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VP_4 gene specific RT- PCR for detection of bovine group A rotaviruses

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ABSTRACT

RT-PCR assay was standardised with partial length primers specific to VP_4 gene of bovine group A rotaviruses. The double stranded RNA (dsRNA) was extracted from MA 104 cell culture grown prototype rotaviruses by GIT lysis method. The presence of dsRNA in the extracts was confirmed by RNA-polyacrylamide gel electrophoresis (RNA-PAGE) with silver staining. To develop the RT-PCR assay, dsRNA was first converted into complimentary DNA (cDNA) by reverse transcriptase enzyme and subsequently amplified by Taq DNA polymerase. The oligonucleotide primers used in RT-PCR assay were specific to both 3' and 5' ends of VP_4 gene, which is highly, conserved among group A rotaviruses. The presence of expected 876 bp long PCR product was visualised by agarose gel electrophoresis. The primer pair used in present study specifically amplified VP_4 gene. The results of present study suggested that RT -PCR assay could be used for detection of rotaviruses.

Key words: Bovine rotaviruses detection, Genome segment 4, RNA-PAGE, RT-PCR

Amongst different serogroups of rotaviruses, group A is presently the most frequently isolated in the cases of rotaviral diarrhoea in bovines. Since isolation of rotaviruses from faecal samples is often difficult, the most widely used diagnostic techniques for rotavirus detection in faecal samples include immunoassays, such as ELISA, latex agglutination test and electron microscopy (EM). The assays aimed at detecting the nucleic acid of the viruses include RNA- PAGE and nucleic acid hybridisation (Flores *et al.* 1983, 1989). However, these techniques have limitations of low sensitivity and have generally not proven to be substantially more sensitive than optimised immunoassay procedures (Wilde *et al.* 1990). The aim of the present study was to develop and use RT-PCR assay as a diagnostic tool for detection of group A bovine rotaviruses using genome segment 4 (VP_4 gene) specific primers.

MATERIALS AND METHODS

Bovine rotavirus reference strains

The bovine rotavirus reference strains used in the present study were B223 (G10P11 type) UK strain (G6P5 type) NCDV (G6P1 type) and cell culture adapted field isolates 685 (G6P11) and 129 (G10P11). Foetal rhesus monkey kidney, an established cell line (MA 104), originally obtained from National Facility on Animal Tissue Culture Collection, Pune, was used for the cultivation of the bovine rotavirus reference strains.

Cultivation of the reference and field rotavirus strains

Standard procedure was followed to cultivate reference strains of rotavirus in MA 104 cells grown in milk dilution tissue culture glass bottles using M199 medium supplemented with 10% new born calf serum. The virus was harvested when the cells showed 75% CPE by 3 cycles of freezing and thawing.

Extraction of the viral nucleic acid

The viral dsRNA was extracted from cell culture grown reference rotaviruses by guanidine isothiocynate (GIT) lysis method (Chomczynski and Sacchi 1987) with some modifications. All the samples were mixed with equal volume of GIT lysis buffer containing 25g guanidine isothiocynate in 29.3 ml diethyl pyrocarbonate (DEPC) treated water; 1.76 ml sodium citrate (0.75 *M*, pH 7.0); 2.64 ml 10% sarcosyl; and 0.36 ml β-mercaptoethanol and vortexed. Subsequently, 0. 1 ml of 2*M* sodium acetate was added in each sample and vortexed again. The sample was then extracted twice with equal volume of phenol and chloroform mixture. Following centrifugation at 12 000 rpm for 20 min, the aqueous phase was collected and to this equal volume of isopropanol was added and kept at –20°C over night. RNA was pelted out by centrifuging at 12 000 rpm

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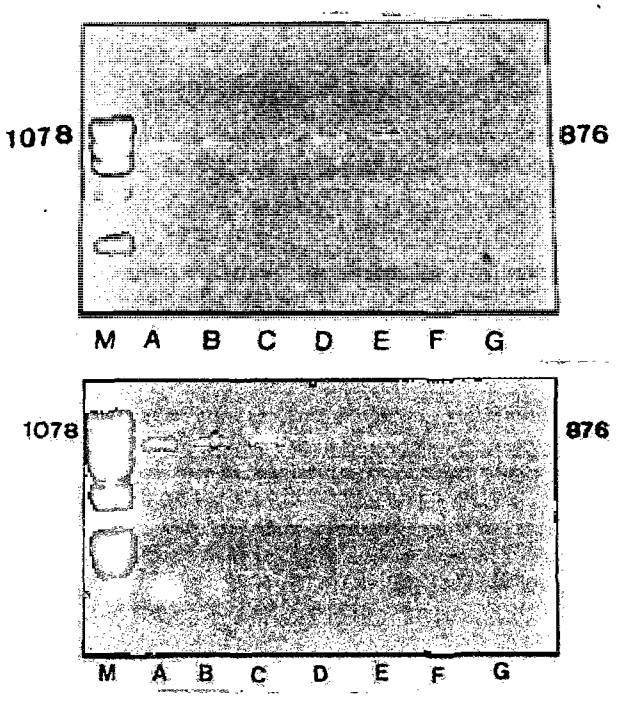

Figs 1-2. 1. RT-PCR of genome segment 4 (VP4 gene) of cell culture grown bovine group A rotavirus (BRV) strains. Lanes: A-C: RT -PCR of BRV reference strains (NCDV, UK, B223), Lanes D & E: RT-PCR of cell culture grown field strain, Lane F& G: negative controls of water without nucleic acid and heterologous ds RNA of bluetongue virus, respectively. Lane M: Hae III cut markers of f × 174 DNA. The 876 represents size of RT-PCR product in base pairs (bp). 2. Sensitivity of RT-PCR of genome segment 4 of bovine rotavirus. Lanes: A-F: different dilutions of ds RNA of BRV reference strain NCDV (A: 200 pg, B: 20 pg, C: 2 pg, D: 200 fg, E: 20 fg, F: 2 fg), Lane M: Hae III cut markers of f× 174 DNA. The 876 represents size of RT-PCR product in base pairs (bp).

for 30 min at 4°C. The pellet was washed with prechilled 0.5ml of ethanol (70%) and centrifuged at 12 000 rpm for 20 min to remove extra salts. Finally, the pellet was air dried and suspended in 30 µl of DEPC treated water and stored at –20°C till further used.

RNA-polyacrylamide gel electrophoresis (RNA- PAGE)

The RNA pellet was directly dissolved in 30 µl of RNA sample buffer to perform RNA-PAGE. The RNA samples were heated at 56°C for 5-10 min to dissolve the pellet completely. Subsequently the samples were loaded into the wells of 5% stacking and 7.5% resolving gels according to the method of Laemmli (1970) and the gel was run at constant voltage of 101 V till the dye came out of the gel (normally for 5-6 hr) using tris-glycine electrode buffer (pH 8.3). The silver staining of the gel was done as described by Svensson *et al.* (1986). The gel was kept in the stop solution for 15 min. The stained gel was photographed and stored in 10% ethanol.

Reverse transcription and polymerase chain reaction for genome segment 4 (VP4 gene)

Primers: A pair of partial length primers specific for genome segment 4 (VP4 gene) was used for RT-PCR amplification as described by Gouvea et al. (1994). The 5' end of the primer contained highly conserved sequences for group A rotaviruses. The published sequences of the primers are given in Table 1.

Table 1. Oligo- primers for RT-PCR amplification of bovine rotaviruses

#	Primer	Sequences (5'-3')	Position
1	Con3	TGGCTTCGCTCATTTATAGACA	11-32
2	Con2	ATTTCGGACCATTTAATAACC	868-887

The primers were got synthesised from GIBCO-BRL, USA.

Reverse transcription (RT): The RNA extracted from cell culture grown bovine rotavirus reference strains was used to standardise the reaction conditions for reverse transcription. The extracted RNA (5 µl) was added to 0.2 ml thin walled PCR tubes (Perkin-Elmer) containing 1.5 µl of dimethyl-sulfoxide (DMSO). The RNA was denatured at 97°C for 5 min and chilled on ice for 5 min. To the denatured RNA added 18.5 µl of reverse transcription reaction mix containing 100 ng of primers Con2 and Con3, 200 mM each of dATP, dCTP, dGTP and dTTP, 1× RT buffer and 5 units of AMV reverse transcriptase. The contents were mixed and the tubes were spun in a microcentrifuge briefly. The reaction was carried out at 46°C for 45 min. The enzyme reverse transcriptase was inactivated at 94°C for 2 min.

Polymerase chain reaction (PCR): The optimum concentrations of $MgCl_2$ (1.5 mM), DMSO (7%) and primers (100 ng) were used. An aliquot of 5 ml of cDNA along with 3 ml DMSO was denatured at 97°C for 5 min and chilled on ice for 5 min. To this added 42 µl of PCR mix containing 200 mM each of dATP, dCTP, dGTP and dTTP, 1× Taq buffer, 1.5 mM $MgCl_2$, and 2.5 units of Taq DNA polymerase .Thirty cycles of amplification were carried out in Perkin Elmer, 2400 thermocycler. A protocol of denaturation at 94°C for 1 min, annealing at 45°C for 2 min, and extension at 72°C for 2 min was followed with the final extension at 72°C for 5 min.

Sensitivity and specificity of assay

To determine the specificity of the primers and reaction, conditions, various controls like heterologous ds RNA of bluetongue virus, negative control (without nucleic acid), were used and subjected to the similar conditions of RT-PCR. To find the threshold of sensitivity for detection of rotavirus ds RNA, the extracted nucleic acid was serially diluted and subjected to RT-PCR protocol.

The amplified products were analysed in 2% agarose prepared in TBE buffer, visualised under UV transilluminator.

and photographed (UVP, GDS 5000).

RESULTS AND DISCUSSION

Preliminary screening by RNA-PAGE indicated that all the viruses yielded similar long electrophoretic migration pattern typical to group A rotaviruses. Conventionally RNA-PAGE with silver staining has been evaluated as a preliminary screening test for detection of group A rotaviruses (Fijtman *et al.* 1987, Singh and Pandey 1988). In the present study all isolates of group A rotaviruses used had long RNA genome segment pattern, which suggested that different G and P genotypes in combination might have similar 'long' RNA migration patterns. In contrast, the studies in human rotavirus infection have indicated that different G and P genotype combinations may have different RNA patterns. Hussian *et al.* (1996) reported that G1P8, G3P8 and G1P6 genotypes isolated from humans had 'long' RNA patterns whereas G2P4 genotype had short RNA pattern.

All the samples including reference strains positive by RNA-PAGE could be amplified by these two primers and an expected PCR product of 876 bp was observed in agarose gel electrophoresis (Fig. 1). No amplification was observed with heterologous nucleic acid from bluetongue viral dsRNA using rotavirus VP4 gene specific primers indicated that the primers used were specific for group rotaviruses. Similarly these VP4 gene specific primers (Con2 and Con3) for partial length and a cocktail of pNCDV, pUK and pB223 along with Con2 were successfully used for VP4 specific detection and P typing of bovine rotaviruses in cow calf faecal samples by Gouvea *et al.* (1990, 1994).

The lower detection limit of RT-PCR as determined by 10 fold dilutions of dsRNA from NCDV strain was 20 fg (Fig. 2) which is equal to 20 virus particles. The threshold sensitivity of PCR for gene segment 9 with genomic RNA of human rotavirus reference strain Wa, was 500 fg which is approximately equivalent to 2×10^4 genome copies (Hussain *et al.* 1996).

The genome segment 4 of group A rotavirus encodes vp4 specific neutralising antigen. The result obtained with RT-PCR suggested that primers complimentary to 5' end of segment 4 are suitable for development of RT-PCR assay. The assay may further be evaluated for detection of rotavirus infection directly in faecal samples of diarrhoeic calves and for nested PCR for genotyping of rotaviruses by using type specific primers and to generate DNA probes to develop nucleic acid hybridisation assays for epidemiological surveys of rotaviruses in the field. The work in this regard is in progress. The availability of such a sensitive diagnostic test will be very useful in epizootiological studies. The specificity, sensitivity and speed of RT-PCR assay would facilitate application of the system for diagnosis of rotavirus infection in animals.

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Serological survey for the prevalence of bluetongue virus antibodies in cattle by competitive enzyme linked immunosorbent assay

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ABSTRACT

A serogical survey for the presence of bluetongue virus (BTV) antibodies in cattle was conducted at selected sites in Andhra Pradesh, Karnataka and Tamil Nanu. Group-specific antibodies to BTV were estimated by a competitive enzyme linked immunosorbent assay (cELISA) using polyclonal antibodies. Results revealed that seroprevalence of BTV in AP, KS and TN were 65.19% and 79.51% and 80.95% respectively. The variation in per cent prevalence of antibodies to BTV infection according to the age and bread type was discussed.

Key words: Bluetongue virus, Cattle, Competitive ELISA, Seroprevalence

Bluetongue infection in cattle is important, as the infected cattle, serve as carriers due to prolonged viremia and thus are reservoir source for the BTV (Gard and Melville 1992). The information on the status of infection and distribution of the disease in different geographical areas are essential to understand the spread of BTV. BTV infection in different species of animals and its distribution in India was reviewed by Prasad *et al.* (1992) and Sreenivasulu *et al.* (1995). Information on seroprevalence of BTV in indigenous, crossbred and exotic breeds of cattle are scarce. In this study competitive ELISA (cELSIA), using polyclonal antibodies was used to estimate the levels of the BTV specific antibodies in cattle at selected sites in Andhra Pradesh (AP), Karnataka (KS) and Tamil Nadu (TN) states, India.

MATERIALS AND METHODS

Collection of serum samples

A serum samples from 350 cattle was collected from Banavasi, AP; Lamfarm, Guntur district, AP: Rajampet, Cuddapah district, AP; Venkatagiri, Nellore district, AP; slaughterhouse, Chennai, TN; slaughterhouse, Bangalore, K.S. All the serum samples were inactivated at 56°C for 30 min and stored at -20°C.

Antigen and polyclonal antisera

A BHK_{21} cell culture adopted BTV (serotype 2) maintained in the Department of Microbiology, College of Veterinary Science, Tirupati, was used as antigen (5.5 $\log_{10}$ $TCID_{50}$/ml). The polyclonal antisera produced against purified BTV in rabbit was used in cELISA (Dayakar *et al.* 1999). Cattle serum containing antibodies to BTV in high titer, low titer and BTV negative (available in Department of Microbiology, Tirupati) were used as controls in cELISA.

Competitive enzyme linked immunosorbent assay

A method of Hamblin *et al.* (1989) with slight modification was followed for detection of group-specific antibodies to BTV. Polyvinyl 96 well ELISA plates were coated with 50µl of BTV antigen diluted in tris-buffered saline pH 9.6 and incubated overnight at 4°C. The plates were washed 3 times with phosphate buffered saline, pH 7.2 containing 0.01% Tween (PBS-T). Each serum sample diluted to 1:10 in antibody buffer (PBS-T containing 0.1% skim milk), was tested in duplicate wells. Polyclonal BTV specific antibodies (50µl) were added simultaneously to all the wells and incubated for 1 hr at 37°C in a moist chamber. A high tited positive serum, low titred positive serum, low titred positive serum and negative serum were used as controls. The plates were washed and 50µl of anti rabbit IgG HRPO conjugate atl:5000 was added to all the wells and incubated for 1 hr at 37°C. The plates were washed with PBS-T and 50µl of tetra methyl benzidine (TMB) substrate was added and incubated

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at room temperature for 10 min. The reaction was stopped by the addition of 50µl of 1 M sulphuric acid to all the wells of the test plates and readings were taken at 450 nm in an ELISA plate reader.

Results of the test sera were determined by expressing them as per cent inhibition (Pl) calculated as show below. The data were analysed statistically as per Snedecor and Cochran (1967). Sera samples were considered positive if the Pl value is >50. The test was repeated if the low titred positive control sera has less <40.Pl, or the negative controls fall outside the 1.1-1.6 OD range.

$$\text{Per cent inhibition} = \frac{100-(100\times\text{mean OD of test serum})}{\text{Mean OD negative control serum}}$$

RESULTS AND DISCUSSION

Competitive ELISA using polyclonal antibodies was used to assess prevalence of antibodies to BTV in cattle. Sera collected from the selected sites of 3 states indicated that, out of 350 samples tested 250 were positive (Table 1). Prevalence of BTV antibodies in AP, KS and TN, were 65.19, 79.51 and 80.95% respectively. In AP, samples collected from Cuddapah and Guntur districts, recorded high seroprevalence rate with 34/40 (97.5%) and 37/49 (90.24%) cattle tested were positive to BTV. Based on age of the animal the 350 sera samples were assorted into 8 groups, starting with 0-1 to over 7 years (Table 2). The prevalence of BTV antibodies varied from 61.11 to 86.66% in different age groups, with the lowest of infection in above 7 years age groups. Cattle between the age of 0-12 months was the most susceptible for BTV infection. The 350 cattle from which sera was collected consisted of 4 breeds, namely, Holstein Friesian, Jersey, Murrah, Ongole and non-descriptive native breed (Table 3). The prevalence of BTV antibodies among these groups ranged from 57.84 to 81.25%. Among the different breeds, Ongole's appears highly susceptible (81.25% positive), followed by Jersey (80.88%), native-breed (76.59%) and Holstein Friesian (67.92%). Buffaloes appear least infected of the tested animals with 59/102 (57.8%). There is no significance difference ($P<0.01$) between the different breeds with regard to seroprevalence of BTV antibodies, and all of the popular breeds and native animals appear to be acting as reservoir hosts to BTV.

