was developed to estimate the body weight, carcass weight and chevon yield with the help of measurements. The multiple correlation coefficient (R) and simple correlation coefficient (r) were highly significant (P<0.01). The coefficient of determination (R^2) value was high enough to predict the body weight, carcass weight and meat yield from all body measurements with high accuracy. However, from practical point of view the prediction equation for body weight using heart and paunch girth and for hot carcass weight or chevon yield using heart girth and thigh circumference would be preferred as only two body measurements are involved for each estimation with similar reliability (Table 2). These 2 traits can be measured easily for prediction and are handy for practical use under field conditions to fix price of the live Barbari kids per unit live weight. Moreover, the butcher would be able to predict the chevon yield and accordingly estimate the return at the existing price. In areas where weighing facilities are available, carcass weight or chevon yield of Barbari kids at an average age of 10 months can also be predicted with high accuracy from only the body weight (Table 2).

SUMMARY

Data on body measurements, body weight, hot carcass weight and chevon yield were recorded from Barbari male kids to establish relationship among these traits. High and positive correlation were established between body measurements and body weight or carcass weight or chevon yield. The most handy prediction equations for estimating body weight ($Y=-32.954 + 0.774 X_4 + 0.10 X_6$), carcass weight ($Y=-14.134 + 0.239 X_4 + 0.305 X_5$) and chevon yield ($Y = -15.033 + 0.257 X_4 + 0.318 X_5$, where X_4, X_5 and $_6$ are heart girth, thigh circumference and paunch girth respectively) at an average slaughter age of 10 months were developed with over 90.5 per cent coefficient of determination (R^2) value.

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Composition, squamation chronology and regeneration of the elasmoid scales of *Oreochromis mossambicus* (Perciformes)

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ABSTRACT

The elasmoid scale of the teleost *Oreochromis mossambicus* (Perciformes) was studied to understand its composition, origin, development and regeneration. Analysis of organic constituents demonstrated the presence of higher percentage of protein compared to that of carbohydrates and lipids. Inorganic elemental composition like K, Na, Ca, P, Cu, Pb, Mg, and Zn with reference to factors like size and habitat indicated that these factors influenced the distribution of elements. Further, scales are suggested to function as a 'store house' besides acting as a 'biological sink' for detoxification. The study on squamation development revealed the time, sequence in the origin of scales and structures like radii, circuli, ctenii and tubulations. Observations on scale regeneration highlighted the role of epi-, hypo-and marginal scleroblasts and scale pocket lining in the development of scales from the dermis.

Key words: Composition, Elasmoid scales, *Oreochromis mossambicus*,Regeneration,Squamation Chronology, Structure

Scales are primarily protective structures, and their probable biological functions were not given due attention. Therefore, biochemical studies on the scales have often been neglected (Seshaiya *et al.* 1963, Saur and Watabe 1989), Rudneva and Obozhin 1992). Studies on the biochemical constituents in relation to size and habitat have not been carried out.

The sequence of scale development in primary and secondary sites in a predetermined time in the fry called squamation chronology was studied by Bilton (1988) and Sire and Arnulfe (1990).

Scale regeneration with emphasis on calcification process was demonstrated by Watermann (1970) and Sire *at al.* (1990), but details regarding the origin of scale and scale regenerating cells are still scarce.

The present study was undertaken using *Oreochromis mossambicus* (Pisces: Perciformes) to get detailed information on the composition, squamation chronology, origin of scale structures and scale associated cells during regeneration.

MATERIALS AND METHODS

Live *Oreochromis mossambicus* from fresh- and brackish-water habitats were grouped into 2 size groups, viz. S1 and S2 based on their total length. S1 and S2 size groups had an average total length of 9.00 and 21.00 cm respectively. Scales from the dorsolateral region and behind the pectoral fin of these fishes were used for various observations. or estimation of the amount of scales (% by weight), the wet weight of fish before and after removal of scales was taken and then the weight of the scales was expressed as percentage of the total weight of fish.

The water content of scales was determined gravimetrically by drying in a hot-air oven to constant dry weight (approximately 48 hr). The results are expressed as percentage.

The crude protein content of the scales was estimated by the microkjeldahl method (AOAC 1965). The carbohydrate and the total lipids were estimated following Seifter *et al* (1950) and Braydon (1951).