Table 1. Prevalence of BTV antibodies in different areas

Region	Total sample tested	No. positive	No. negative	% Positive
Banavasi, Kurnool	88	56	32	36.63
Rajampet, Cuddapah	49	37	12	90.24
Lamfarm, Guntur	40	34	6	97.5
Venkatagiri, Nellore	27	6	21	22.22
Slaughterhouse, Bangalore	83	66	17	79.51
Salughterhouse, Chennai	63	51	12	80.95

Table 2. Prevalence of BTV antibodies in different age groups

Region	Total sample tested	No. positive	No. negative	% Positive
0-1 year	15	13	2	86.66
1-2 years	36	22	14	61.11
2-3 years	22	16	6	72.22
3-4 years	69	46	23	66.66
4-5 years	59	40	19	67.79
5-6 years	70	55	15	78.57
6-7 years	33	26	7	78.78
Above years	46	29	17	63.04

Table 3.Breed-wise prevalence of BTV antibodies

Region	Total sample tested	No. positive	No. negative	% Positive
Holstein Friesian	53	36	17	67.92
Jersey	68	55	13	80.88
Native breed (Non descript)	79	60	19	75.94
Ongole	48	39	9	81.25
Murrah	102	59	43	57.84

For estimating antibodies to BTV, competitive ELISA using monoclonal antibodies is widely used (Gustafson and pearson 1992). But monoclonal antibodies are not available in our country. Further, monoclonal antibodies produced against group-specific VP7 protein failed to react with all 24 BTV serotypes (Afshar *et al.* 1991, White 1992). Thus replacement of monoclonals with polyclonal antibodies was advocated in Australia (Geoff Gard, personal communication). Moreover, number of BTV serotypes exists in India (Sreenivasulu *et al.* 1995) and an assay system that can detect all the serotypes is useful. recently, cELISA using polyclonal antibodies was developed and standardized for estimating group-specific antibodies to BTV (Dayakar *et al.* 1999). This system was used to estimate BTV specific antibodies in serum indicated high prevalence to BTV in all the states. This suggests that these regions could be endemic to BTV and the infected cattle might be serving as persistent virus sources leading to several BTV outbreaks. Prevalence of high levels of BTV antibodies in cattle of 0-1 year age groups also suggests that the virus is in constant circulation in these areas. Prevalence of BTV antibodies in cattle were recorded in various regions in India by several workers (Prasad *et al.* 1997, Oberoi *et al.* 1988, Prasad *et al.* 1992, Naresh and Prasad 1995). They reported 4.2 to 70% seroprevalence of BTV in cattle. All these studies suggests that the BTV is endemic in India. Therefore application of any control measures to prevent BT infection in sheep, should also target cat-

tle, which acts as source for virus. Further studies are required to understand the conditions that favour BTV outbreaks in the rainy season.

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In vivo immunosuppression in broiler chickens with spontaneous infectious stunting syndrome

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ABSTRACT

Cellular and and humoral immune response were assessed in broiler chickens with spontaneous infectious stunting syndrome (ISS). Cellular immunity was measured by delayed hypersensitivity response to 2, 4- dinitrochlorobenzene (DNCB) and phytohaemagglutinin- purified (PHA-p) tests. In DNCB test, the mean increase in skin thickness and induration sites were significantly lower in infected birds. Similarly, the mean increase in skin thickness and induration sites were also significantly lower in infected birds than those of controls in PHA-p test. In ISS- infected birds, marked reduction in size of all lymphoid organs was observed with lymphocytolysis and/or depletion of lymphoid cell population. The mean antibody titres in the stunted birds immunized with Ranikhet disease virus as antigen, were significantly lowered. The results clearly demonstrated that the causative agent(s) of ISS, produced immunosuppression in broiler chickens.

Key words: Chicken, Immunosuppression, Infectious stunting syndrome

Broiler farming has suffered from infectious stunting syndrome (ISS), characterized by high early mortality, stunting, poor feathering and leg weakness (Olsen 1977, Kouwenhoven *et al.* 1978). This syndrome causes marked reduction in size and weight of bursa of Fabricius and thymus because of absolute depletion of lymphoid cells and/or lymphocytolysis (Bracewell and Randall 1984, El- Ballal and El- Zanaty 1994). Because ISS has been established to affect adversely immunobiological organs in broiler chickens, an investigation of immune response seemed warranted. The specific objective of this study was to assess the effect of syndrome on the status of cell-mediated immunity (CMI) by delayed hypersensitivity response and also to measure the humoral immunity (HI) of stunted birds against an antigen to establish the immunosuppressive effect of ISS.

MATERIALS AND METHODS

The diagnosis of ISS in 3.75% broiler chickens in 17 commercial farms of West Bengal was based on epizootiology and clinical signs (Bhattacharya and Bhowmik 1998). All stunted birds were segregated by a partition from their normal penmates. ISS- infected broiler chickens (35), aged 3 weeks and having 50% lowered average body weight (264.7±2.84g) as compared with birds growing normally, were procured from 3 farms and divided into 3 unequal groups. Two groups of such stunted birds, 10 in each were used to assess CMI by delayed hypersensitivity response to 2, 4- dinitrochlorobenzene (DNCB) test (group 4) and phytohaemagglutinin-purified (PHA-p) test (group 5). Group 4 stunted birds (15) were used to measure HI response to Ranikhet disease vaccine as antigen. Three week-old broiler chickens (30) with normal body weight (400.6±7.19g) obtained from a ISS-free farm were also divided into 3 equal groups (1,2,3) of 10 each, serving as controls for 3 experimental groups (4,5,6). Birds were housed group-wise in non-heated cages at an ambient temperature of 28±2°C. Continuous lighting was provided by fluorescent bulbs. All groups of birds were provided with standard commercial broiler ration and water *ad lib.*

Assessment of CMI response

2,4-DNCB test: Ten each of ISS-infected (group 4) and normal broiler chickens (group1), aged 3 weeks were selected for DNCB test (Malaviya 1993). A relatively featherless areas over 2 cm diameter of both right and left breasts were shaved and disinfected. The birds were primarily sensitized by a single application of 0.1 ml of 2% DNCB in a vehicle consisting of acetone in the right side of breast. The left breast received only the vehicle, serving as control. After 14 days, all sensitized birds, were challenged with 0.1 ml of 0.05% DNCB at the sensitized area, while the left side again received vehicle only.

Thickness of skin was measured at both sides by a mi-

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crometer at 9, 18, 27 hr post challenge. The diameter of reacting area (cm) was also measured at the same intervals with a scale. The overall MST was obtained by taking the mean of individual birds within the group. Likewise, mean increase of diameter was measured.

PHA-p test: The CMI response was also evaluated by measuring the blastogenesis response of T-lymphocytes inducted by PHA-p *in vivo*, and the methods described for chickens were followed (Bridger and Taxton 1982). As with DNCB test, 10 each of ISS-infected (group 5) and normal broiler chickens (group 2) were selected. The wing web of both right and left wings were defeathered and disinfected. Ten micrograms of PHA-p in 0.1 ml of normal saline were injected subcutaneously in a defined areas of right wing of each bird. Similarly, the defined sites of left wing were given 0.1 ml normal saline, serving as controls. Thickness of each wing was measured by a micrometer at 9, 18, 27 and 36 hr after the injections. Delayed hypersensitivity response was calculated as follows

$$\Delta\% = \frac{T_t - T_0}{T_0} \times 100$$

: where, Δ%=per cent changes in wing web thickness, T_t=thickness (mm) at each time period after PHA-p or saline injections, and t_0=thickness (mm) prior to PHA-p and saline injections (Bridger and Thaxton 1982).

Cytopathology

At the end of DNCB test, all birds of groups 1 to 4 were sacrificed by cervical dislocation and bursa of Fabricius, spleen and thymus were collected in 10% formalinsaline at necropsy. In PHA-p test, skin biopsies were taken from each bird of groups 2 and 5 at 9, 18, 27 and 36 hr after injections. Formalinized tissues of all groups were processed for paraffin sectioning (5μ) and stained with haematoxylin and eosin for histopathological changes.

Assessment of HI response

The ISS-infected (15) and normal broiler chickens (10) were used to measure HI response. Birds of groups 3 and 6, already vaccinated with Laosta F_1 strain in farms, were revaccinated on 28 day with F_1 strain. Blood samples were collected from both normal and stunted birds on 0 day (prior to vaccination), and thereafter on 7, 14, 21 and 28 day of post-vaccination. Blood samples were collected, the sera separated were activated at 56°C for 30 min and preserved at 4°C until use. Considering HI as a specific test to measure antibody titre of Ranikhet disease, it was conducted on collected sera samples of groups 3 and 6 birds, using method of Cunningham (1966).

Student's test was applied to test the mean values of DNCB and PHA-p tests, and humoral antibody titres of normal and ISS-infected birds for significant differences (Snedecor and Cochran 1968).

RESULTS AND DISCUSSION

CMI response

Application of challenge dose of DNCB produced ery-

Table 1. Delayed hypersensitivity response of control and ISS broiler chicks to DNCB test

Post hours challenge	Breast site	Croups		"t" values		
		Control (1) n=10	ISS-infected (IV) n=10	Right vs left of control	Right vs left of ISS-birds	Right vs left control, ISS-birds
9	Left (control)	0.18±0.01	0.14±0.02			
				42.34**	36.78**	22.94**
	Right (challenge)	2.37±0.05	1.14±0.02			
18	Left (rontrol)	0.12±0.01	0.08±0.01			
				18.14**	20.58**	12.19**
	Right (challenge)	1.14±0.02	0.71±0.03			
27	Left (control)	0.09±0.02	0.04±0.01			
				3.72**	0.65**	9.52**
	Right (challenge)	1.14±0.02	0.25±0.03			
			Skin diameter (cm)			
9	Left (control)	1.93±0.03	1.44±0.03			
				35.19**	19.10**	23.16**
	Right (Challenge)	3.70±0.04	2.43±0.04			
18	Left (control)	1.17±0.02	1.11±0.02			
				21.66**	9.76**	16.07**
	Right (challenge)	2.11±0.04	1.41±0.02			
27	Left (control)	0.40±0.03	0.38±0.04			
				16.74**	3.86**	13.50**
	Right (challenge)	1.15±0.03	0.56±0.03			

Significant (P<0.05), **highly significant (P<0.01); n, No. of birds.

Table 2. Delayed hypersensitivity response of control and ISS broiler chicks to PHA-p test

Groups	Wing web S/C injection	Post-injection hours (mean±SE)				Pooled treatment (mean±SE)
		9	18	27	36	
Second						
Control birds	Left (Saline)	24.20±0.66^{A}c	14.80±0.86^{B}c	7.20±0.66^{D}c	12.35±0.64c	
n=10	Right (PHA-p)	229.08±1.77^{B} A	256.0±3.50^{A}a	214.60±2.04^{c}a	190.0±1.7^{D}a	226.0±2.18a
Fifth						
ISS birds	Left (Saline)	21.67±0.95^{A}c	11.17±0.75^{B}c	442.0±0.49^{C}	2.0±0.19^{D}c	9.82±0.57c
n=10	Right (PHA-p)	170.75±1.7^{B}b	195.92±1.72^{A}b	154.42±1.70^{C}b	129.0±1.65^{D}b	162.52±1.67b

Mean in a row having different superscripts differ significantly (P<0.01), and means in a column having different subscripts differ significantly (P<0.01). Saline means a injection with 0.1 ml saline, whereas PHA-p means an injection of 0.1 ml of saline containing 10 μg PHA-p. Mean±SE are the wing web responses expressed as per cent change from 0 time, i.e. prior to injection.

thema, swelling, vesiculation, induration and scab formation at the sites of skin. These changes were more pronounced in control broiler chickens than in ISS-infected birds. The mean increase in skin thickness and diameter in control birds was significantly (P<0.05, 0.01) higher than those of stunted birds (Table 1). However, the reaction attained its peak at 9 hr post-challenge, and then gradually declined at 18 and 27 hr (Table 1). The reduced contact skin sensitivity suggested a significant suppression of CMI response in stunted birds.

Delayed hypersensitivity response to PHA-p test is presented in Table 2. The mean increase in thickness and induration sites of stunted birds was significantly (P<0.01) lesser than that in controls at 9, 18, 27 and 36 hr post-challenge. However, the reaction attained peak at 18 hr, and then started to decline from 27 hr post-challenge (Table 2). This further confirmed that CMI response was lower in ISS-infected birds as compared to controls.

Histopathology

The bursal follicles of ISS- infected birds showed reduction in size because of marked necrosis and/or depletion of lymphoid cells and increase on interfollicular connective tissue, accompanied with cysts formation. There was also no distinction between white and red pulp of spleen, and cortex and medulla of thymus with evidence of lymphocytolysis.

Nine hours after injection of PHA-p, the histopathological changes comprised haemorrhage, capillary congestion, oedema and perivascular cuffing of lymphocytes with infiltrations of lymphocytes and few macrophages in the skin. The vascular changes remained the same at 18 hr post-challenge, but the cellular changes were massive in degree. At 27 and 36 hr, the intensity of vascular and cellular changes were less evident. The cytopathological changes were suggestive of delayed cutaneous hypersensitivity reaction.

In this study, the skin reactions against DNCB and PHA-p tests were significantly lesser in ISS- infected birds as compared to controls. Results on DNCB contact sensitivity reaction and delayed hypersensitivity to PHA-p clearly demonstrated that CMI response was affected in birds with spontaneous ISS. The suppression of CMI in ISS-infected birds is attributed to interference or poor rate of lymphoid cell proliferation, which is further substantiated by the fact that the stunted birds also histologically showed marked reduction of lymphoid cells due to depletion and/or necrosis of lymphocytes in the bursa of Fabricius, spleen and thymus. Thus, in ISS-infected birds the suppression of CMI response might be due to injury to T-lymphocytes.

HI response

The mean antibody titres on 0, 7, 14, 21 and 28 day post-immunization were significantly (P<0.05, 0.01) lower in ISS-infected birds than those of controls (Fig.1). Such low levels of antibody titres clearly indicated the development of immunosuppression in the stunted birds.

Although a definitive explanation of immuno-suppressive effect is not currently known, several possibilities such as binding of μ-globulin *in vitro*, causing reduction in antibody formation and reduction in specific reactivity of the circulating antibody molecules might be accounted for reo-virus induced immunosuppression.

Data of this study clearly demonstrated that ISS exerts a

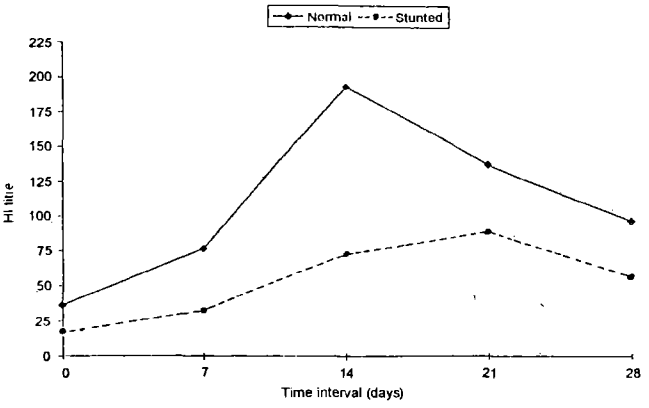

Fig. 1. Antibody titre in normal and stunted birds after RD vaccination.

decreased function of CMI and also interferes in the formation of normal circulatory levels of antibodies, producing immunosuppression in broiler chickens.

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Bacteria-induced increased vascular permeability in the chicken skin

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ABSTRACT

An increase in vascular permeability induced by the intradermal inoculation of bacterial suspensions in the chicken was assessed quantitatively and microscopically. Using *Streptococcus pyogenes* and *Escherichia coli* a triphasic pattern of permeability was observed. This is in marked contrast to the biphasic response obtained hitherto with non-biological agents.

Key words: Bacteria, Chickens, Vascular permeability

Bacterial diseases in poultry are mostly inflammatory in nature. Whereas, some information on the vascular response to non-living agents is known, the response to pathogenic bacteria in birds remains unexplored. The present study deals with the increase in vascular permeability of chicken skin induced by *Streptococcus pyogenes* and *Escherichia coli*.

MATERIALS AND METHODS

White Leghorn chickens, of either sex, aged 10-16 weeks, were used. Evans blue was used as a freshly prepared 1.5% w/v solution in normal saline @ 15 mg^{-1} body weight to estimate an increase in vascular permeability (Katiyar *et al.* 1992a, b). A colloidal suspension of carbon was used intravenously @ 0.1 ml/100 g body weight to delineate the leaky vessels (Katiyar *et al.* 1992 a, b).

Freeze-dried cultures of *Streptococcus pyogenes* (MTCC No. 442) and *Escherichia coli* (MTCC No. 723) obtained from Institute of Microbial Technology, Chandigarh, were subcultured and bacterial suspensions were prepared. The concentration of *S. pyogenes* and *E. coli* ml^{-1} of the suspension were determined as 3.0 and 1.9 thousands of millions respectively.

The sites of cutaneous reactions, on lateral thoracic regions of birds, were prepared (Katiyar *et al.* 1992a, b).The birds were given 0.02 ml bacterial suspension intradermally.

Increased vascular permeability

Dye technique: Each bird on each side of the lateral thoracic region was inoculated with the respective bacterial suspension for any 2 of the 8 time intervals (Table 1). Evans blue solution was then injected slowly into the wing vein. The dye was allowed to remain in the circulation up to 30 min and birds were then exsanguinated (Katiyar *et al.* 1992a, b). The increase in vascular permeability was quantitated by measuring the exuded dye per lesions spectrophoto-metrically (Pillai *et al.* 1988a).

Colloidal carbon technique: In another group of birds, a colloidal suspension of carbon was injected slowly into the wing vein. The birds were killed 30 min later, the time when carbon cleared from the circulation. Eight skin lesions with sufficient surrounding tissue for each time-interval were obtained and processed for topographical study (Katiyar *et al.* 1992a, b).

Statistical analysis: The experimental design used was

Table 1. Quantitative assessment of an increase in vascular permeability

Time interval	Permeability response (dye (µg/lesion) (Mean± SE)		
	Normal saline	*S. pyogenes*	*E. coli*
0-2 min	2.18 ± 0.048^{c}	7.65 ± 0.091^{c}	6.41 ± 0.127^{b}
30 min	2.56 ± 0.034^{b}	9.44 ± 0.046^{a}	6.65 ± 0.188^{a}
1 hr	3.22 ± 0.082^{a}	7.65 ± 0.091^{c}	6.20 ± 0.170^{b}
3 hr	2.04 ± 0.096^{cd}	6.29 ± 0.119^{d}	5.82 ± 0.117^{c}
6 hr	1.77 ± 0.079^{d}	4.86 ± 0.066^{e}	4.31 ± 0.028^{e}
12 hr	1.54 ± 0.086^{e}	8.62 ± 0.063^{b}	6.66 ± 0.037^{a}
1 day	1.29 ± 0.109^{e}	4.56 ± 0.071^{e}	4.58 ± 0.080^{d}
2 day	1.27 ± 0.058^{e}	9.17 ± 0.093^{a}	5.89 ± 0.018^{d}

Values with similar superscripts indicate nonsignificant differences; those with different superscripts significant differences ($P<0.05$).