Potassium and sodium were estimated by using a flame photometer. Kramer-Tisdall method was followed for calcium (Oser 1965). Total phosphorus was determined as per Fiske and Subba Row (1925). Other metallic elements like Cu, Pb, Fe, Mg and Zn were analysed by using an emission spectrometer with minitorch and the values of unknown element were obtained from a standard graph.

Squamation development was studied in *O. mossambicus* (Park and Kim 1984). Fry stocked in the laboratory were sampled at intervals of 1 week until their bodies were completely covered with scales. Total length of specimens ranged from 1.0 to 3.8 cm. The fry for observations were fixed in 10% formalin for 24 hr. After removing the pigments using alka-

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line H_2O_2, specimens were stained in alkaline 0.1% alizarin red S. Finally, the fry were cleared in absolute glycerol for 7 days.

To study scale regeneration, specimens of 5 to 6 cm in length were used. After removing 15 to 20 scales covering an area of 0.5 cm^2 from behind the pectoral fin, fishes were periodically observed for regeneration of scales. The study was continued for 20 days using skin samples (size 5mm × 5mm). The excised skin was processed following routine histological procedures (Mittal and Banerjee 1974).

RESULTS AND DISCUSSION

Scale composition

The average amount of scales was 3.89% of the total weight (Table 1). Comparatively, the amount of scales was more in smaller size groups than in large size group. The average water content was 27.4%. Van Oosten (1957) has reported the water content of scales in Menhaden as 20.6% in contrast to 80-85% in the whole fish (Love 1957). The poor water content bound with structural proteins such as collagen and ichthylepidin (Holmes and Donaldson 1969) might be due to the low turnover rates of metabolites and solutes.

The study demonstrated that scales were rich in proteins (74.05%) compared to carbohydrates and lipids. Saraswat (1978) had estimated the amount of protein to range from 72.18 to 74.00 g/100 g in *Channa punctatus*. In general there was a relative decrease in the amount of protein, carbohydrate and lipid in the scales of size group 2. Further, the amount of lipid was comparatively low in brackish water forms and is suggested to have a relationship with the habitat.

In the present investigation, the amount of alkaline elements K and Na was low compared to other tissues (Sastry and Aradhana 1991). The calcium content was high (0.23 mg/100 mg). Van Oosten (1957) reported $Ca(PO_4)_2$ to vary from 1.93% in *Cololabris* sp. to 98.34% dry weight of scales in *Alosa* sp. The amount of P was highest in the scales of *O.mossambicus*. Van Oosten (1957) reported that P_2O_5 amounted to 13.12% in carp and 18% in Pike. The high amount of Ca and P noted in scales was perhaps an indication of the function of scales as a storehouse of these elements as suggested by Yamada and Watabe (1979).

The elevated concentration of havy metals Pb, Cu, Fe, Mg and Zn in the scales of brackish water *O. mossambius* compared to freshwaters indicated the influence of the environment in the incorporation of these metals in scales. The brackish water habitat of the fish is known to contain high amount of heavy metals. The accumulation of heavy metals like Zn, Mn, Co, Sr and Pb in scales had been reported in fishes like *Salmo trutta* (O' Grady and Abdulla 1985); and minnows (Rudneva and Obozhin 1992). It may be suggested that the enhanced presence of heavy metals in scales could be used in the identification of local fish populations or stocks and in monitoring heavy metal pollution. Based on the concentration of heavy metals, Salmon population have been identified by Lapi and Mulligen (1981).

Generally, excess amounts of elements are excreted by fish via the kidney of chloride cells or gills (Crespo *et al.* 1981). Further, excess of metal content induce the synthesis of metallothioneins which bind, transport or detoxify the metal ions in vertebrates (Noel-Lambot *et al.* 1978). But Brown and Parson (1978) explained that the animal's ability to synthesize metallothioneins was limited. Hence, as Saur and Watabe (1984) suggested, the fish might have alternate ways of detoxification of these elements. The evolution of calcification (mineralization) in hard tissues like scales arose due to the necessity of Ca elimination or detoxification (Halstead 1969). Saur and Watabe (1989) had confirmed the role of detoxification and removal of metals through fish scales. From the observations of the present study, it may be inferred that

Table 1. Amount of scales, water content and the organic and inorganic constituents in the scales of two size groups (S1 and S2) of *Oreochromis mossambicus* from fresh- and brackish-water habitats

	Fresh water		Brackishwater	
	Size group 1 T.L=9.4cm (mean±S.D)	Size group 2 T.L=21.0cm (mean±S.D)	Size group 1 T.L.9.5cm (mean±S.D)	Size group 2 T.L=12.0cm (mean±S.D)
Amount of scales (% by weight)	4.47±0.33	3.99±0.72	4.02±0.62	3.10±0.43
Water content (%)	27.74±4.8	28.47±2.7	26.38±1.7	27.04±1.1
Organic constituents				
Total protein (%)	74.81±1.06	73.87±2.65	74.93±2.81	72.62±3.6
Carbohydrate (%)	0.25±0.16	0.24±0.27	0.3±1.12	0.23±0.43
Total lipid (%)	1.82±0.08	1.67±0.01	1.43±0.26	1.47±0.009
Inorganic constituents				
Potassium (mg/100mg)	0.03±0.001	0.04±0.0	0.04±0.0	0.03±0.002
Sodium (mg/100mg)	0.21±0.01	0.24±0.01	0.23±0.01	0.26±0.008
Calcium (mg/100mg)	25.71±7.02	31.43±4.49	23.65±7.5	25.12±3.34
Phosphorus (mg/g)	55.0±10.0	61.67±4.71	107.0±4.0	136.6±6.2

Table 2. Comparison of copper, lead, iron, magnesium and zinc content in the scales of *O. mossambicus* from fresh- and brackish-water habitats

Habitat	Mean length and size range	Concentration of metals (mg/g dry weight) in scales (SD) (mg/g dry weight) (mean S±D)				
		Copper	Lead	Iron	Magnesium	Zinc
Fresh water	14.82 (14.3-15.7)	0.01±0.004	0.02±0.02	0.45±0.23	2.27±0.07	3.65±1.35
Brackish water	15.26 (13.6-16.6)	0.02±0.005	0.03±0.002	1.80±0.64	2.64±0.26	6.34±1.15

the large accumulation of elements in scales may be related to mineralization or detoxification of elements and the subsequent elimination of the excess elements through scale shedding. Thus, scales could also act as a 'biological sink'.

Squamation chronology and origin of scale structures

In the fry of *O. mossambicus,* the scales appeared initially on the midlateral sides anterior to the caudal peduncle approximately 2 weeks after hatching (Fig. 1A). This is the primary locus. The scales numbering 6 to 8 were found arranged in a row. Such scales showed 1 or 2 circuli, but not structured like radii, ctenii or granulations or tubulations. In the third week, new scales were continued to be laid along the midlateral body region up to one third from the tip of the snout anterior to the primary locus (Fig. 1B).

By the end of the third week, new scales appeared as small clusters above and below the already formed single row of scales and they extended forward (Fig. 1C). In addition 2 or 3 circuli were noted.

With the start of the fourth week, fresh additions of scales continued both vertically and horizontally (Fig. 1D). Though the total number of scales increased, they showed no overlapping owing to their small size. Scale formation towards the belly region was slower compared to other body regions.