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randomized block design with hierarchical classification. The treatment was the main effect, and intervals were tested within treatment.

RESULTS AND DISCUSSION

The pattern of the permeability response was more or less similar for both the organisms. Visually, the dye started exuding from the vessels within 5-7 min of the intradermal bacteria. Quantitatively, in the *S. pyogenes* - induced reaction, 7.65±0.09 μg of dye had exuded in 0-2 min lesions (Table 1). The blueing increased thereafter and was maximal at 30 min. In *E. coli* injury also, maximal concentration of the dye was obtained in 30 min lesion (Table 1). The increased concentrations of exuded dye were again obtained at 12 hr and 2 days for both the bacteria.

The permeability curve following intradermal bacterial suspension exhibited first peak at 30 min. The permeability factors such as histamine, and 5-hydroxytryptamine (5-HT) increases the vascular permeability only for 30 min in the chicken (Awadhiya *et al.* 1980, Ito and Bohm 1986, Jain *et al.* 1995). It is probable that in the bacterial injury also, the early phase is mediated by histamine and 5-HT.

The second peak of the permeability curve in bacterial injury was noticed at 12 hr as a part of the delayed phase. Ito and Bohm (1986) and Jain *et al.* (1995) suggested that prostaglandins may be important in the delayed phase of the permeability response. It is suggested that in the chicken in bacterial injury also, prostaglandins and leukotrienes may participate in the second phase of the inflammatory response.

An interesting feature of this study was a third rise obtained at 2 days. Such a triphasic permeability response has hitherto been unreported in the chicken, and needs further investigation.

S. pyogenes and *E. coli* lesions revealed deposition of carbon in small segments of venules at 0-2 min. The venular labelling increased gradually thereafter, and a triphasic pattern of increased vascular permeability, similar to that observed by dye technique, was also recorded by colloidal carbon.

The permeability at all stages in bacterial injury was confined to the venules and small veins only. This is in agreement with the observations recorded in acute inflammation in the chicken induced by different stimuli (Awadhiya *et al.* 1980, Pillai *et al.*1987, 1988b, Sharma *et al.* 1993). It is now well established that the action of all known mediators of permeability is restricted to venules and small veins only (Vegad and Katiyar 1995). The present venular response suggests its mediation by chemical mediators like histamine and 5-HT.

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Performance index based evaluation of anticoccidials against field strains of *Eimeria tenella* in broiler chickens

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ABSTRACT

Amprolium, clopidol, nitrofurazone+ furazolidone and sodium sulphadimethylpyrimidine were evaluated for their performance against 4 field strains of *Eimeria tenella* isolated from severe outbreaks of coccidiosis at 4 different broiler farms, 2 each at Hisar and Jind districts in Haryana state. Day-old hybrid chicks of either sex were challenged with 1×10^5 sporulated oocysts of *E. tenella* at 3 weeks of age. The drugs were administered to the chicks 24 hr prior to challenge for ascertaining their prophylactic activity. The medication was continued up to 7 days post-challenge. The comparative efficacy of the anticoccidials was evaluated on the basis of a performance index (PI) based on ratio of mean weight gains, survival and faecal scores of chickens. While the order of drug efficacy at Hisar and Jind districts was sodium sulphadimethylpyrimidine followed by amprolium, clopidol and nitrofurazone + furazolidone, the same for Jind district was clopidol followed by amprolium, sodium sulphadimethylpyrimidine and nitrofurazone + furazolidone.

Key words: Anticoccidials, Chickens, *Eimeria tenella*

Caecal coccidiosis remains a predominant cause of mortality in all ages of chickens. The problem of drug-resistance to different anticoccidials under field conditions has left poultry producers in a dilemma regarding choice of suitable drugs for the control of this disease at their farms. There is ample documentation (Basu *et al.* 1990, Guha and Mishra 1990 and Thyagarajan and Narahari 1996) on the efficacy of different anticoccidials commercially available in different parts of India. No such information, however, seems to exist for this region of the country. A study was, therefore, conducted to assess the efficacy of some commonly used anticoccidials under field conditions in some parts of Haryana.

MATERIALS AND METHODS

Chicks

Day-old hybrid chicks of either sex raised under coccidia-free environment in horizontal battery brooders with raised wire-netting floor were used in the experiment. Coccidiostat-free chick mash and water were provided *ad-lib.* to the chicks throughout the period of experiment.

Anticoccidial drugs

The following drugs, used at recommended levels under field conditions, in Haryana were evaluated for their performance:

(a) Amprolium (20% w/w) @ 30g / litre of water ×7 days
(b) Clopidol (50% w/w meticlorpindol) @ 250 g / 1000 g of feed × 7 days
(c) Nitrofurazone (25% w/w) + furazolidone (3.6% w/w) @ 125 g / 250 kg of feed ×7 days
(d) Sodium sulphadimethylpyrimidine (12.5% w/v) @ 30 ml / 4 litre of water × 2 days followed by 15 ml / 4 litre of water × 5 days

Field strains

Four highly pathogenic field strains of *Eimeria tenella*, 2 each from semi-arid Hisar and Jind districts of Haryana state; designated HI-1, HI-2 and JI-1, JI-2, respectively, were used as challenge strains. Isolation was made from broiler chickens brought for necropsy at the Central Disease Investigation Unit of the University.

Experimental design

The experimental chicks of nearly same body weights were randomized at 3 weeks of age into 6 groups of 10 chicks each. Four groups served as infected medicated controls (IMCs) while 2 groups served as infected unmedicated control (IUC) and uninfected unmedicated control (UUC) each. Four chicks from each group, except UUC were kept individually in pens of a vertical battery brooder in an isolated place exclusively for recording faecal scores. Freedom of the chicks from adventitious coccidial infection was ascertained prior

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to challenge by examining their faeces with the centrifugal salt floatation technique (Davies *et al.* 1963). Each chick was administered 1×10^5 sporulated oocysts, intracrop, at 3 weeks of age. The drugs were tested for prophylactic activity with the medication commencing 24 hr before challenge. The medicaton was continued up to 7 days post-challenge (PC).

The anticoccidial efficacy of a particular drug was calculated by constructing a performance index (PI) as per Morehouse and Baron (1970). The parameters for PI included:

$$\text{(a) Per cent ratio of mean gains} = \frac{\text{Average wt. gain in IMC}}{\text{Average wt. gain in UUC}} \times 100$$

$$\text{(b) Per cent survival ratio} = \frac{\text{Total chicks survived}}{\text{Total chicks challenged}} \times 100$$

$$\text{(c) Per cent faecal score} = \frac{Z-X}{Z} \times 100$$

Where,

Z is summation of the highest score for each chick in IUC; X,summation of the highest score for each chick in IMC.

The PI was calculated as

$PI = a + b + c$

RESULTS AND DISCUSSION

Among chickens of the infected groups, viz. IMCs and IUC, the first signs of the adverse effects of the disease were recorded on day 5 PC, when most of the chicks weighed less in comparison to their pre-infection body weights and to the body weights of the UUC. Bedrnik (1983) established that the reduced efficacy of an anticoccidial has an adverse effect on weight gain and/or on feed conversion efficiency of chickens. Changes in body weight provide a more sensitive indirect measurement of the severity of infection (Mukkur and Bradley 1969). The feed and water intake of the UUC group was normal with the chickens exhibiting a steady increase in weight gains during the experiment. The maximum average weight gains in the chickens challenged with HI-1 and HI-2 were observed in those administered sodium sulphadimethylpyrimidine followed by those with amprolium, clopidol and nitrofurazone + furazolidone. The order of efficacy based on weight gains was clopidol followed by amprolium, sodium sulphadimethylpyrimidine and nitrofurazone + furazolidone in the chickens challenged with JI-1 and JI-2. The effect of anticoccidials on per cent ratio of mean gains of the chickens following experimental infection has been depicted in Fig. 1. Mortality in the infected chickens started from day 4, with the highest rate of mortality observed between days 5 and 7 PC. No mortality was observed in the UUC chickens. The survival ratio is the major criterion of PI and revealed that both amprolium and clopidol provided an absolute protection against a challenge with HI-1 and HI-2 (Fig. 2). There is ample documentation on the effectiveness of amprolium (Guha and Mishra 1990, Thyagarajan and Narahari 1996) in reducing or preventing clinical signs of coccidiosis due to drug sensitive

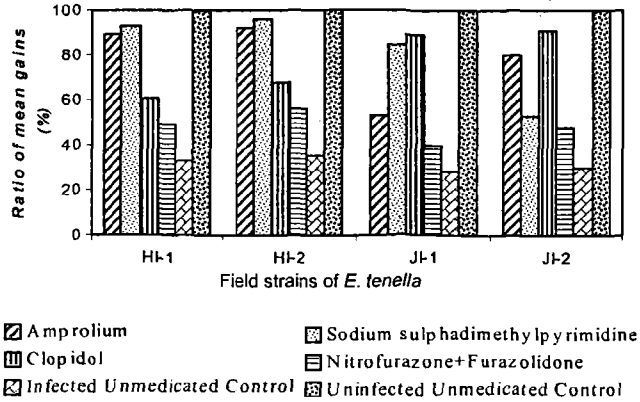

Fig. 1 Post-challenge effect of anticoccidials on ratio of mean gains in broiler chickens.

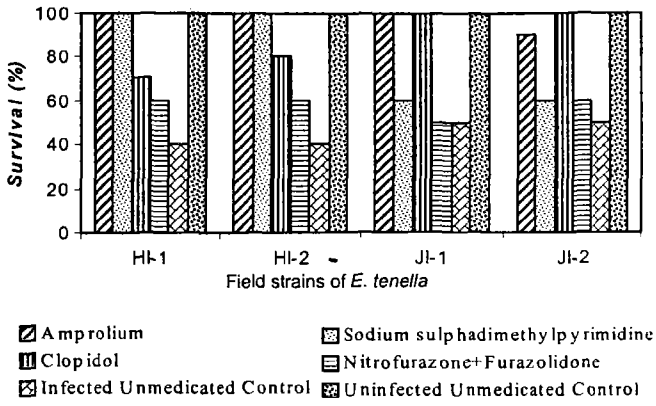

Fig. 2. Post-challenge effect of anticoccidials on survival in broiler chickens.

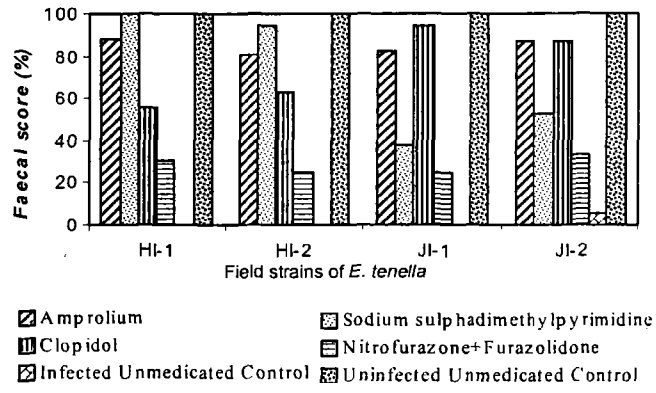

Fig. 3. Post-challenge effect of anticoccidials on faecal scores in broiler chickens.

Table 1. Performance index of anticoccidials against Hisar strains of *Eimeria tenella* in broiler chickens

Parameters	HI-1						HI-2					
of PI/field strains	A**	N	S	C	I	U	A	N	S	C	I	U
Ratio of mean gains (%)	89.20	49.12	92.85	60.79	32.77	100.00	92.30	56.01	95.92	67.61	35.66	100.00
Survival (%)	100.00	60.00	100.00	70.00	40.00	100.00	100.00	60.00	100.00	80.00	40.00	100.00
Faecal score (%)	87.50	31.25	100.00	56.25	0.00	100.00	81.25	25.00	93.75	62.50	0.00	100.00
PI	276.70	140.37	293.85	187.04	72.77	300.00	273.55	141.01	289.67	210.11	75.66	300.00

**A - Amprolium; N - nitrofurazone + furazolidone; S - sodium sulphadimethylpyrimidine; C - clopidol; I- infected unmedicated control; U,uninfected unmedicated control.

strains of *E. tenella*. The group administered sodium sulphadimethylpyrimidine exhibited absolutely normal faecal scores followed by those medicated with amprolium and clopidol against a challenge with HI-1. The order of normalcy of faecal scores for the groups challenged with HI-2 was more or less same as with HI-1. Clopidol and amprolium proved quite effective in preventing infection with JI-1 and JI-2, while nitrofurazone + furazolidone was least effective in this regard against all the 4 strains. Absolutely normal faecal droppings were observed in UUC chickens throughout the experiment.

while sodium sulphadimethylpyrimidine and nitrofurazone + furazolidone provided 60 and 50% protection respectively (Table 2). Clopidol was effective (Basu *et al.* 1990) in prevention and control of *E. tenella* infection in chickens. Against JI-2, clopidol and amprolium provided 100 and 80% protection, respectively, in the IMCs against an infection which killed 50% of IUC chickens while sodium sulphadimethylpyrimidine and nitrofurazone + furazolidone provided 60% protection each. Low PI values for clopidol and sodium sulphadimethylpyrimidine against Hisar and Jind strains,

Table 2. Performance index of anticoccidials against Jind strains of *Eimeria tenella* in broiler chickens

Parameters of PI/field strains	JI-1						JI-2					
	A	N	S	C	I	U	A	N	S	C	I	U
Ratio of mean gains (%)	84.73	40.26	53.18	89.34	28.52	100.00	79.62	47.40	52.74	91.11	30.37	100.00
Survival (%)	100.00	50.00	60.00	100.00	50.00	100.00	80.00	60.00	60.00	100.00	50.00	100.00
Faecal score (%)	81.25	25.00	37.50	93.75	0.00	100.00	86.66	33.33	53.33	86.66	6.25	100.00
PI	265.98	115.26	150.68	283.09	78.52	300.00	246.28	140.73	166.07	277.77	86.62	300.00

Post-challenge effect of anticoccidials on per cent faecal scores in chickens is illustrated in Fig. 3. PI results with HI-1 revealed that sodium sulphadimethylpyrimidine and amprolium absolutely protected IMCs against an infection which killed 60% of the IUC chickens while clopidol and nitrofurazone+furazolidone provided 70 and 60% protection, respectively (Table 1). Reports are available on the anticoccidial efficacy of sodium sulphadimethylpyrimidine (Thyagarajan and Narahari 1996). Against HI-2, PI results indicated that sodium sulphadimethylpyrimidine and amprolium gave absolute and 80% protection, respectively, against an infection which killed 50% of the IUC chickens while clopidol and nitrofurazone + furazolidone provided 80 and 60% protection respectively. This surprisingly high efficacy of sodium sulphadimethylpyrimidine in broilers needs further studies to exploit the use of this drug in broilers. PI results with JI-1 revealed that amprolium and clopidol absolutely protected IMCs against an infection which killed 50% of IUC chickens, respectively, and for nitrofurazone+furazolidone against all the 4 strains may be due to limited anticoccidial activity of these drugs possibly owing to the development of resistance against them by the field strains. Comparative high values of mortalities observed in these groups may be attributed to insufficient drug efficacy (Bedrnik 1983) and is the most reliable sign of anticoccidial resistance for highly pathogenic *E. tenella*.

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In-vivo assessment of drug resistance in field isolates of *Eimeria tenella*

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ABSTRACT

Four field isolates of *Eimeria tenella* designated HI-1, HI-2, JI-1 and JI-2 isolated from severe outbreaks of caecal coccidiosis at broiler farms in some semi-arid regions of Haryana state lying in the sub-tropical belt of India, were assessed for the development of resistance to anticoccidials, viz. amprolium, clopidol, nitrofurazone + furazolidone and sodium sulphadimethylpyrimidine. The drugs were administered to the chicks 24 hr prior to oral challenge with 1×10^5 sporulated oocysts at 3 weeks of age. Infected unmedicated control (IUC) and uninfected unmedicated control (UUC) were also maintained simultaneously. A relative anticoccidial index of each isolate of *E. tenella* against an anticoccidial was determined to ascertain the relative drug-resistance of coccidia. It was compared with another criterion of drug-resistance, i.e. there was an at least 50% difference in the average dropping pan score (ADPS) of the chicks in IMCs and IUC. These criteria correlated in establishing resistance of all the 4 isolates to nitrofurazone+ furazolidone, some of the isolates to clopidol and sodium sulphadimethylpyrimidine and none of the isolates to amprolium.

Key words: Anticoccidials, Chickens, Drug-resistance, *Eimeria tenella*

Coccidiosis continues to be one of the most important diseases causing significant economic losses to the poultry industry despite the availability of latest anticoccidial drugs. Many anticoccidial drugs were introduced but sooner or later resistance to all these products developed in *Eimeria* species (Chapman 1993). In this scenario, the determination of a relationship between the anticoccidial being used and the level of resistance acquired against it in a given area assumes paramount significance. The present study was taken up with the objective of assessing the development of drug-resistance by field isolates of *E tenella* to some anticoccidials being used in some parts of Haryana.

MATERIALS AND METHODS

Field isolates

Four field isolates of *E. tenella*, 2 each from Hisar and Jind districts of Haryana, designated HI-1, HI-2, JI-1 and JI-2, respectively, were used for assessing the development of drug-resistance against a panel of anticoccidials. Isolations were made from broiler chickens brought for necropsy at the Central Disease Investigation Unit of the university. Identification of *E. tenella* was based on the examination of gross lesions, size of intesine affected, size of oocysts, sporulation time and appearance of characteristic schizonts and gametocytes in fresh smears of caecal mucosa. Subsequently, harvesting of oocysts was done by salt floatation technique to isolate the oocysts. The requisite number of oocysts per unit volume of suspension were counted in a McMaster Chamber (Davies *et al.* 1963).