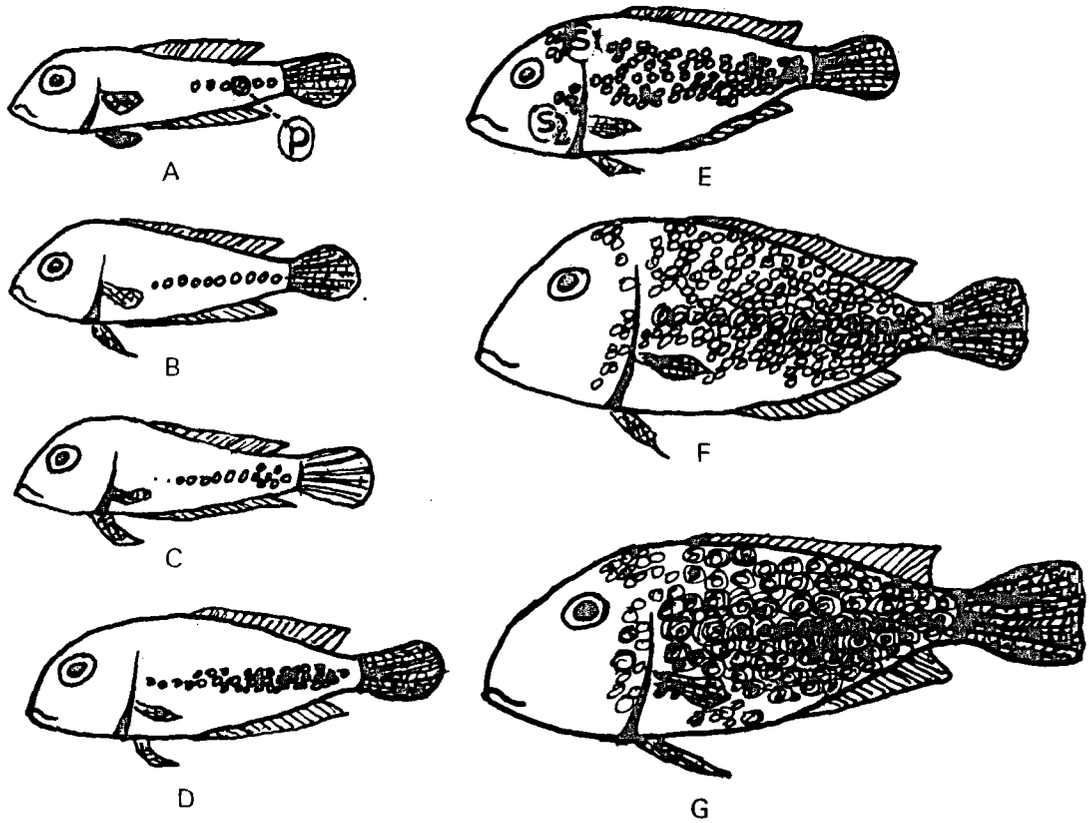


Fig. 1 A-G. The squamation chronology showing sequence of scale development in the fry of *O. mossambicus* (freshwater). P=Primary locus; S1=secondary locus at parietal region; S2=secondary locus at opercular region.

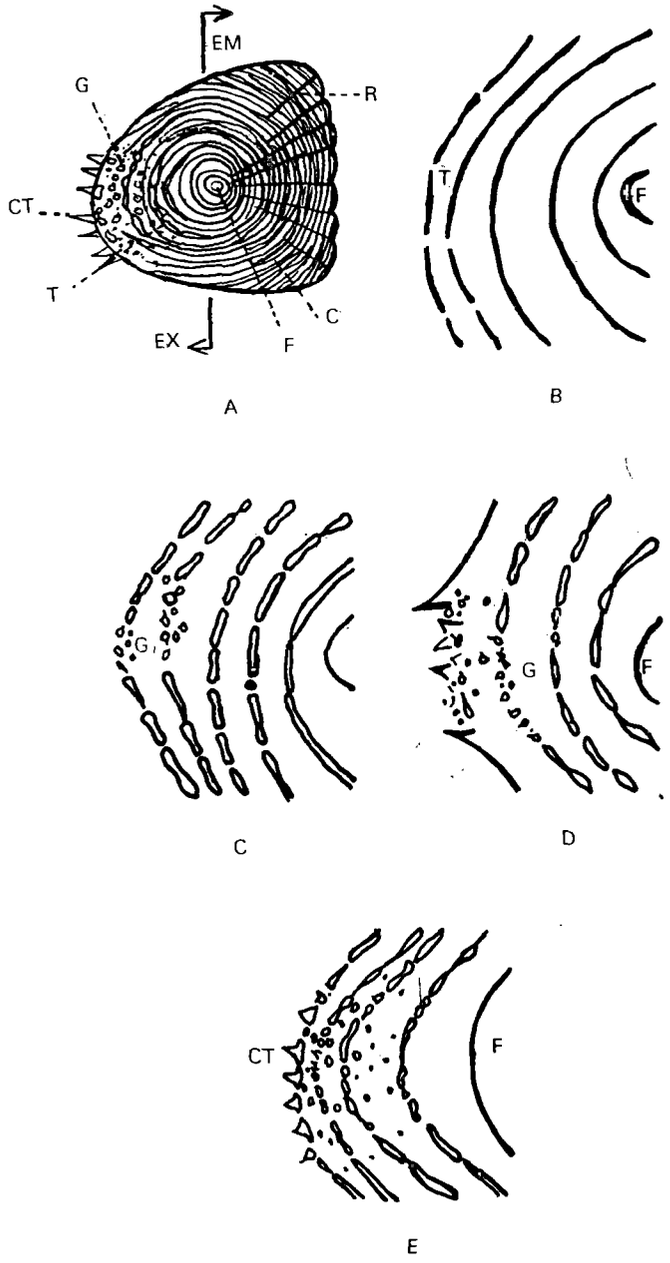


Fig.2 A-E. The origin of scale structures like ctenii, tubulation and granulation from the circuli in the exposed region of scales in *O. mossambicus*. A-A typical midlateral scale: B-the concentrically arranged ridge like circuli in the exposed region in the newly formed scale: C-the circuli in the exposed region are being disrupted into tublations and some of which later get disintegrated into fine granulations: D-posteriormost circuli being transformed into weak ctenii: E-the weak ctenii finally getting transformed into stout ctenii: C-circuli: CT-ctenus: EM-embedded region: Ex-exposed region: F-focus: G-granulation: R-radii: T-tubulation.

By the end of the fourth week, scales began to appear in the parietal and the opercular regions. These are the secondary locii and appeared more or less simultaneously. The new scales appearing from this secondary locii soon covered the operculum and head. In all respects, the new scales were alike the existing ones except in size. Previous studies by Fugita (1971) had pointed out that there was only a single locus of squamation development which is caudal peduncle in different *Tilapia* spp. However, Sire and Arnulfe (1990) had demonstrated the existence of 3 sites of squamation development in other cichlids, viz. caudal peduncle, pectoral peduncle and parietal region. Thus, operculum is reported as a new secondary site of scale develoment instead of pectoral peduncle known in other cichlids. Hence, it is possible that the sites of scale development can show variation in different species of same family as suggested by Sire and Arulfe (1990).

In the fifth week, scales began appearing toward the belly as a continuation of the primary locus when the fry were 1.6 cm in total length (Fig.1 G). Additional scales were also laid on the head and opercular region from the secondary locii. At this stage of growth, the major area of the body surface was covered by scales except in the breast, caudal fin and infraorbital regions.

During the subsequent growth of fry, development of different scale structures was observed. When the fry was 1.8 cm, the scales of midlateral region attained the maximum size together with 6 grooves like incomplete redii in the process of formation. The number of circuli added reached 14.

As the fish attained a size of 2.3 cm, the ctenii started appearing as ciliated spines on the posterior margin of midlateral scales only, by the transformation of the circuli (Fig.2) as also observed by Coburn and Gaglione (1992). There were 5 complete and 3 incomplete radii on an average. The mean circuili number counted was 23. Another notable feature was development of scale structures like granulations and tubulations as disintegration or disruption of the circuli in the exposed region of the scale similar to the observations of Lippitsch (1990).

The development of stout ctenii was complete when the fish grew to 3.8 cm in total length. The ctenii numbered 6 to 8. The circuli number was around 30. The circuli in the exposed (posterior) region was extensively disrupted into tubulations and granulations. An average of 5 complete radii was noted. The ctenii bearing scales, referred to as 'ctenoid' and others reffered to as 'cycloid' of this small sized fish resembled the scale of large adult fish except in size, number of ctenii, circuli and radii. The scales of midlateral region alone got modified into ctenoid. So, both ctenoid and cycloid scales are present in the adults as reported among other cichlids (Lippitsch 1990).

The development of scale sturctures like radii, tubulations, granulations and ctenii during earlier stages of growth in *O.mossambicus* in a definite sequence in various body positions indicated that it is a genetically programmed event taking place during development. Further, as scale structures are derived subsequently with growth of scale, it appeared that cycloidy was a neotenous condition whereas ctenoidy was an advanced state as also suggested by Lippitsch (1990).