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Chicks

Day-old chicks of either sex were raised under coccidia-free environment in horizontal battery brooders with raised wire-netting floors. Coccidiostat-free commercial chick mash and water were provided *ad lib.* to the chicks throughout the period of experiment.

Anticoccidial drugs

The following drugs at recommended dose regimen were tested to study the development of resistance against each:

Amprolium (20% w/w) @ 30g / litre of water × 7 days

Clopidol (50% w/w) @ 250 g / 1000 g of feed ×7 days

Nitrofurazone (25% w/w) + furazolidone (3.6% w/w) @125 g / 250 kg of feed × 7 days

Sodium sulphadimethylpyrimidine (12.5% w/v) @ 30 ml/4 litre of water × 2 days followed by 15 ml / 4 litre of water × 5 days

Experimental design

At 3 weeks of age, the chicks were randomized into 6 groups of 10 chicks each. Four groups, each corresponding to the test anticoccidial served as IMCs while 2 groups served

as IUC and UUC each. Freedom of the chicks from adventitious coccidial infection was ascertained prior to challenge by examining their faeces using centrifugal salt floatation technique. At 3 weeks of age, each chick was directly administered 1×10^5 sporulated oocysts into the crop. The drugs were given to the chicks in feed or water 24 hr prior to challenge. The medication was continued up to 7 days post-challenge (PC).

Drug-resistance

ADPS of the chicks was calculated 4-7 days PC according to the method of grading devised by Morehouse and Baron (1970). A relative anticoccidial index (I) of each isolate of *E. tenella* against the panel of anticoccidial drugs was calculated as per the method of Jeffers and Challey (1973) with slight modifications and expressed as,

$$I = \frac{\text{Average pen weight day 7}}{\text{Average pen weight day 0}} - \frac{\text{ADPS}}{10}$$

The scale used to indicate relative drug-resistance of coccidia was as under:

I = 1.35 to 1.60 ± sensitive

I = 1.10 to 1.35 ± moderately resistant

I = < 1.10 ± highly resistant

This was compared with another criterion of drug-resistance (Jeffers 1974) of an isolate, i.e. the ADPS of the chicks calculated 4-7 days PC in IMCs was at least 50% of that in the IUC.

RESULTS AND DISCUSSION

With the isolation of first resistant field strain of *E. tenella* among broiler chickens (Waletzky *et al.* 1954) research efforts have continuously been focussed on identification of existing and emerging strains of *E. tenella* resistant to in-use anticoccidials in order to know the magnitude of the problem of drug-resistance.

Amprolium, clopidol, nitrofurazone + furazolidone and sodium sulphadimethylpyrimidine have been used since long for the prevention and control of coccidiosis in chickens. The Hisar isolates of *E. tenella* were resistant to clopidol with an anticoccidial index of 1.14 each (Table 1). Williams (1969) and McDougald *et al.* (1987) reported on resistance of strains of *E. tenella* to meticlorpindol. The reduction in ADPS of chickens in these groups to 50% *vis-a-vis* IUC chickens supported the resistance of these isolates to *E. tenella.*

The group of chickens challenged with Jind isolates revealed a better efficacy of clopidol with the isolates exhibiting a sensitivity to this compound and having AIs as 1.35 and 1.48. Absolutely normal pan score of JI-1 *vis-a-vis* IUC chickens supported this finding while only 25% reduction in ADPS of chickens challenged with JI-2 implied a sensitivity of Jind isolates to this compound (Table 2). The drug might have facilitated better inhibition of the mitochondrial respiration by disrupting electron transport in mitochondrial cytochrome system of the parasites. Since the drug exerts maximum effect around first day of life cycle of the parasites,

Table 1. Anticoccidial index based drug-resistance pattern of *E. tenella* strains

Group	Strain	Average pen weight (g)		ADPS*	Anticoccidial index (I)	Remarks
		Day 0 (A)	Day 7 (B)	(Z)	I=(B/A-Z/10)	
Amprolium						
	HI-1	221.6	334.0	1	1.40	Sensitive
	HI-2	213.0	317.3	1	1.38	Sensitive
	JI-1	219.0	320.0	1	1.36	Sensitive
	JI-2	221.5	329.0	1	1.35	Sensitive
Nitrofurazone+	HI-1	217.6	279.5	2	1.08	Highly resistant
furazolidone	HI-2	211.3	274.6	3	0.99	Highly resistant
	JI-1	221.0	269.0	3	0.91	Highly resistant
	JI-2	220.0	284.0	3	0.99	Highly resistant
Sodium	HI-1	224.0	341.0	0	1.52	Sensitive
sulphadimethylpyrimidine	HI-2	207.0	315.4	0	1.52	Sensitive
	JI-1	223.6	287.0	2	1.08	Highly resistant
	JI-2	223.0	294.2	2	1.11	Highly resistant
Clopidol	HI-1	219.4	296.0	2	1.14	Moderately resistant
	HI-2	220.5	296.9	2	1.14	Moderately resistant
	JI-1	217.5	324.0	0	1.48	Sensitive
	JI-2	215.0	338.0	1	1.47	Sensitive
Infected	HI-1	221.5	262.8	4	0.78	-
unmedicated control	HI-2	222.7	263.0	4	0.78	-
	JI-1	220.0	254.0	4	0.75	-
	JI-2	222.0	263.0	4	0.78	-

*Average dropping pan score.

Table 2. Average dropping pan score based resistance pattern of *E. tenella* isolates

Group	Isolates of *E. tenella*			
	HI-1	HI-2	JI-1	JI-2
Amprolium	1	1	1	1
Nitrofurazone+ furazolidone	2*	3	3	3
Sodium sulphadimethylpyrimidine	0	0	2	2
Clopidol	2	2	0	1
Infected unmedicated control	4	4	4	4

*Bold figures indicate drug-resistant isolates.

therefore, better pan scores were observed in the group medicated with this drug. Stock *et al.* (1967), Singh *et al.* (1984) and Basu *et al.* (1990) have reported the efficacy of this drug. A remarkably high degree of resistance of all the 4 isolates to nitrofurazone+furazolidone with AIs ranging from 0.91 to 1.08 was observed. This was further supported by a reduced grading in the ADPS of these chickens to 75% of that in the IUC. This drug acts on second generation schizonts around fourth day of life cycle by interfering with energy metabolism of parasites. Poor pan scores in all the groups recorded 4-7 days PC reflected the resistance of all the 4 isolates to this drug. Joyner (1957) observed field strains of *E. tenella* in chickens that had developed resistance to nitrofurazone+furazolidone. Sodium sulphadimethylpyrimidine effectively protected groups of chickens infected with Hisar isolates of *E. tenella*. Both these isolates exhibited an anticoccidial index of 1.52 each. Absolutely normal ADPS of the chickens in these groups supported the sensitivity of *E. tenella* isolates to this drug. No mortality was observed in these groups of chickens which was in close agreement with the findings of Ramprakash and Krishnaiah (1973). Thyagarajan *et al.* (1987) concluded that sulphadimidine was closely followed by amprolium as the most effective and cheapest drug tested against caecal coccidiosis. Davies and Kendall (1954) reported that sulphadimidine in drinking water proved markedly effective over nitrofurazone+furazolidone in controlling mortality from caecal coccidiosis.

The sensitivity of *E. tenella* isolates may be because of the marked effect of the drug against second generation schizonts of *E. tenella* (Wehr and Farr 1947) around 4-6 days of the life cycle of parasite. This drug acts by blocking the synthesis of tetrahydrofolic acid, an essential vitamin in the development of parasite. The success of this drug was evident in the perfectly normal pan scores observed in these groups. Among Jind isolates, JI-2 was moderately resistant and JI-1 was highly resistant to sodium sulphadimethylpyrimidine with isolates having low AIs of 1.11 and 1.08 respectively. These findings were supported by a reduction in ADPS of the chickens in these groups to 50% of that in IUC. These findings correlated with those of Waletzky *et al.* (1954) who reported strain of *E. tenella* isolated from a flock that failed to respond to treatment with sulphadimidine. All the 4 isolates of *E. tenella* were quite sensitive to amprolium. The values of AIs in these groups ranged from 1.35 to 1.48. This drug provided protection against weight loss, mortality and development of lesions against infection with the sensitive strains of *E. tenella*. These findings are in confirmation with those of Cuckler *et al.* (1960) and Thyagarajan and Narahari (1983). The data on the drug sensitivity and anticoccidial activity of drugs indicated that the highest protection of chickens was afforded by amprolium and the lowest protection by nitrofurazone + furazolidone. The results on evaluation of drug-resistance based on the 2 parameters suggested a good correlation. The phenomenon of development of drug resistance under field conditions appears to be very strange. Some of the resistant strains may appear suddenly as new mutations, while other strains may have been primarily present but appear after having been selected out by long continued use of an anticoccidial in a given area.

The mechanism by which certain drug-sensitive *E. tenella* strains perpetuate themselves during very long periods of exposure to highly efficacious anticoccidials have not been established (Jeffers 1974). No *E. tenella* isolates were recovered which could not be effectively controlled by a commercially available anticoccidial as no single isolate had mutiple resistance to all of the anticoccidial tested. The findings suggest that a complete characterization of the drug resistance spectra of isolates is important for determination of the anticoccidial drugs that would control them most effectively.

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Effect of praziquantel on histopathology in experimental porcine schistosomiasis

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ABSTRACT

Indigenous piglets, 2-month old and of either sex were infected each with 1500 cercariae of *Schistosoma incognitum* by tail method and were divided into 2 groups. The first group was treated with praziquantel @ 25 mg/kg body weight on 21st DPI while second group was kept to study infected untreated control. Additionally, 2 piglets from same litter were kept as uninfected untreated healthy control. The animals were sacrificed on 47-52 DPI and studied for gross pathology; tissues were processed in acetone-benzene paraffin series and 5 μ sections were studied after staining with haematoxylin and eosin.

Grossly, lungs of infected untreated animals showed hyperaemia and emphysema. The liver was enlarged, congested with grayish foci over the surface while severe congestion of mesenteric blood vessels was observed in the intestine. The treated animals showed either mild or absence of these features. Microscopic studies revealed inflammatory reactions, distension and rupture of alveoli, hyperplasia of peribronchial lymphoid tissues in untreated infected animals while treated animals showed mild emphysematous changes with absence of peribronchial hyperplasia. The liver of infected untreated animals had perivascular infiltration and degenerative changes in the hepatocytes which were absent in the treated animals with subsiding inflammation in perivascular area. A chronic inflammation with sloughing of intestinal epithelium was observed in untreated, infected animals while no such reaction could be observed in treated animals.

Key words: Chemotherapy, Histopathology, Porcine schistosomiasis, Praziquantel, *Schistosoma incognitum*

Effect of a drug, under experimental conditions, is routinely tested by counting the number of surviving helminths after chemotherapy. In field cases recovery from clinical symptoms is the main criterion along with EPG estimation. Both these have been followed in schistosomiasis in India (Agrawal and Shah 1989, Agrawal and Alwar 1992). However, these criteria do not reflect total effect of the drug as revealed by Jain *et al.* (2000) where praziquantel had favourable effect on haemoglobin and total erythrocyte count in the pigs, despite nonsignificant blood fluke reduction by the drug. Likewise, faecal EPG in porcine schistosomiasis was influenced significantly by praziquantel, though blood fluke reduction was nonsignificant (Jain *et al.* 2000). Therefore, it was considered worthwhile to extend such studies by observing effect on gross and histopathology of lungs, liver and intestine of pigs which were treated with praziquantel for experimental *Schistosoma incognitum* infection.

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MATERIALS AND METHODS

About 2-month old indigenous piglets (16) of either sex were infected each with 1500 cercariae of *S. incognitum* by tail immersion method (Shames *et al.* 2000). Of these, 6 died within 21 day post- infection (DPI). Remaining animals were equally divided into 2 groups—one was treated with praziquantel, while the other was kept as infected, untreated control. Additionally, 2 piglets of the same litter were kept as uninfected, untreated normal control.

Animals were administered praziquantel 50mg/piglet (20-25 mg/kg body weight) *per os* on 21 DPI and observed clinically and for other parameters (Jain *et al.* 2000). The animals were sacrificed on 47-52 DPI or 26-31day post treatment (DPT) by intra cardiac injection of saturated magnesium sulphate solution and were perfused as reported by Shames *et al.* (2000). Prior to perfusion, the organs were examined grossly for any pathological changes. After perfusion, the tissues from the lungs, liver and intestine were taken in 10% formalin and processed in acetone benzene paraffin series. Sections of 5μ thickness were made, stained with haematoxylin and eosin, and studied microscopically.

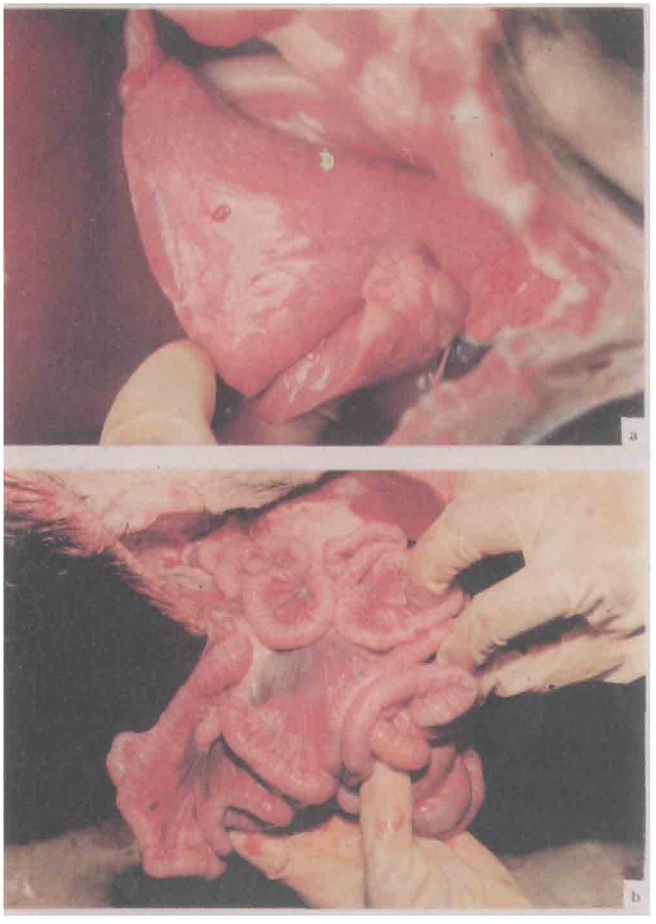

Fig.1. Photographs of organs of infected untreated piglets showing, (a) emphysema in the lungs, (b) congestion of mesenteric blood vessels.

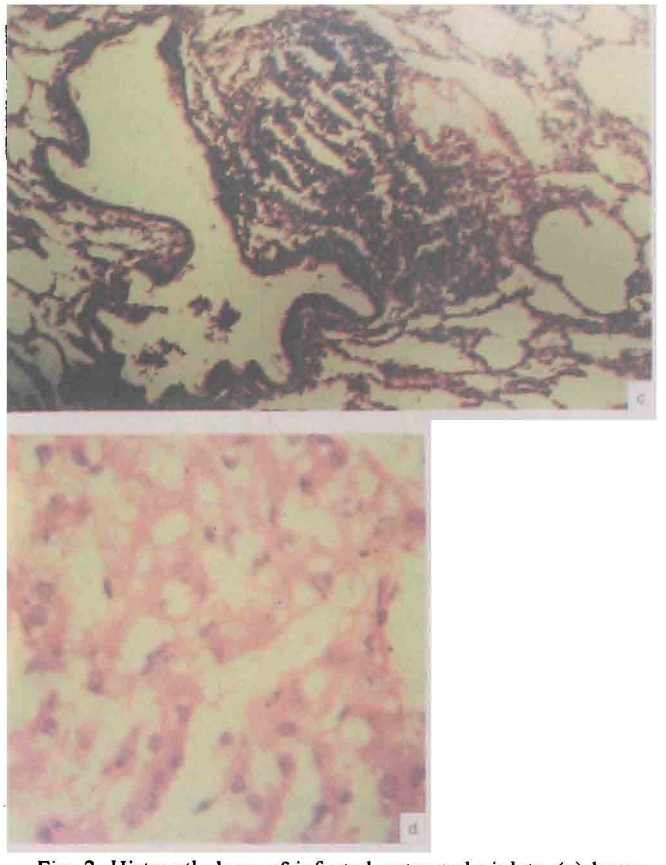

Fig. 2. Histopathology of infected untreated piglets, (c) lungs showing hyperplasia of peribronchial tissues. (100×), (d) liver showing degenerative changes in the hepatocytes (400×).

RESULTS AND DISCUSSION

Gross pathology

Necropsy of the animals showed gross pathological changes mainly in lungs, liver and intestine. The lungs of the infected untreated group showed hyperaemia and emphysema (Fig. 1a), whereas only mild emphysema was seen in treated piglets with apparently normal lungs in uninfected control group. The liver of the infected untreated piglets was slightly enlarged, congested with numerous grayish foci scattered over the surface. No marked abnormalities were observed in treated group while healthy group showed apparently normal liver. Severe congestion and dilatation of mesenteric blood vessels with presence of enteritis was observed in infected untreated piglets (Fig. 1b). Such changes could not be detected in other groups.

Histopathology

The histopathology of lungs of infected, untreated animals revealed inflammatory reactions in the alveoli with infiltration of macrophages, epitheloid cells and lymphoid cells. There was over distension of alveoli with their rupture at places confirming emphysema. Hyperplasia of peribronchial lymphoid tissues was observed (Fig. 2c), while other areas showed catarrhal exudate consisting of desquamated cells and mucus; enfolding of tunica intima of bronchi with prominent mucosal folds was also observed. The treated animals also revealed emphysematous changes but of lower intensity. Peribronchial hyperplasia or excessive enfolding of bronchial mucosa or significant inflammatory reaction could not be observed in treated (Fig. 3e). No appreciable histopathological changes could be observed in the uninfected control group.