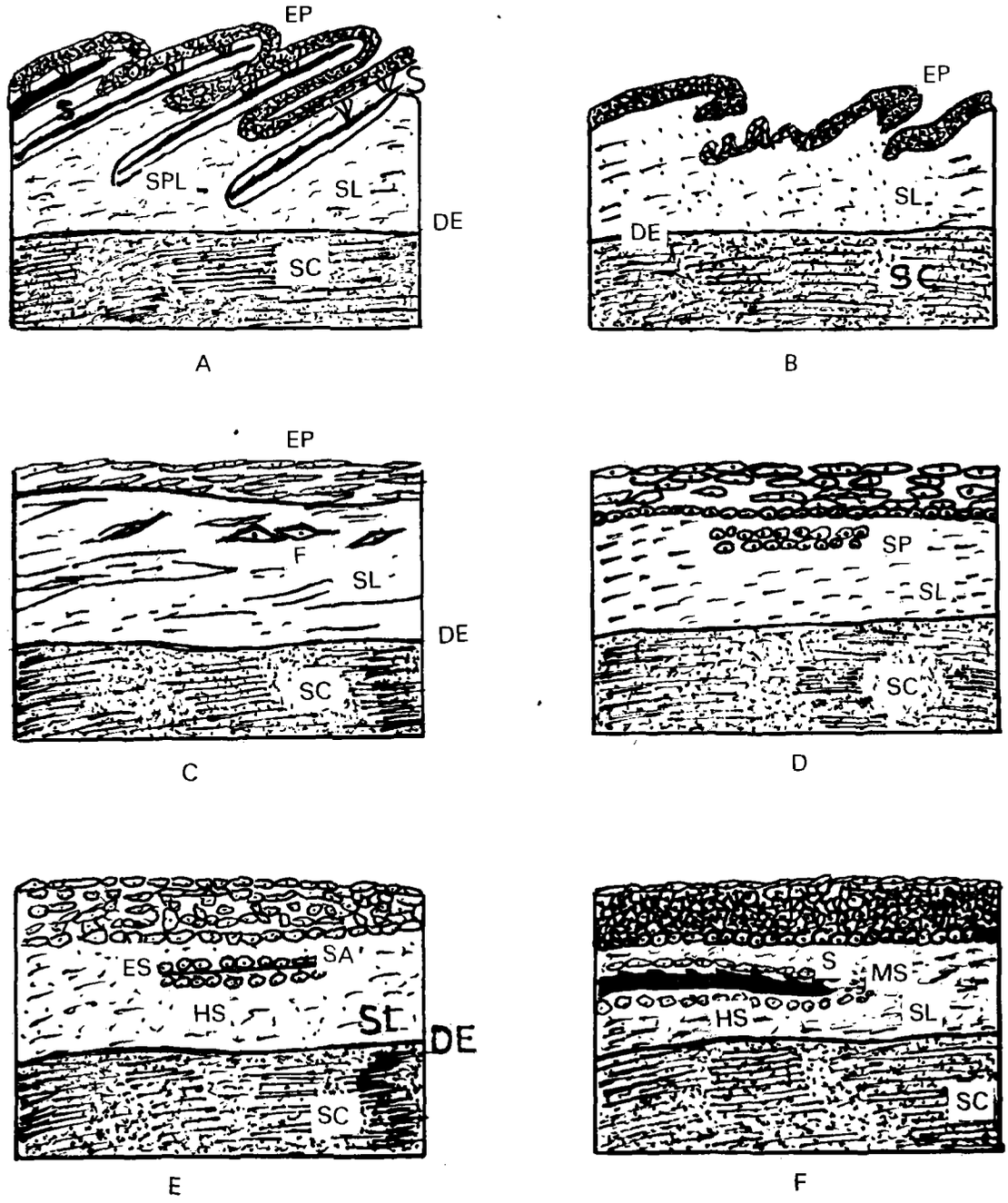


Fig. 3. Normal structure of the skin and various stages of scale regeneration in *O mossambicus*. A-Skin with scales lodged in it: B-skin sturcture after scale removal: C-wound closure (stage I): D- formation of scale papilla (stage II): E-scale anlage formation (stage III): F-completion of regeneration (stage IV). DE-dermis: EP-epidermis: ES-episquammal scleroblasts: F-fibroblasts: HS-hyposquammal scleroblasts: MS-marginal scleroblasts: S-scales: SA-scale anlage: SC-stratum compactum: SL-stratum laxum: SP-scale papilla.