In liver of infected untreated animals, hepatocytes showed degenerative changes (cellular swelling and fatty chages) (Fig. 2d) with infiltration of mononuclear cells and eosinophils in portal triad area, perivascular area and around bile duct. A subsiding chronic inflammation with absence of cellular swelling and fatty changes in hepatocytes was seen in infected treated piglets (Fig. 3f). The liver of normal animals did not reveal any microscopic alteration in this study.

Histopathological examination of intestine of infected untreated animals showed chronic inflammation characterized by infiltration of lymphocytes, macrophages and

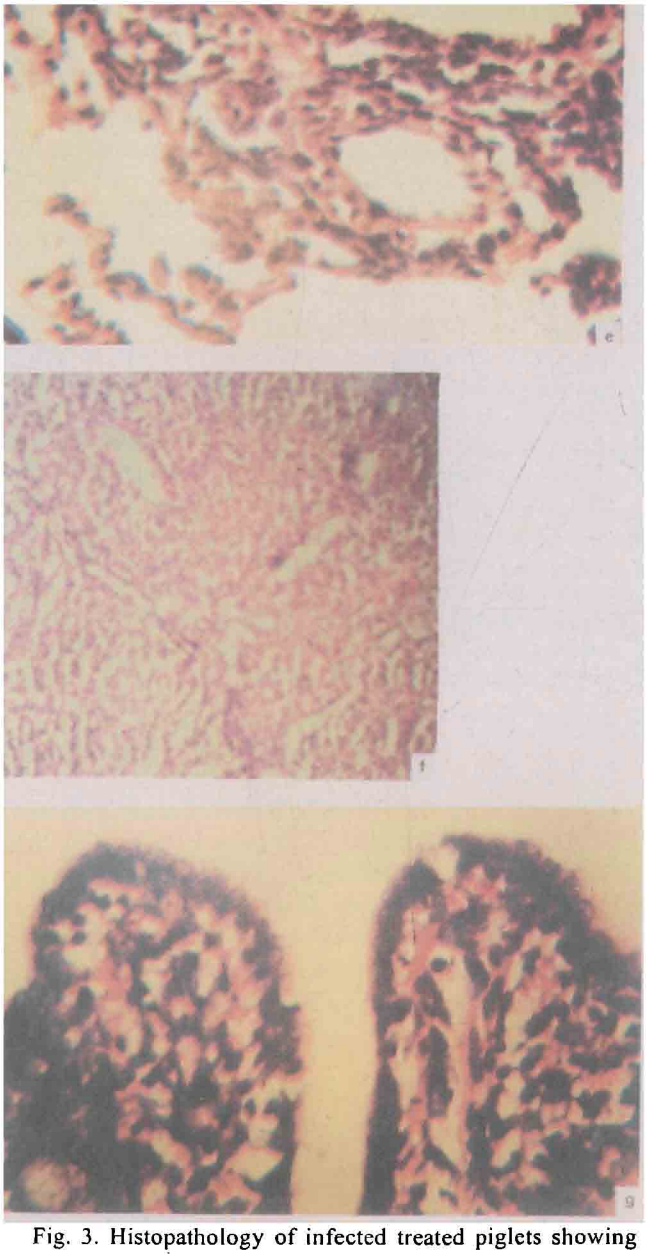

Fig. 3. Histopathology of infected treated piglets showing (e) absence of peribronchial hyperplasia in the lungs (100×), (f) absence of degenerative changes in the hepatocytes (100×), (g) absence of inflammatory reactions in the intestine (400×).

eosinophils at the base of intestinal crypts and mucosa. At some places excessive sloughing of intestinal epithelium was seen suggesting severe degenerative and necrotic changes. In few sections goblet cells were prominent and swollen. In the treated group of animals, chronic inflammatory reaction and sloughing of intestinal mucosa appeared absent (Fig. 3g). The intestine of normal piglets did not reveal any histopathological changes.

It may be pointed out that no well formed granuloma could be observed in any organ in the present studies. Nevertheless, there were patches of chronic inflammation showing epitheloid cells, a precursor of granuloma stage.

Agrawal and Shah (1989) and Agrawal and Alwar (1992) have assessed the effect of schistosomicides either counting number of survived blood flukes or disappearance of clinical symptoms. However, Shrivastava and Agrawal (1999a, 1999b) and Shames *et al.* (2000) have tried to identify effect of schistosomicides on male, female, mature and immature blood flukes and claimed varied lethal effects according to sex or age of the blood flukes. The drugs also had suppressing effect on egg production resulting in significant faecal egg reduction despite nonsignificant blood fluke reduction (Jain *et al.* 2000).

Jain (1999) claimed that values of haemoglobin, total erythrocyte count, packed- cell volume, body weight increased in porcine schistosomiasis by treating the animals with praziquantel. In continuation, the present work suggested beneficiary effect of the drug on micropathology of tissues and organs of infected and treated animals. Though in this case the blood fluke reduction (60.06%) was statistically nonsignificant (Shames *et al.* 2000), yet histopathology of treated animals revealed great improvement over untreated cases making the tissues apparently normal. Obviously this was the effect of the treatment but animal may further develop micro pathology as yet a significant number of blood flukes survived. Our observations supported clinical recovery of animals from schistosomosis by the treatment although a sizable number of blood flukes still remained in the animals.

Various Indian workers have described histopathology and immunopathology in some cases, of *Schistosoma* infections in pigs, mice, monkeys, dogs, rabbits and other animals (Agrawal and Shah 1989, Agrawal and Alwar 1992, Agrawal and Southgate 2000). However, we could not find any reference describing effect of chemotherapy on histopathology of schistosomosis in India. Nevertheless histopathology of praziquantel treated and untreated schistosomosis cases were described in other countries (El-Haway *et al.* 1986, Bishara and Said 1991, El-Matarawi and Kamel 1991, Khalil *et al.* 1995. Johansen *et al.* 1996) with ameliorative pathology in *S.mansoni* infections. Nonetheless, praziquanted was able to kill 80-100 % *S.mansoni* flukes, whereas, the drug killed only 60% *S.incognitum* in this case (Shames *et al.* 2000). Obviously long-term effect of the drug on the host may be different in these 2 cases. Additionally, Johansen *et al.* (1996) have drawn attention to pathological consequences of praziquantel treatment in African dwarf goats experimentally infected with *S.bovis* where the treatment exaggerated hepatic pathology instead of its recovery warranting caution of treating animals with higher worm load or concomitant liver diseases.

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Disposition kinetics and distribution of ciprofloxacin in biological fluids of goats after intravenous administration

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ABSTRACT

Pharmacokinetics and distribution of ciprofloxacin in various biological fluids were undertaken post i.v. dose (4 mg. kg^{-1}) in goats. Peak concentrations of 2.53 ± 0.07, 12.23 ± 0.82 and 198.50 ± 25.89 $\mu g.ml^{-1}$ were attained at 0.083, 1 and 1 hr in plasma, milk and urine respectively. The therapeutic concentration (≥0.12 $\mu g.ml^{-1}$) were maintained up to 10, 12 and 36 hr, respectively, in plasma, milk and urine. Distribution half life ($t_{1/2}\alpha$) and elimination half life ($t_{1/2}\beta$) of 0.32 ± 0.05 and 4.71 ± 0.67 hr were noted in the present study. Vd_{area} of 4.22 ± 0.51 $L.kg^{-1}$ obtained for ciprofloxacin in goat denotes its wide distribution in the body. The present investigation suggests a loading dose (D*) of 4.4 $mg.kg^{-1}$ followed by a maintenance dose (Do) of 3.8 $mg.kg^{-1}$ at the dosage interval (γ) of 12 hr for maintaining therapeutic concentration (MIC) of 0.12 $\mu g.ml^{-1}$.

Key words: Ciprofloxacin, Disposition kinetics, Goat

Pharmacokinetic studies on ciprofloxacin, an antimicrobial agent, were carried out in dogs (Abadia *et al.* 1994, 1995), calves and piglets (Nouws *et al.* 1998), ponies (Dowling *et al.* 1995) and buffalo calves and goats (Raina 1991). So far, it seems that no such studies have been conducted with ciprofloxacin in goats, particularly with reference to its distribution in milk and urine. Hence, the present study has been undertaken.

MATERIALS AND METHODS

Healthy lactating goats (6) of non-descript breed weighing between 20 to 25 kg body weight, ciprofloxacin was injected intravenously (i.v.) @ 4 mg. kg^{-1} body weight in each goat. The samples of biological fluids (plasma, milk and urine) were collected before and at 0.083, 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 10 and 12 hr after administration of the drug. The samples of milk and urine were also collected even beyond 12 hr at 24, 30, 36 and 48 hr after i.v. administration of the drug. For collection of urine, a Foley's balloon catheter was introduced into the bladder through urethra and kept in position by inflating the balloon by giving 20 ml of water. The biological samples collected prior to administration of the drug were used for preparing drug standards in the respective biological fluid.

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The concentration of ciprofloxacin in biological samples was estimated by microbiological assay technique (cylinder plate diffusion method) using *Escherichia coli* (ATCC 25922) as the test organism (British Pharmacopoeia 1980). The sensitivity of the method is 0.05 $\mu g.ml^{-1}$. Pharmacokinetic parameters were calculated from log plasma drug concentration versus time profile by using 2-compartment open model as described by Gibaldi and Perrier (1975), Baggot (1977) and Notari (1980).

Appropriate loading doses (D*) and maintenance doses (D_0) for maintaining minimum therapeutic concentration of 0.12 $\mu g.ml^{-1}$ ($Cp\infty$ min = MIC) at dosage interval (r) of 6, 8 and 12 hr were also derived (Notari 1980).

RESULTS AND DISCUSSION

The present study revealed that peak concentrations of drug were attained at 0.083, 1 and 1 hr in plasma, milk and urine respectively. The therapeutic concentration (≥0.12 $\mu g.ml^{-1}$) was maintained up to 10, 12 and 36 hr, respectively, in plasma, milk and urine after i.v. administration of ciprofloxacin @ 4$mg.kg^{-1}$ (Table 1).

Distributions rate constant (a) of 2.551 ± 0.470 hr^{-1} and distribution half life ($t_{½}\alpha$) of 0.32 ± 0.05 hr were obtained in goat. The value of $t_{½}\alpha$ noted in the present study is more or less in agreement with the value of 0.28 hr obtained in cow-calf (Nouws *et al.* 1988), lower than that in piglets (Nouws *et al.* 1988) and higher than that in Beetal-goat (Raina 1991), ponies (Dowling *et al.* 1995), dog (Abadia *et al.* 1994) and

Table 1. Concentrations of ciprofloxacin in various biological fluids after a single i.v. dose (4 mg.kg^{-1})

	Value in μg.ml^{-1} (mean ± SEM) n = 6		
Time (hr)	Plasma	Milk	Urine
0.083	2.53 ± 0.07	0.75 ± 0.18	61.58 ± 7.25
0.25	1.89 ± 0.10	4.27 ± 0.15	96.00 ± 2.59
0.50	1.33 ± 0.12	6.71 ± 0.17	128.50 ± 3.60
0.75	1.11 ± 0.12	11.80 ± 2.55	153.67 ± 9.11
1	0.97 ± 0.11	12.23 ± 0.82	198.50 ± 25.89
2	0.69 ± 0.10	7.83 ± 0.22	164.17 ± 15.26
3	0.52 ± 0.10	6.08 ± 0.08	126.25 ± 7.50
4	0.44 ± 0.09	4.92 ± 0.16	101.92 ± 5.46
6	0.32 ± 0.10	3.99 ± 0.08	78.23 ± 5.34
8	0.25 ± 0.09	2.41 ± 0.26	64.22 ± 4.26
10	0.12 ± 0.09	1.54 ± 0.21	38.50 ± 3.90
12	N D	0.66 ± 0.17	17.32 ± 1.65
24		0.07 ± 0.05	3.87 ± 0.65
30		N D	0.96 ± 0.30
36			0.17 ± 0.06
48			N D

N D, Non-detectable.

buffalo calf (Raina 1991). Lower $t_{1/2}\alpha$ obtained in goat in this study may denote that the drug is expected to be distributed rapidly in peripheral compartment. This might be the reason that the drug appeared immediately (0.083 hr) in milk and reached its peak concentration comparatively earlier at 1 hr.

Mean elimination rate constant (ß) of 0.159 ± 0.017 hr^{-1} and elimination half life ($t_{½}$ ß) of 4.71 ± 0.67 hr were noted for goat (Table 2). The $t_{½}$ ß observed in this investigation is higher in contrast to those in Beetal-goat (Raina 1991), dog (Abadia *et al.* 1994), cow calves (Nouws *et al.* 1988), piglets (Nouws *et al.* 1988), pony (Dowling *et al.* 1995) and buffalo calf (Raina 1991) after i.v.administration. Correspondingly, lower total body clearance (Cl_B) of 11.19 ± 1.55 ml.kg^{-1}.min^{-1} was observed in this study in goat as compared to higher ClB values in Beetal-goat and buffalo calf (Raina 1991) and further higher Cl_B value in pony (Dowling *et al.* 1995).

The value of rate constant of drug transfer from central to peripheral (K_{12}) and peripheral to central (K_{21}) were calculated to be 1.355 ± 0.356 and 0.880 ± 0.151 hr^{-1}, in the present investigation for goat (Table 2). Higher K_{12} and K_{21} values in Beetal goat and chicken were reported (Raina 1991).

A high value of Vd area obtained in the present study may be attributed to its wide distribution in the body since ciprofloxacin is highly lipophilic with low protein binding (Neer 1988). This is supported by higher approximate tissue to plasma concentration ratio (T ≈ P) of 2.02 ± 0.50 (Table 2) as well as higher concentrations of ciprofloxacin obtained in milk at various time intervals post i.v. injection (Table 1).

The main aim of conducting kinetic study of an antimicrobial agent is to compute rational dosage regimen for treating various diseased states caused by susceptible

Table 2. Kinetic parameters of ciprofloxacin after a single i.v. dose of 4 mg.kg^{-1}

Kinetic parameters		Value (mean±SEM) n=6
Zero time concentration (μg.ml^{-1})		
Distribution	(A)	1.97 ± 0.21
Elimination	(B)	0.87 ± 0.12
C^0_p = A + B		2.84 ± 0.17
Rate constant (hr^{-1})		
Distribution	(α)	2.551 ± 0.470
Elimination	(ß)	0.159 ± 0.017
Half life (hr)		
Distribution	($t_{½}$ α)	0.32 ± 0.05
Elimination	($t_{½}$ ß)	4.71 ± 0.67
Micro-rate constant for drug transfer (hr^{-1})		
Central to peripheral compartment	(K_{12})	1.355 ± 0.356
Peripheral to central compartment	(K_{21})	0.880 ± 0.151
Elimination from central compartment	(Kel)	0.475 ± 0.079
Area under plasma concentration time curve		
AUC (mg.L^{-1}.hr)		7.11 ± 1.73
Fraction of drug available for elimination from central compartment		
Fc		0.37 ± 0.04
Tissue to plasma concentration ratio		
T ≈ P		2.02 ± 0.50
Volume distribution		
Vd_{area} (L.kg^{-1})		4.22 ± 0.51
Total body clearance		
Cl_B (ml.kg^{-1}.min^{-1})		11.19 ± 1.55

microorganisms. The calculated loading (D*) and maintenance (Do) doses for maintaining therapeutic concentration (Cp^{∞} min = MIC) of 0.12 μg.ml^{-1} at various dosage interval (γ) of 6, 8 and 12 hr for i v route are presented in Table 3. The attainable maximum plasma concentration (Cp^{∞} max) during the above dosage regimen is also shown in Table 3.The results of the present study revealed a mean D* of around 4.4 mg. kg^{-1} and Do of 3.8 mg. kg^{-1} at r of 12 hr may be used for

Table 3. Calculated dosage regimen of ciprofloxacin for intravenous route in afebrile goats for maintaining Cp^{∞} min (MIC) of 0.12 μg. ml^{-1}

	Value (mean±SEM)		n=6
	γ=6 hr	γ=8 hr	γ=12 hr
Loading dose (D*) in mg.kg^{-1}	1.60 ± 0.23	2.25 ± 0.34	4.42 ± 0.75
Maintenance dose (D_o) in mg.kg^{-1}	1.00 ± 0.16	1.64 ± 0.27	3.82 ± 0.70
C_p^{∞}max in μg.ml^{-1}	0.31 ± 0.03	0.45 ± 0.06	0.89 ± 0.17

C_p^{∞} min (MIC), minimum therapeutic concentration in plasma (minimum inhibitory concentration); C_p^{∞} max, attainable maximum concentration in plasma; γ, dosage interval.

treating systemic infections in goat. On the contrary, Raina (1991) calculated higher D* of 5.4 mg. kg^{-1} and Do of 5.0 mg. kg^{-1} at a short r of 8 hr in beetal goat. Since therapeutic concentration of ciprofloxacin was maintained for 12 hr in milk, the drug (5 mg. kg^{-1}) can also be effectively used for treating mammary gland infections by parentral route every 12 hr. The drug can be administered @ 4 mg. kg^{-1} every 24 hr or 36 hr by parental route for treating urinary tract infections caused by ciprofloxacin susceptible microorganisms.

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Effect of follicular fluid on acrosomal integrity of buffalo spermatozoa*

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ABSTRACT

This study was conducted to evaluate the effect of bubaline follicular fluid (FF) on acrosomal integrity of buffalo spermatozoa. Steroid free and whole FF from buffalo ovaries at the concentration of 1% alone and in combination with cysteine HCl (0.1%) and EDTA (0.1%) were used as additive in tris dilutor for preservation of washed and unwashed buffalo spermatozoa at 4°C and -196°C. The intact acrosome and abnormal acrosome at different stages of preservation were assessed. The effect of FF on acrosome was significant ($P<0.01$), and inclusion of FF as additive in tris dilutor exerted a protective effect on the acrosome during refrigerated preservation and freezing. This is an indicative of some synergistic action of FF along with other cryoprotectants in the dilutor.