Scale regeneration

Normal skin structure (Fig.3 A): The skin of *O. mossambicus* had the structure of a typical teleost. The scales were lodged in scale pocket bound by a scale pocket lining located in the outer zone of the dermis known as stratum laxum. The scale pocket lining was formed of minute spindle shaped cells arranged in 2 rows. On the outerside of scales were present special small cells identified as episquammal scleroblasts. Similarly, beneath the scales was present hyposquammal scleroblasts. Minute cells were also present in the marginal zone of scales referred to as marginal scleroblasts. In cross section, scales had a basal lamellated

fibrous layer and an upper nonlamellated bony layer. Many anchoring fibres extended between membrane of epidermis and the scales, helping in anchoring the epidermis to the scales below.

Skin structure after scale removal (Fig.3 B): Following scale removal, the epidermal tissue over the exposed portion of scales and peripheral portion of stratum laxum including fibroblasts were lost. Scale pocket and scale pocket lining was shattered. Many scale associated cells, viz. the scleroblasts were lost.

The regenerative process after scale removal could be categorized into 4 stages as detailed below.

Stage I. Wound closure (Fig.3 C). Twelve hours after scale removal, a basement membrane differentiated over the existing stratum laxum. A thin overlying epidermal coat was then formed. In the stratum laxum, no restorative change was noted.

Stage II. Formation of scale papilla (Fig.3 D). Twenty four hours after scale removal, a proliferation of fibroblasts was evident in the stratum laxum. These fibroblasts transformed into potential scale forming cells, the scleroblasts, which appered as a cluster of horizontally arranged cells just beneath the epidermis. This organization of scleroblasts was identified as scale papilla.

Stage III. Scale anlage formation (Fig.3 E). By the third day, the appearance of the scale primordium took place. It was thin long plate, also termed as scale anlage formed in between the potential scale forming scleroblasts. Apparently, the scleroblasts along the upper surface known as episquammal scleroblasts and those on lower surface named as hyposquammal scleroblasts were responsible for laying of the upper osseous and lower fibrillary layer of scale respectively. The marginal scleroblasts helped in the increase of scale diameter. Thus, the present study showed that scale primordium of anlage of scale pocket lining which had remained intact after scale removal. Further, the observation that upper osseous layer and lower fibrillary layers of scales are formed from epi and hyposquammal scleroblasts are in consistence with the view of Zylberg *et al.* (1992).

Stage IV. Completion of regeneration (Fig.3 F). The initial scale anlage was transformed into a distinct scale after 4 to 5 days. Subsequent increase in thickness of scale was mainly due to the further addition of fibrils which were added layer by layer in the basal fibrillary plate. Approximately 9 to 10 days after scale removal, the scale pockets got well organized and the entire scale was surrounded by scleroblasts inside a scale pocket lining, the whole unit being referred to as a scale bag. By the end of 20 days, the regenerated scales attained the size of a normal scale. Many fibres were formed anchoring the epidermis to the scale surface. The regenerated scales exhibited many deformities. More than half of the concentrically arranged circuli found in the centre of scales were disorganized so that the focus was much enlarged. Inside this focus, many granulations and ridges were present. This might have happened as a consequence of the faster scale developmental process compared to the normal ontogeneic development at larval stages. Earlier, Sire and Geraudie (1984) and Sire (1986) designated scale with similar ornamentation as regenerated scales in *Hemichromis bimaculatus*.

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Short Communication

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Mycotic involvement in trout fishes of Himachal Pradesh

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Key words: Fungus, Trouts

The fish, being poikilothermic, suffers more due to subtle changes in its immediate surroundings. The fungal infections are not acute in intensity but still does result in reduced aquaculture productivity. Therefore, a study was carried out to assess the prevalence of various mycotic diseases in trouts.

Samples (76) of trouts were procured from different places of Himachal Pradesh. These were identified as rainbow trout *(Salmo giardneri)*, brown trout (*Salmo truttafario)* and snow trout *(Schizothorax richardsoni)*. Superficial and internal lesions in the affected fishes were closely examined and samples were processed for fungal isolation. The inoculum was inoculated onto sabouraud's agar plate and incubated at room temperature (12°-15°C) for 1-2 weeks. The characteristic growth along with the presence of aerial mycelium, sexual, asexual spores and hyphae were considered for their identification. In all 29 fungal isolates emanated from the 76 samples of trouts and the maximum fungal isolates were isolated from the fins and kidneys. The species of *Aspergillus, Penicillium, Rhizopus, Trichosporum* and *Cladosporium* were identified in the present study (Table 1).