Key words: Acrosome, Buffalo, Follicular fluid, Spermatozoa

Spermatozoal motility is the most frequently examined characteristic, however, spermatozoa could be highly motile but not fertile, owing to the acrosomal damage. The damage to acrosome may occur during dilution, cooling, freezing and thawing processes (Tasseron *et al.* 1977). A number of additives have been used to reduce the acrosomal damage during preservation of buffalo semen (Tuli *et al.* 1986, Kumar *et al.* 1995). Follicular fluid (FF) stimulates sperm motility (Lee *et al.*1992) but no report is available on its effect on acrosomal integrity. Hence this study was undertaken to find out the effect of FF alone and in combination with certain additives on acrosomal integrity of buffalo spermatozoa during preservation at 4°C and -196°C.

MATERIALS AND METHODS

FF was collected from buffalo ovaries obtained from local abattoir, immediately after slaughter of animal. The FF was aspirated from follicles of >3mm diameter under aseptic condition; centrifuged (3000 rpm for 20 min) and preserved in refrigerator at subzero temperature until use. FF (1%) was used as an additive in tris dilutor in 2 ways. One part was made steroid free, i.e. steroid free FF (SFFF) by adding activated charcoal (Wallace and Neilly 1985) and the other part was used as such, i.e. whole FF (WFF). Tris dilutor was prepared according to Davis *et al.* (1963). The WFF and SFFF were used, either alone or in combination with 0.1% cysteine HCl and 0.1% EDTA. Thus, 9 dilutor combinations were used (Table 1) in this experiment.

Eighteen ejaculates (6 each) from 3 Murrah bulls maintained under uniform feeding and management conditions were used. After evaluation for physical characteristics, ejaculates were divided into 2 parts. One part was washed with tris buffer after centrifugation at 3 000 rpm for 30 min then the sperm pellets were re-suspended in equal volume of tris buffer and the second part was used as unwashed.

Washed and unwashed semen was extended into 9 dilutor combinations so as to keep 60 million motile spermatozoa per ml of extended semen. Each dilutor combination again divided into 2 parts. One part was frozen as per the Sahani and Mohan (1988) and the second part was refrigerated (4°C). The per cent (%) intact and abnormal acrosome were estimated at 24 and 48 hr of refrigeration and after thawing (37°C for 30 sec) as per Watson (1975). Abnormal acrosomes were categorized as detached, ruffled and swollen. The mean and standard error were calculated and data was analyzed for ANOVA test (Snedecor and Cochran 1967).

RESULTS AND DISCUSION

Observations on the per cent intact and abnormal acrosome of washed and unwashed semen samples at different hour of refrigeration and after thawing are given in Table 1. Analysis of variance revealed that intact acrosome varied significantly due to dilutor combinations ($P < 0.01$) at 48 hr of refrigeration and post-thaw level, and

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Table 1. Mean (± SE) per cent intact and abnormal acrosome of unwashed and washed buffalo spermatozoa at different hours of refrigeration and post-thaw in different dilutor combinations

Dilutor combinations	At 24 hr of refrigeration % abnormal acrosome				At 48 hr of refrigeration % abnormal acrosome				Post-thaw % abnormal acrosome			
	% intact acrosome	Detached	Ruffled	Swollen	% intact acrosome	Detached	Ruffled	Swollen	% intact acrosome	Detached	Ruffled	Swollen
Unwashed semen												
Tris	88.4 ±1.0	3.3 ±0.4	7.6 ±0.8	0.7 ±0.2	84.9 ±0.8	3.7±0.2	10.6 ±0.7	0.8±0.2	68.4 ±1.5	7.6 ±0.6	22.1 ±1.2	2.0 ±0.4
Tris + WFF	91.1 ±0.9	2.1 ±0.3	6.1 ±0.3	0.6 ±0.6	81.3 ±0.8	3.6 ±0.3	14.8 ±0.8	0.8 ±0.2	75.2 ±0.8	6.4 ±0.3	17.2 ±1.9	1.2 ±0.2
Tris + SFFF	90.0 ±0.9	2.7 ±0.3	6.4 ±0.7	0.8 ±0.2	82.4 ±0.8	3.7 ±0.3	13.1 ±0.8	0.8 ±0.2	82.4 ±0.8	6.4 ±0.5	16.9 ±1.8	1.2 ±0.3
Tris + cysteine HCl	89.2 ±0.9	3.2 ±0.3	7.0 ±0.8	0.6 ±0.4	80.6 ±0.7	3.5 ±0.2	14.8 ±0.9	1.1 ±0.2	80.6 ±0.7	5.9 ±0.5	18.1 ±1.7	1.4 ±0.2
Tris + cysteine HCl + WFF	88.7 ±1.0	2.8 ±0.3	7.7 ±0.7	0.8 ±0.2	83.8 ±0.9	3.2 ±0.2	12.1 ±0.8	0.9 ±0.3	83.8 ±0.9	5.7 ±0.4	16.2 ±1.7	1.1 ±0.2
Tris + cysteine HCl + SFFF	89.8 ±0.9	2.5 ±0.3	7.2 ±0.7	0.4 ±0.2	79.2 ±0.8	3.8 ±0.4	15.2 ±0.9	1.4 ±0.5	79.2 ±0.8	6.0 ±0.4	16.4 ±1.5	1.1 ±0.3
Tris + EDTA	89.0 ±1.0	3.2 ±0.3	7.1 ±0.9	0.7 ±0.2	88.1 ±1.0	3.4 ±0.2	11.9 ±1.0	0.6 ±0.2	88.0 ±1.0	7.0 ±0.5	15.6 ±1.7	1.1 ±0.2
Tris + EDTA + WFF	86.2 ±1.0	4.1 ±0.5	8.9 ±0.8	0.8 ±0.2	83.0 ±0.7	3.1 ±0.3	13.1 ±0.8	0.7 ±0.2	83.0 ±0.7	6.0 ±0.5	16.4 ±1.9	1.2 ±0.4
Tris + EDTA + SFFF	88.3 ±1.1	3.4 ±0.3	7.8 ±0.3	0.5 ±0.1	86.2 ±1.0	2.6 ±0.2	10.5 ±1.0	0.7 ±0.2	86.1 ±1.0	5.9 ±0.5	15.2 ±1.7	0.8 ±2.1
Washed semen												
Tris	90.3 ±0.9	2.8 ±0.3	6.6 ±0.7	0.3 ±0.1	81.8 ±1.0	3.8 ±0.4	14.2 ±1.1	0.3 ±0.1	80.1 ±1.2	8.8 ±0.6	10.6 ±0.8	0.4 ±0.1
Tris + WFF	86.7 ±0.9	3.9 ±0.3	9.0 ±0.8	0.4 ±0.1	85.9 ±0.9	2.7 ±0.3	12.1 ±0.8	0.3 ±0.1	85.1 ±1.1	6.9 ±0.4	07.8 ±0.8	0.4 ±0.1
Tris + SFFF	89.6 ±1.2	3.0 ±0.4	6.9 ±0.7	0.2 ±0.1	83.1 ±1.0	3.4 ±0.3	13.1 ±1.0	0.4 ±0.1	82.7 ±1.0	7.4 ±0.4	09.6 ±0.7	0.3 ±0.1
Tris + cysteine HCl	89.0 ±1.0	3.0 ±0.4	7.6 ±0.6	0.4 ±0.1	85.6 ±1.0	2.8 ±0.4	11.3 ±1.0	0.3 ±0.1	79.4 ±1.0	8.8 ±0.6	11.1 ±0.7	0.6 ±0.2
Tris + cysteine HCl + WFF	87.6 ±1.1	3.5 ±0.4	8.6 ±0.8	0.4 ±0.1	80.3 ±1.0	3.6 ±0.3	15.7 ±0.8	0.4 ±0.1	84.1 ±1.1	6.7 ±0.5	08.8 ±0.8	0.4 ±0.1
Tris + cysteine HCl + SFFF	90.7 ±1.1	2.7 ±0.4	6.4 ±0.7	0.2 ±1.0	82.0 ±0.9	2.9 ±0.3	14.8 ±0.9	0.3 ±0.1	84.9 ±1.1	6.1 ±0.6	08.6 ±0.7	0.4 ±0.1
Tris + EDTA	89.4 ±1.1	3.1 ±0.3	7.2 ±0.9	0.3 ±0.1	80.3 ±1.0	3.4 ±0.2	15.7 ±1.0	0.6 ±0.2	83.9 ±1.1	6.3 ±0.5	09.3 ±0.8	0.4 ±0.1
Tris + EDTA + WFF	90.0 ±0.9	2.8 ±0.3	6.7 ±0.7	0.4 ±0.1	82.7 ±1.1	2.9 ±0.3	14.1 ±1.1	0.3 ±0.1	80.8 ±1.1	8.1 ±0.6	10.8 ±0.9	0.4 ±0.1
Tris + EDTA + SFFF	88.4 ±1.1	2.9 ±0.4	8.1 ±0.8	0.4 ±0.1	78.6 ±1.1	3.8 ±0.3	17.3 ±1.1	0.3 ±0.1	85.3 ±1.3	6.1 ±0.6	08.2 ±0.8	0.4 ±0.1

due to washing ($P < 0.01$) at post-thaw level only. Among the abnormal acrosome swollen acrosome varied significantly due to washing ($P < 0.01$) and nonsignificantly due to dilutor combination at all storage level. Detached acrosome varied significantly due to washing ($P < 0.01$) and dilutor combinations ($P < 0.01$) at post-thaw level and nonsignificantly at 24 and 48 hr of refrigeration. Ruffled acrosome varied significantly due to washing and dilutor combinations at 48 hr of refrigeration and post-thaw level, while nonsignificantly at 24 hr of refrigeration.

Out of these 9 dilutor combinations tris + WFF, tris + cysteine HCl + SFFF, tris + EDTA + SFFF performed better in reducing the acrosomal abnormalities in both unwashed and washed semen samples at all storage intervals. Out of the studied acrosomal abnormalities the majority were of ruffled type in both unwashed and washed spermatozoa both after refrigeration and freezing. The results obtained in this study agree fairly well with those of Rao *et al.* (1989), Singh *et al.*(1992) and Kumar *et al.*(1995) but direct comparisons are difficult. The values for intact acrosome are higher in this study that may be due to inclusion of these additives to the dilutor.

No report is available on the effect of FF on acrosomal integrity of buffalo spermatozoa. However, a number of reports show that FF induces acrosome reaction, fusion of sperm plasma membrane with outer acrosomal membrane, in a number of mammalian species (Revelli *et al.* 1995). Our results show some synergistic action of FF along with other cryoprotectants in the dilutor in reducing the damage to sperm acrosome. The source of this inconsistency is not known. It may be due to species differences or some other factors such as maturity of the follicles at the time of follicular aspiration; treatment, storage, concentration of FF used; and processing and treatment of spermatozoa. A variety of chemicals such as metal ions, proteins, enzymes, steroids, hormones and prostaglandins are present in FF (Edwards1974) which may help the spermatozoa in one way or the other during preservation. The addition of purified lipid preparation to sperm significantly reduces cold shock and freeze thaw damage (Graham and Foote 1987). Albumin of FF carries twice as much lysophosphatydilcholine (LPC) as plasma albumin because FF does not contain any red cells, which harbor the enzymes like lisophospholipase and acyl transferase responsible for metabolism of LPC (Lepage *et al.* 1993). The high contents of LPC associated with FF might also contribute to the beneficial effect of this fluid to reduce acrosomal damage caused by low temperature during preservation of spermatozoa.

The present study thus indicates that inclusion of FF as additive in tris dilutor exerts a protective effect on the acrosome during refrigerated preservation as well as freezing. This requires further detailed studies to confirm the present results.

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Ovarian antral follicular activity and serum estradiol-17β concentrations in buffaloes during different periods of the year*

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ABSTRACT

Ovarian antral follicular activity in buffaloes was studied from November to April 1998-99 (cooler and warmer periods of the year), using the ovaries collected from civil slaughterhouse, Bangalore. Antral follicular population on the surface of the ovary was studied and simultaneous serum estradiol-17β was assayed by radioimmunoassay to determine the ovarian activity in the animals. Total number of follicles on the surface of the ovary, number of medium and large sized follicles, and number of functional corpora lutea reduced significantly ($P<0.05$) as the ambient temperature increased. The serum concentration of estradiol-17β was significantly lesser ($P<0.05$) in animals without large follicle than those with large follicle, and it reduced significantly with increase in ambient temperature. The study revealed that the buffaloes show variability in the ovarian activity during different periods of the year. The activity was higher during the colder months. The results indicate sensitivity of buffaloes to higher ambient temperature.

Key words: Ambient temperature, Antral follicle, Estradiol-17β, Corpora lutea, Ovary

Seasonal calving patterns in buffaloes were reported by Deen and Talwar (1988) and Vale *et al.* (1990). Female buffaloes are not as sexually active during the hot summer months as during the winter (Pandey and Razada 1979, Tailor and Jain 1993). Kanai and Shimizu (1983) reported that there was no significant seasonal variation in the estrus characteristics in swamp buffaloes. Taneja *et al.* (1995) also observed higher number of small and medium size follicles, and sooner and higher turnover of medium to large follicles during hot and dry months in superovulated buffaloes, which could be attributed to the superovulatory treatment. Thus results of various reports of studies on seasonal variation in the reproductive activity of buffaloes varied. Seasonality was studied by monitoring FSH and LH levels in sheep (McLeod *et al.* 1996), progesterone levels in goats (Lee *et al.* 1985) and buffaloes (Takkar *et al.* 1983), and antral follicular activity by ultrasonography in buffaloes (Taneja *et al.* 1995). This, study was aimed to look at the ovarian antral follicular activity and serum estradiol-17β levels in buffaloes during different periods of the year.

Monitoring estradiol-17β levels, as a support to assessing the reproductive activity of an animal by gross examination of antral follicles on the ovary is more logical as virtually all estradiol in circulation is secreted from the largest follicle of the ovary (Bjersing *et al.* 1972).

MATERIALS AND METHODS

The experiment was conducted from November 1998 to April 1999, in Bangalore (latitude 13° 0' N, longitude 77° 37' E) which is located at a height of 899 meters from sea level. Animals that were brought to slaughterhouse and were apparently healthy, middle aged and had calved at least once (by looking for the well-grown udder and teats), were selected for collection of ovaries and blood. The data of 25 animals/ month were pooled. Blood was collected at the time of slaughter in 50ml test tubes and the serum separated was stored at –20°C until assayed for estradiol-17β. Both the ovaries were excised and transferred to marked-containers with phosphate buffer saline maintained at ice-cold temperature. Then the ovaries were transported to the laboratory within 2 hr of slaughter and were subjected to follicular population study.

Follicles were grouped, according to their surface diameter, into 3 groups, viz., small (3-6mm), medium (6-10mm) and large (>10mm). Number of follicles, falling into each group, on the surface of both the ovaries was noted down. Presence

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of functional corpus luteum, if any, was also noted. Animals were grouped into categories namely, those with large follicle on either of the ovaries and those without large follicle on both the ovaries. Serum estradiol-17β concentration was assayed by radioimmunoassay (Raghava *et al.* 1997).

Regression analysis and 2-way ANOVA (Snedecor and Cochran 1968) were used for statistical analyses of follicular activity and serum estradiol-17β concentrations respectively.

RESULTS AND DISCUSSION

Follicular activity

The number of small follicles did not vary (P>0.05) significantly with the different periods of the year (Table 1), as evidenced by the Pearson's correlation coefficient (r = +0.0089; P>0.05) indicating follicular turnover irrespective of the time of the year. Similar reports were made in sheep with follicular growth in smaller group both in breeding and non-breeding seasons (Ravindra and Rawlings 1997), and in prepubertal cattle with follicular activity in smaller groups (Spicer and Echternkamp 1986). The number of follicles in medium and large groups reduced significantly (r = -0.944 and r = -0.973: P<0.05) as the monthly average mean temperature increased from colder months (December 21.20°C, and January 20.95°C) to hotter months (March 25.75°C, and April 27.20°C). This indicated that the follicles entered from small group (basal follicular activity) into the medium and large groups, in accordance with cold or hot months. This is in contrast to the results of Taneja *et al.* (1995) that have shown, by ultrasonographic study in FSH-superovulated buffaloes, that more number of medium and large follicles was available in hotter months than that in colder months. The difference could be attributed to the small sample size and the experimental method in that study. In this study, there were significantly more number of buffaloes (P<0.05) with corpora lutea in colder months than that in hotter months.

Table 1. Ovarian follicular activity during different periods of the year (25 animals/ month)

Month	Average mean temp. (°C)	Total no. follicles	Small	Medium	Large	No. of functional CL
Nov	22.40	212	61	89	62	12
Dec	21.20	238	69	97	72	16
Jan	20.95	243	63	103	77	14
Feb	22.75	207	66	87	54	10
Mar	25.75	173	59	79	35	7
April	27.20	178	69	76	33	8

Regression analysis with independent variable, monthly average mean temperature

Dependent variable	Correlation coefficient	Variation (%)	P
Total no. of follicles	-0.947	89.77	0.004
Small	+0.0089	00.01	0.987
Medium	-0.944	89.16	0.004
Large	-0.973	94.73	0.001
No. of CL	-0.892	79.49	0.017

Source (temperature): Department of Agro-meteorology, GKVK, UAS, Bangalore.

These results indicate that the ovarian follicular activity varied significantly with different periods of the year and that the ambient temperature is having a significant influence on ovarian follicular activity in buffaloes. This is in agreement with Madan and Raina (1985), Deen and Talwar (1988) and Vale *et al.* (1990).

Serum estradiol-17β

The effect of month and presence of large follicle was significant (P<0.05), and the interaction effect was nonsignificant (P>0.05). The serum estradiol-17β concentration reflected the ovarian follicular activity of the animals, as it was significantly affected (P<0.05) by the presence of large follicle (Table 2). Serum concentration of estradiol-17β in buffaloes increases during the proestrus (Ravindra and Narayana 1984) when there would be large ovulatory follicle. In other words, buffaloes with larger follicles were reproductively more active as compared to those without large follicles. Similar results were shown in cattle (Spicer and Echternkamp 1986).

Table 2. Serum concentration of estradiol-17β (ng/ml; mean±SE) in the animals with or without large follicle in different months

Month	With large follicle	Without large follicle
Nov	16.33 ± 4.67^{aw}	09.5 ± 0.01^{bw}
Dec	34.32 ± 8.77^{ax}	21.0 ± 3.67^{bx}
Jan	19.22 ± 0.67^{ay}	16.0 ± 1.64^{by}
Feb	10.85 ± 3.16^{awz}	07.7 ± 1.70^{awz}
Mar	08.67 ± 2.29^{az}	06.2 ± 0.65^{az}
April	09.50 ± 1.80^{az}	06.8 ± 0.24^{bz}

Two-way ANOVA; 1. Effect of month: P<0.05; 2. Effect of presence of large follicle: P<0.05; 3. Values with different superscript within a row (a, b) and within a column (w, x, y, z) differ significantly (P<0.05).

The serum estradiol-17β concentration was significantly lesser (P<0.05) during warmer months compared to colder months (Table 2). This confirmed that the follicular activity was reduced in warmer months. The serum estradiol-17β was significantly (P<0.05) lesser in the warmer months in animals of both the groups, i.e., with and without large follicle. The ambient temperature (month) had independent effect on the serum estradiol-17β concentration, as the interaction effect was not significant (P>0.05).

It may be speculated from these results that there could be some other mechanism, in addition to affecting the size to which a follicle grows, through which the ambient temperature

reduced the follicular ability to secrete estradiol-17β. This additional mechanism could be through reduction in the FSH availability (McLeod *et al.* 1996) and sooner and higher turn over of medium to large follicles during hotter months when superovulated with FSH supplementation in buffaloes (Taneja *et al.* 1995)

In conclusion, the present study revealed that variability existed in the ovarian antral follicular activity and the serum estradiol-17β concentration during different periods of the year in the buffalo, with highest activity during the colder months like December and January.

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Pretreatment effect of human chorionic gonadotropin and estradiol-17β on ovarian response, embryo production and endocrine profile in cattle superovulated with FSH-P*

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ABSTRACT

Pretreatment effect of hCG and estradiol- 17β on superovulatory response by exogenous administration of FSH was studied in 15 adult cyclic crossbred cows randomly divided in 3 groups. Group 1 animals received 40 mg FSH-P in 6 doses of constant 3 days schedule from day 11 of cycle; 25mg of $PGF_2\alpha$ was injected with last FSH injection. Group 2 animals received 500 IU hCG (im) on day 9 and 2mg estradiol (sc) on day 10 followed by superovulation on day 11. Group 3 animals received 250 IU hCG in place of 500 IU hCG. Other treatments were similar to that of group 2 animals. Animals were inseminated at 0, 12 and 24 hr after onset of estrus. The ovarian response and embryo quality was recorded after nonsurgical collection on day 8. Blood plasma collected during the experiments was subjected to FSH, progesterone and estradiol estimation.

Ovulation rate did not differ significantly among the different treatment group 3. However, comparatively higher number of transferable embryos were recorded from the 250 IU hCG and estradiol pretreatment (4.75±0.94) in comparison to control (1.60±1.12) or 500 IU hCG (2.00±1.37). Increased FSH, estradiol level at estrus and progesterone on day of flushing was positively correlated with transferable embryo.

It may be concluded that pretreatment of low dose of hCG and estradiol prior to superovulatory treatment probably develops more follicles and ovulate synchronously, thus enhances transferable embryo production.

Key words: Cattle, Embryo, FSH,Steroid hormone, Superovulation

Several superovulation protocols failed to overcome variability and unpredictable responses in ovulation rates and the recovery of transferable embryos in cattle (Hahn 1992, Armstrong 1993, Boland and Roche 1993, Adams 1994). The superovulatory response is influenced by the genetic composition (Bindon *et al.* 1986), presence of gonadotropin responsive follicles (Monniaux *et al.* 1984), the types of superovulatory agent used (Murphy *et al.* 1984) and the stage of estrous cycle at which superovulation treatment began (Lindsell *et al.* 1986). Follicle development in cattle occurs in 2 or 3 waves during an estrous cycle (Ginther *et al.* 1989). Each wave of follicles has an emergence, selection and dominance phase. The presence of dominant follicle during superovulation decreased the superovulatory response, yield of embryo (Bungartz and Niemann 1994) and reduced the number of more than 7mm diameter follicles (Grasso *et al.* 1989) or alter the maturation process of recruitable follicles and their response to gonadotropin stimulation (Guilbault *et al.* 1991).

Elimination of dominant follicle by electrocautery using ultrasonic guidance (Bergfelt *et al.* 1994) or hormone induced demises such as hCG (Rajamahendran and Calder 1993) or progesterone in combination with estradiol (Bo *et al.* 1995) or superovulation of cattle in the absence of dominant follicle (Hahtinen *et al.* 1992) altered the follicular growth and increased superovulatory response. Higher dose of hCG followed by estradiol causes silent ovulation and adversely affected superovulation and embryo recovery in goat (Tiwari 1995) and cattle (Khanna 1996). However, low dose of hCG though do not cause ovulation of dominant follicle but leaches out FSH receptors from gonadotropin responsive follicle (Majumdar *et al.* 1997) and estradiol-17β subsequently enhances formation of FSH receptor in the follicle (Majumdar and Sharma 1999).

Thus, the ability to synchronise the follicular wave

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emergence in a group of randomly cycling cattle before start of superovulation treatment with gonadotropin may have some important implication for the success of embryo transfer programme. Therefore, in the present experiment effect of different doses of hCG in combination with estradiol on synchronization of follicular development, ovulation embryo development and endocrine response to superovulation was studied in a group of crossbred cattle.

MATERIALS AND METHODS

Treatment

Crossbred cyclic cows (15) weighing between 350 to 450 kg were housed in a well managed shed and fed a maintenance ration of concentrate and green fodder, allowed water *ad lib.* and monitored for estrous behavior, morning and evening. The cows were randomly allocated to 3 groups. Group 1 (n = 5) animals received 1 ml saline (im) on day 9 and 1 ml olive oil (sc) on day 10 as placebo. The animals in group 2 (n = 5) and group 3 (n = 5) were injected with 250 IU and 500 IU of human chorionic gonadotropin intramuscularly on day 9 of the estrous cycle and 2 mg estradiol-17β in olive oil subcutaneously on day 10. Animals of all groups were superovulated on day 11 of the estrous cycle with 40 mg FSH-P injected twice daily (6.5/6.5, 6.5/6.5, 6.5/6.5) at 0700 and 1800hr. Lutalyse (25 mg) was administered at the sixth injection of FSH-P. The estrus was detected twice daily with the help of teaser bull and visual observation. All animals were inseminated 3 times at 12 hr interval starting from standing estrus. Ovaries were palpated per rectally and number of palpable CL and large follicles were recorded prior to flushing. Embryos were collected on day 8 following standing estrus and classified according to the criteria of Shea (1981).

Blood sampling

Blood samples were collected on day -7 (before hCG pretreatment), day -6 (before estradiol pretreatment), day -5 (before FSH injection), day -4, day -3, day -2 (PG), day -1, day 0 (superovulatory estrus), day 1, day 2, day 3, day 4 and day 8 (day of flushing) post estrus in heparinized glass tube using 18 G hypodermic needle by jugular vein puncture. Immediately after blood collection, samples were centrifuged at 3 000 rpm for 15 min.. Plasma was separated out and stored in to glass vials at -20°C until analysis.

Hormone analysis

Plasma FSH levels were determined by enzyme linked immunosorbant assay kit. The sensitivity of the kit was 2.5 mIU/ml.

Plasma progesterone concentrations were measured by radio immunoassay method (Sufi *et al.* 1983). The sensitivity of the assay was 0.05 ng/tube and recovery of 3H progesterone after extraction was 84.80%. Interassay and intra-assay coefficients of variations were 14.0% and 8.4% respectively. Plasma estradiol concentration was measured using a commercially available estradiol coat-a-count kit. The sensitivity of the assay was 8 pg/ml. Interassay and Intra-assay coefficients of variations were 8.1% and 7.0% respectively.

Data pertaining to ovulation rate, embryo recovery, embryo quality and hormonal profile was analyzed using Duncan's multiple range test. Correlations between ovulation rate, embryo recovery, embryo quality and hormonal profile at different stages of superovulation were examined by calculating correlation coefficients. Differences between means were compared using student's 't' test.

RESULTS AND DISCUSSION

The results of this study demonstrated convincingly that the pretreatment of low dose of hCG and estradiol-17β before gonadotropin stimulation influences markedly the superovulatory response in the crossbred cows.

The animals responded to superovulation were 100% in groups 1 and 2 but in group 3 two animals did not express estrus symptom after superovulatory treatment. One animal in group 2 showed exceptionally higher number of embryos, thus was not included in the analysis (Table 1). However, hormonal profile of this animal did not differ significantly with the other animals. Thus, data pertaining of FSH, progesterone and estradiol was included in this group. There was a trend of increased ovulation rate in group 2 as compared to groups 1 and 3 animals. The total number of ova embryo found and progesterone level on day 8 after superovulatory estrus both indicate the difference of higher ovulation rate in group 2 as compared to groups 1 and 3. The embryo recovery was slightly better in group 3 than that of control. Two animals of group 3 did not express estrus symptoms and if these animals are eliminated, the mean number of transferable embryo would further increased. A significant correlation was observed between ovulation rate and embryo recovery in (r = 0.99) and between embryo recovery and transferable embryo (r = 0.99) in group 3. No decrease in fertility or embryo quality was noted due to increase in ovulation rate. On the contrary, the percentage of unfertilized oocytes and degenerating embryos were lower in the group of cows treated with low dose of hCG and estradiol before gonadotropin treatment. The recovery of ova and transferable embryos in group 2 was comparatively higher than previous reports (Khanna 1996).

The improvement in superovulation parameter in group 2 may be due to leaching out of FSH receptors due to hCG pretreatment from the different stages of follicles as has been reported that LH release the proteases in the follicle (Parr 1975) which digests/leaches out the surface protein (gonadotropin) receptors (Arsenis *et al.* 1985) from gonadotropin responsive follicles followed by synchronous formation of FSH receptors in all the follicle due to administration of estradiol. Exogenous administration of

Table 1. Pretreatment effect of hCG and estradiol-17β on ovulation rate, embryo recovery and number of transferable embryos using FSH-P

Treatment group	Sl. No	No. of palpable CL	No. of palpable follicle	No. of ova/embryo	No. of transferable embryo
	1	4	2	2	0
	2	5	2	3	0
Group 1	3	11	3	0	0
	4	4	0	1	1
	5	14	1	21	6
X±SE (n=5)		7.60±2.06	1.60±0.50	5.40±3.93	1.60±1.12
	1	10	2	2	2
	2	8	1	9	6
Group 2	3	9	0	6	6
	4	5	1	5	5
	5*	15	1	32	28
X±SE (n=4)		8.40±1.08	1.00±0.40	5.50±1.44	4.75±0.94
	1	10	3	7	7
	2	0	3	0	0
Group 3	3	5	2	3	3
	4	0	0	5	3
	5	3	1	1	0
X±SE (n=5)		3.60±0.44	2.00±0.44	2.20±1.31	2.00±1.37

*Animal showing exceptionally high value was not included in the analysis.Means do not differ significantly (P>0.05).

estradiol has been reported to results in the formation of FSH receptors (Richards *et al.* 1976) in all follicle. In this case also probably estradiol-17β injection followed by low dose of hCG injection developed a batch of gonadotropin follicle synchronously. Further Majumdar and Sharma (1999) in their *in vitro* follicle culture studies showed that LH leaches out FSH receptor that reappeared again when the follicles were treated with estradiol-17β. On exogenous FSH administration at this stage might have resulted in synchronous growth of all follicles present in the ovary. Beneficial effects of pretreatment of low dose of hCG and estradiol on ovulation rate, egg recovery and transferable embryos have been observed in goat by Majumdar *et al.* (1997).

The unovulated large follicles palpated on day of flushing were higher in group 3 as compared to control and group 2. It seems that in group 3 two animals ovulated and formed CL on hCG injection and after superovulatory treatment injection of PG could not cause luteolysis of these newly formed CL in proper time as has been seen earlier that PG fails to cause luteolysis of newly formed CL (Hansel and Convey 1983). Further the progesterone level remained comparatively elevated in these 2 animals. Similar observation was made in goat when 500 IU hCG showed almost similar effect and surgical observation confirmed newly formed CL in these cases (Tiwari 1995).

Mean FSH concentration on day -7 was 34.60±2.51, 32.75±1.23 and 31.20±5.23 mIU/ml in groups 1, 2 and 3 respectively (Fig.1). The FSH level at the initiation of superovulation treatment were significantly correlated with the ovulation rate and transferable embryos as previously observed (Kweon *et al.* 1987, Lussier and Carruthers 1989). FSH level increased after the gonadotropin injection and then there was a slight decrease before the preovulatory FSH peak which is in agreement with the previous reports (Lussier and Corruthers 1989, Roberge *et al.* 1995 and Desaulnier *et al.* 1995). However, Kweon *et al.* (1987) observed a decline in FSH level from gonadotropin injection until before the preovulatory surge. The mean plasma FSH concentration was

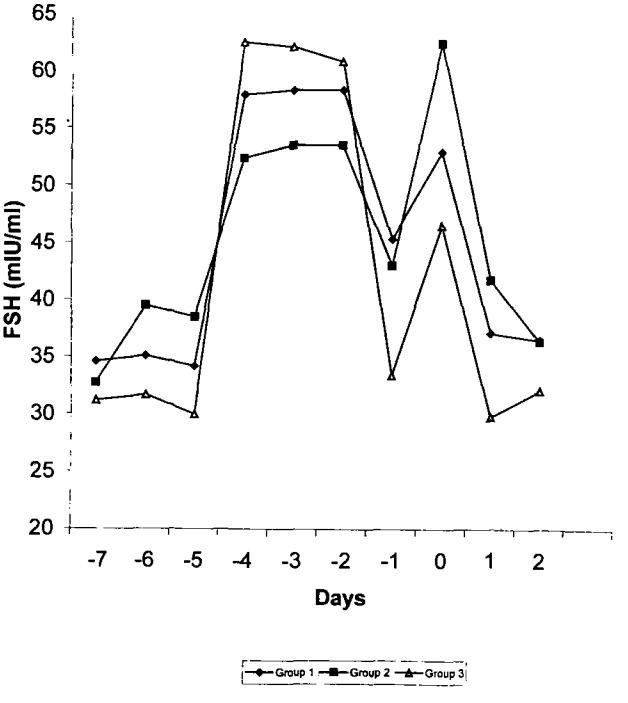

Fig. 1. Mean plasma FSH profile of cattle superovulated with FSH-P.

higher on day of superovulatory estrus in group 2 (62.37±2.38) than groups 1 (50.90±5.49) and 3 (46.50±4.07). Transferable embryo recovery was found to be significantly correlated with FSH concentration on day 0 in group 2 (r = 0.92). It was observed in this study that those animals having more ovulations or produced more number of embryos had comparatively low FSH in the circulation during gonadotropin treatment. The responder animals also had a higher peak of FSH at estrus as compared to non-responders. This is in agreement with Desaulniers *et al.* (1995).

Mean plasma progesterone concentration before start of treatment (day -7) was slightly higher in group 2 than other groups. The concentration of progesterone at the initiation of superovulation treatment was not significantly correlated with subsequent ovulation rate and number of embryos recovered as previously reported (Greve *et al.* 1983, Lindsell *et al.* 1986 and Agarwal *et al.* 1992). There was a significant ($P < 0.05$) increase in progesterone concentration after hCG pretreatment in groups 2 (2.55±0.49ng/ml) and 3 (2.74±0.52ng/ml) as compared to group 1 (1.26±0.09). A significant increase in progesterone concentration following hCG treatment has also been reported in other studies (Rajamahendran and Sianangama 1992; Rajamahendran and Calder 1993). The progesterone concentration after hCG injection were significantly higher ($P < 0.05$) in groups 2 (2.55±0.49ng/ml) and 3 (2.74±0.52ng/ml) as compared to group 1 (1.26±0.09 ng/ml). The increased plasma progesterone concentration might have resulted from hypertrophy of luteal cells in spontaneous CL (Helmer and Britt 1986). Luteinization of granulosa cells of unovulated follicles (Booth *et al.* 1975, Opavsky and Armstrong 1989) and the formation of accessory C.L. following ovulation of dominant follicle (Rajamahendran and Calder 1993) may also contribute towards increase in progesterone concentration after hCG treatment but under such cases the animal did not responded to PG treatment and failed to show estrus symptom at proper time as PG can not cause luteolysis of early CL (Hansel and Convey 1983).

There was a general increase in progesterone concentration after gonadotropin treatment, which might be due to luteotropic effect of FSH-P preparation, which has been shown to contain appreciable LH activity (Murphy *et al.* 1984). However, in group 2 there was a slight decline in progesterone concentration after first gonadotropin injection till the day of PG treatment. This might be due to luteolytic effect of estradiol, which was administered before gonadotropin treatment in group 2, whereas, it was not observed in group 3. This might be due to the fact that the low dose of hCG in group 2, which could not be supported the luteotropic function to the exten to compensate the fall of progesterone concentration. It has been reported that estradiol exerts its luteolytic effect via uterine PGF2α synthesis (Hansel *et al.* 1973).

A sharp decline in progesterone concentration 24hr following $PGF_2\alpha$ injection in all treatment groups was observed. The progesterone level comes down to its basal value (< 1ng/ml) by 48 to 72 hr post $PGF_2\alpha$ injection which correspond to the onset of estrus. However, in animals of group 3 the progesterone level did not decline appreciably and thus did not showed estrus symptom in proper time. The progesterone concentration increased after superovulatory estrus until the day of flushing due to the formation of multiple CL as previously observed (Saumande 1980, Yadav *et al.* 1986, Agarwal *et al.* 1992). The progesterone concentration on day of flushing was higher in group 2 (6.20±1.74 ng/ml) than groups 1 (5.92±1.52 ng/ml) and 3 (4.22±1.11), (Fig.2). A significant correlation was observed between progesterone concentration on day 8 and ovulation rate (r = 0.92), embryo recovery (r = 0.84) and transferable embryo (r = 0.76) in group1.