The presence of *Penicillium* spp., *Aspergillus flavus* and *Aspergillus* spp. in this study finds support with the work done by El-Hissay *et al.* (1980), who recovered *Aspergillus* spp. and *Penicillium* spp. during an investigation with aquatic fungi associated with 7 species of Nile fish. In this study an isolate of *Fusarium* spp. could be recovered from the intestine of a trout. This fungus had been reported to be pathogenic to fishes (Horter 1960). The isolation of *Paecelomyces* spp. in this study are in agreement with Bruno (1989), who documented *Paecelomyces farinosus* from moribund atlantic salmon. Such fishes showed reddening of the ventral area with swollen abdomen, and the swim bladder revealed swelling and a whitish mass.

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Table 1. Fungal species isolated from different parts of trout

Type of organ	No. of samples processed	Positive samples	Fungal isolates
Intestines	24	6	2 *Penicillium* spp. 1 *Aspergillus flavus* 1 *Fusarium* spp. 1 *Aspergillus* spp. 1 Unidentified spp.
Kidneys	59	7	2 *Aspergillus flavus* 1 *Rhizopus* spp. 1 *Aspergillus* spp. 1 Arthrospore and septate hyphae 1 *Trichosporum* spp. 1 *Paecelomyces*
Pooled organs	54	2	1 *Caldosporium* spp. 1 Septate hyphae and arthospore
Muscles	8	4	2 *Penicillium* sp. 2 Unidentified mold
Fins	8	10	3 *Penicillium* spp. 2 *Aspergillus* spp. 3 Unidentified molds 2 Arthospores and septate hyphae

SUMMARY

The prevalence of various mycotic diseases in trouts was studied. In all 29 fungal isolates were isolated are maximum from fins and kidneys.

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Book review

The Mineral Nutrition of Livestock. 1999. 3rd edn. Underwood E J and Suttle N F. 613 pages, pg size 24 cm × 17 cm. CABI Publications, CAB International. Wallingford Oxon, OX108DE, UK.

The mineral nutrition of livestock by Underwood (deceased) and Suttle have been extensively researched and exhaustively reviewed book. It gives an elaborate account of all the mineral affecting and influencing the livestock. The endeavour of the authors to meet the needs of undergraduate, graduate students of Veterinary and Agriculture College is highly successful. It proves to be a ready reference for teachers and research workers of Veterinary and Agricultural Faculty for both developed and developing countries. The authors are highly successful in their efforts.

The book is divided into 19 chapters. The first few chapters deal with macro elements. Here separate account of Ca-Mg-P are given in different chapters i.e. for each element there is a different chapter. It is highly commendable, on the part of authors, to break these elements into separate chapters, which shows such a close interrelationship. Phosphorus and sodium which were usually bracketed with calcium and potassium respectively have been alloted separate chapters. Enhaustive account of micro elements is also elucidated in separate chapters. Other macro elements have been given ample coverage in separate chapters. There is a new chapter under occassionally beneficial elements which includes boron, chromium, lithium, nickel, molybdenum, tin and vanadum. Toxic elements have been given separate chapters and adequate emphasis has been given on various aspects of toxicity. With recent interest on the toxicity aspects of various minerals these days the chapter entitled 'Essentially Toxic Elements' is very handy and will prove to be an immense help for the workers in the field.

The layout of each chapter is lucid, simple and compact, starting with dietary sources, metabolism, function, disease and ending in treatment. Biochemical manifestations and clinical symptoms of various mineral deficiency diseases have been lucidly elaborated. The final chapter on the supplementation trial have great practical utility and application. It shall be of great utility for fieldworkers and policy makers alike.

The review of the literature has been taken up comprehensively by the authors, which shall be of tremendous help to other researchers and workers. The literature cited is broad and comprehensive which shall be extremely useful for worker who want more information on specific topics. The language of the book is simple, organized and lucid.

As such the book is compact ready reference and shall be immensely useful to field veterinarians and students as well.

Authors deserve complements for bringing out elaborate literature on mineral and source, metabolism function, deficiency and application.

M C SHARMA

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