Mean estradiol-17β concentration on day -7 was 10.88±1.21, 9.28±1.18 and 11,60±0.97 pg/ml in groups 1, 2 and 3 respectively. The mean concentration of estradiol-17β at the initiation of superovulation treatment was correlated significantly with ovulation rate, which is contradictory to that of Agarwal *et al.* (1992). The level of estradiol-17β tended to increase following administration of gonadotropin in control animals superovulated without hCG and estradiol pretreatment as previously observed (Saumande 1980. Kweon *et al.* 1987, Agarwal *et al.* 1993, Roberge *et al.* 1995) The increased concentration of estradiol-17β was postulated to be coming from increased stimulation of growth of antral follicles by FSH-P treatment (Schams *et al.* 1979, Roberge *et*

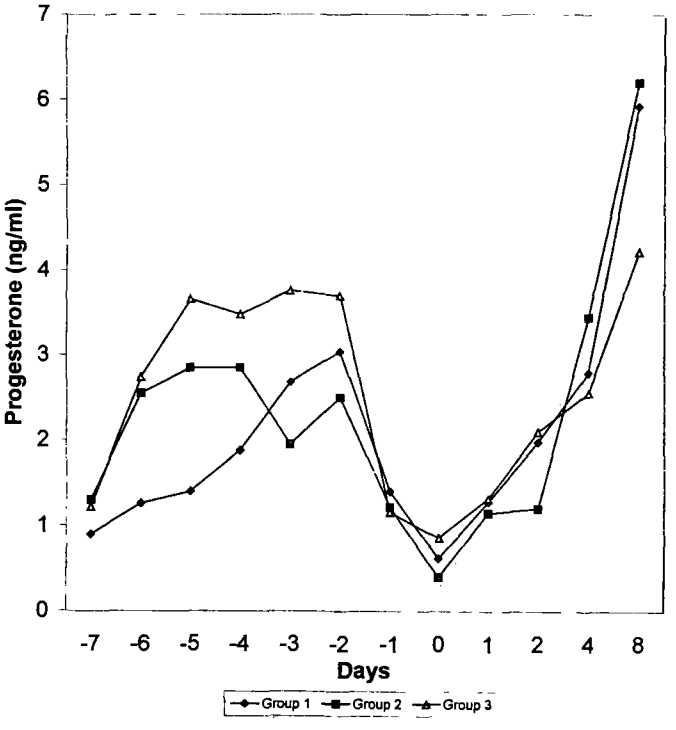

Fig. 2. Mean plasma progesterone profile of cattle superovulated with FSH-P.

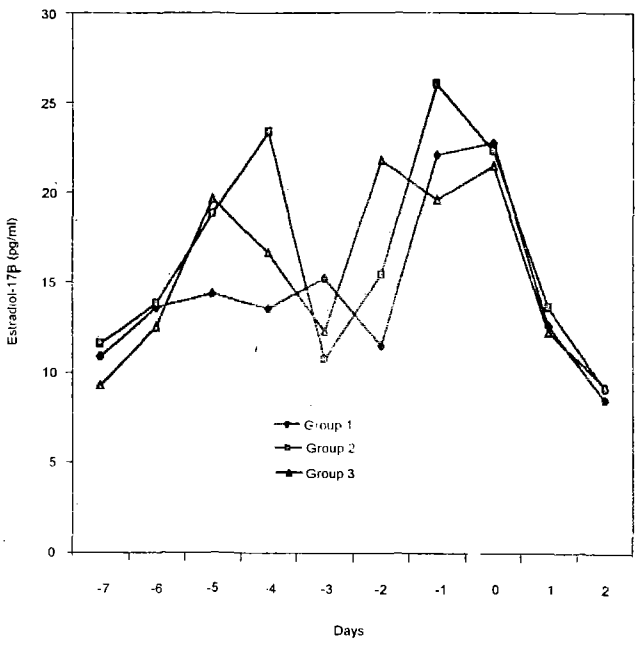

Fig. 3. Mean plasma estradiol-17β profile of cattle superovulated with FSH-P.

al. 1995). The concentration of estradiol-17β increased further and reached to its peak value by 48hr after PGF2α injection. Estradiol concentration increased to a higher level (26.00±2.04 pg/ml) on day -1 in group 2. Whereas the peak level of estradiol in groups 1 (22.76±2.16) and 3 (21.48±3.81) occurred on day 0 (Fig. 3). A significant correlation was observed between ovulation rate and peak level of estradiol on day of estrus in groups 1 (r = 0.94), 2 (r = 0.94) and 3 (r = 0.89). It is generally believed that a sharp increase in the estradiol level is involved in releasing the optimum LH surge at estrus, whereas a fluctuating level of estradiol has been shown to decrease the ovulatory LH surge (Goto *et al.* 1988). Higher level of estradiol at superovulatory estrus was due to development, growth and maturation of more number of follicles contributing to production of higher levels of hormone in this study.

In groups 2 and 3, exogenous administration of estradiol caused increase in plasma estradiol level which then gradually decline to its basal level and it again increased 24hr after gonadotropin treatment till its peak value on the day of expected estrus. It was suggested that the administration of exogenous estradiol could be able to initiate the FSH receptor (Richards *et al.* 1976) in the small and medium follicles making them gonadotropin responsive which upon gonadotropin stimulation produced increased concentration of estradiol till the preovulatory LH surge. The peak level of estradiol-17β in present study was higher than reported by Agarwal *et al.* (1993) and it occurred on day 1 or day of estrus. The adverse effect of high estradiol was not observed in this study, as there was recovery of a quite large number of transferable embryos. However, the peak estradiol level in 2 animals of group 3 which failed to superovulated has occurred without a sharp decrease of progesterone before the injection of $PGF_2\alpha$ which might have interfere in the ovulation of the follicles. These unovulated follicles would have undergone gradual luteinization, which resulted in decreased secretion of estradiol till day 2.

Concentration of estradiol-17β at superovulatory estrus was significantly correlated with ovulation rate, number of embryo and transferable embryo recovered which indicated that animals with higher estradiol-17β at estrus would have higher ovulation rate and yielded large number of total and transferable embryos as previously reported (Saumande *et al.* 1980, Monniaux *et al.* 1983, Lindsell *et al.* 1986, Agarwal *et al.* 1992)

It may be concluded from the study that pretreatment of low dose of hCG followed by estradiol prior to FSH treatment may increase the number of transferable embryos from crossbred cows.

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The use of fast acting antioxidants for the reduction of cow placental retention and subsequent endometritis

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ABSTRACT

Vitamin E either with selenium or vit. A as antioxidants was administered to reduce incidence of retained placenta and uterine infections that could delay subsequent conception. Pregnant multiparous crossbred cows (70) were grouped into 3: animals in group 1 (n=30) injected with 400 mg vit. E and 12 lakh IU vit. A, group 2 (n=20) with 500 mg vit. E and 15 mg selenium at 4 weeks before expected calving and group 3 (n=20) an untreated control. The serum levels of different biochemical parameters were also estimated at prepartum stage. These animals were observed for occurrence of placental retention following parturition. The placental retention 24hr after parturition was 25% in control and reduced to 10 and 15% in treated groups 1and 2 respectively. Endometritis occurred in 100% of the animals following placental retention as compared to 10.17 % of those with normal expulsion of the fetal membranes. A comparison of reproductive indices showed no significant improvement in fertility of treated cows. The results revealed that serum levels of alkaline phosphatase and progesterone, were higher at 250 ± 10 days of gestation in cows which experienced placental retention following calving.

Key words: Antioxidants, Cows, Endometritis, Placental retention

Placental retention (PR) has a multifactorial etiology, which makes difficult to assess the effectiveness of therapeutic treatment (Joosten *et al.* 1991) or to evaluate the advantages of prophylaxis when the risk of disease is variable. An average incidence of PR of 10% was associated with wide fluctuations of 5 and 40 % (Pandey *et al.* 1994) so that the effectiveness and economic advantages of a preventive treatment have been shown only on farms with an above average incidence (Joosten *et al.* 1988).

PR prevention means avoiding all of the factors involved in its etiopathogenesis. At least 3 different kinds of specific intervention for PR prophylaxis have been effective. These are: use of oxytocin during or immediately after calving (Roberts 1971), prostaglandin F2 alpha administration postpartum (Studer *et al.* 1989) and selenium supplementation alone or with vitamin E during the dry period (Hidiroglou *et al.* 1987). Thus, a treatment, which helps placental release, must be aimed at the prevention of complications that could lead to delayed conception and milk yield reduction (Joosten *et al.* 1988).

Oxidative stress (Brazezinska-Slebodzinska *et al.* 1994) and some enzymes (Kankofer *et al.* 1998) affect the etiopathogenesis of PR. Oxidative stress results from imbalance between production of oxygen-centred free radicals also referred as reactive oxygen metabolites (ROM) (Powell 1991) and their safe disposal (Gutteridge and Halliwell 1994). Stress elicit increased ROM production (Nockels 1996) which causes extensive damage to the cell membranes (lipids) DNA and enzymes (Halliwell 1987) through oxidative chain reaction (Buettner 1993). Possible relationship among dietary antioxidants, oxidative stress and PR were investigated in the periparturient dairy cows (Brazezinska-Slebodzinska *et al.* 1994, Nockels 1996). Antioxidants are agents, which break the oxidative chain reaction thereby, reduce the oxidative stress (Nockels 1996). Vit. E, as the primary lipid soluble fast acting antioxidant breaks the oxidative chain reaction function in concert with selenium as a integral component of glutathione peroxide, in minimizing oxidative stress and reducing incidence of PR in dairy cows (Brazenzinska-Slebodzinska *et al.* 1994).

The aim of this study was to evaluate the effects of administration of certain antioxidants before calving as a first step to preventive therapy in reducing the incidence of PR and the occurrence of post PR endometritis to improve reproductive performance.

MATERIALS AND METHODS

Friesian × Sahiwal cows (70) of different grades from the

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Military Dairy Farm, Bareilly, India, were maintained under good managerial conditions. The multiparous pregnant cows aged 4-7 years were divided into 3 groups: animals in group 1(n=30) were injected i/m with 400 mg vitamins E and 12 lakh IU of vit. A and animals in group 2 (n=20) with 500 mg vit. E along with 15mg selenium at 4 weeks before expected date of calving. The remaining animals formed an untreated control group 3 (n=20) with each of them receiving saline injections at an equivalent time.

The blood samples were collected from each animal prior to injections. The blood was allowed to clot and serum was separated by centrifugation. The serum samples were stored at –20°C until used for the biochemical assays. The estimations for different biochemical parameters were carried out by diagnostic kits. The serum immunoglobulins (McEwan *et al.* 1970) and progesterone levels were estimated by radioimmunoassay technique.

All cows were observed for occurrence of retained fetal membranes (RFM) following calving. The number of cows which retained the foetal membranes for 24hr were considered a clinical case of RFM and cows which expelled out their foetal membranes before 24hr were considered a non-RFM.

From 7 to 10 days after delivery, each one the 70 cows underwent a clinical examination to detect endometritis. Its presence was diagnosed on the basis of the presence of a mucopurulent (sometimes fetid) exudate, in the cervicovaginal tract. Those animals with endometritis were treated with 4 intrauterine pessaries a day containing a total of 2.0 g tetracycline hydrochloride.

Cows were observed for estrus twice daily and artificially inseminated at the first estrus detected at least 60day postpartum if palpation per rectum confirmed the presence of a mature follicle and the absence of genital disorders. Artificial insemination (AI) was performed using frozen semen (post-thaw motility> 40%) from tested Friesian bulls. Pregnancy was diagnosed by palpation per rectum about 60day post AI. Open cows with no signs of pathology were not treated and were submitted to AI up to conception or culling. Reproductive indices, such as conception rate (CR), days from calving to first service, days from calving to conception were considered to complete this study.

Statistical analysis

The data obtained in the study were analyzed by using suitable statistical methods (Snedecor and Cochran 1968). A chi-square test was used to study the association between treatment and RFM, between RFM and endometritis and between treatment and CR. For comparison of other biochemical parameters and data of different reproductive indices, Student's t-test for independent samples was used.

RESULTS AND DISCUSSION

Data summarizing the effects of prepartum supplementation of vit E either along with vit A or selenium to reduce the occurrence of RFM are shown in Table 1. Those in Table 2 summarize data relating to the occurrence of endometritis in cows with or without RFM.

Table 1. Effect of prepartum supplementation of vit-E either alongwith vit A or selenium on the occurrence of RFM in crossbred cows

	Treated				Control	
	Group 1 (n=30)		Group 2 (n=20)		Group 3 (n=20)	
	no.	%	no.	%	no.	%
Without RFM	27	90.0	17	85.0	15	75.0
With RFM	3	10.0**	3	15.0*	5	25.0
Total	30	100.0	20	100.0	20	100.0

Treated versus control χ^2 = 10.37 **, P< 0.01; 6.20 *, P< 0.05.

Table 2. Distribution of endometritis with or without RFM in crossbred cows

	Without RFM		With RFM	
	no.	(%)	no.	(%)
Without endometritis	53	89.83	0	0
With endometritis	6	10.17	11	100.0**
Total	59	100.00	11	100.0

**P< 0.01.

The RFM prevalence in the control group was 25% (Table 1) which reduced to 10 and 15% in treated groups 1 and 2 respectively. The results also showed that RFM was associated with a significant increase in the occurrence of endometritis (Table2).

The difference in reproductive indices viz. the number of inseminated cows, conception rate, days to first service and days to conception, between the treated and the control groups (Table 3) was statistically insignificant (P> 0.05).

There were no significant difference (Table 4) in serum levels of cholesterol, glucose, albumin, calcium, phosphorus and immunoglobulins between RFM and non-RFM animals. However, the serum alkaline phosphatase (AKP) levels were significantly higher in animals with RFM as compared to non-RFM. Similarly, increased serum progesterone levels were observed during 250±10 days of gestation in cows which subsequently experienced RFM following calving

Our results showed that administration of vit E either with selenium or vit A at 4 weeks prior to expected date of calving reduced the incidence of RFM following parturition,however, it did not reduce the occurrence of endometritis. This confirmed the findings of Hidiroglou *et al.* (1987). Although our data (Table 3) did not reveal any significant improvement in fertility following treatment, however, in some studies. administration of antioxidant vitamins like beta-carotene (precursor of vit A) (Ascarelli *et al.* 1985) or vit E and

Table 3. Reproductive indices in treatement (T) and control (C) groups

Groups	Cows			CR	Days to first service	Days to conception
	Total (no.)	Inseminated (no.)	Pregnant (no.)	(%)	(Mean±SD)	(Mean±SD)
1 (T)	30	26	14	53.8	80.1±7.8 (66-150)	124.4±14.5
2 (T)	20	17	10	58.8	93.3±10.6 (82-147)	136.8±26.3
3 (C)	20	16	8	50.0	100±15.0 (85-115)	142.2±18.8

Figures in parenthesis show range.

Table 4. Serum levels of different biochemical variables at prepartum stage (250 ± 10 days of gestation)

Parameters	Without RFM (n=21)	With RFM (n=6)
Cholesterol (mg %)	164.1 ± 17.2	134.0 ±14.1
Glucose (mg %)	45.20 ± 6.30	43.57 ± 4.20
Albumin (g %)	5.25 ± 0.50	5.48 ± 0.35
Calcium (mg %)	8.71 ±1.20	10.43 ± 0.50
Inorganic phosphorus (mg %)	4.83 ± 0.33	4.76 ± 0.29
Alkaline phosphatase (IU/litre)	34.01 ± 8.20	47.61 ± 11.30*
Immunoglobulins (mg %)	15.50 ± 1.50	12.99 ± 1.60
Progesterone (ng/ml)	15.60 ±2.75	22.05± 3.50*

Values are given as mean ± SD; * P<0.05.

selenium (Arechiga *et al.* 1994) improved fertility of cattle.

Antioxidant systems include molecules such as beta-carotene and vit E, which act as membrane antioxidants to maintain the integrity of phospholipids against oxidative damage (DiMascio *et al.* 1991). Various enzymes also remove free radicals. Among these, glutathione peroxidase is a selenium-dependent enzyme that utilizes electrons from glutathione and other thiols to convert peroxides to water. Production of free radicals could represent a source of infertility because ovarian steroidogenic tissue (Margolin *et al.* 1990) is sensitive to free radical damage. Thus, increase in the level of these enzymes could be a defense mechanism against the free radicals.

It is not surprising that a single injection of vit E and selenium could cause a positive effect on reproductive function 4 to 5 weeks after the injection because administration of these molecules having long-term effects in cattle. Intramuscular injection of vit E caused elevated amounts in serum for at least 28day (Charmley *et al.* 1992), while injection of selenium increased concentrations of selenium in whole blood and serum for 28day and increased whole blood-glutathione peroxidase activity for at least 84day (Maas *et al.* 1993). Injections of vit E and selenium 3 week before calving increased erythrocyte GSH peroxidase in dairy cows during the first 12 week of lactation (Lacetera *et al.* 1996).

The increased levels of AKP in serum (Table 4) may result from leakage out of necrotic or damaged cells (Dutta and Dugwekar 1982). RFM is commonly associated with small portion of necrotic epithelium between the chorionic villi and the cryptal walls. It has been suggested that the estimation of isozymes of AKP which are tissue specific may improve the diagnostic value of test (Rowel 1971). Although it was not possible in the present experiment to estimate the isozymes of AKP of placental origin, it would be tempting to infer that the pathological changes leading to RFM might have occurred much before parturition. However, further studies would be necessary to draw a conclusion.

This study revealed that during 250 ±10 days of gestation, the serum levels of progesterone were higher in cows which subsequently had RFM. This agrees with earlier finding of Chew *et al.* (1977).

Our findings clearly showed that though there was decrease in the incidence of RFM by prepartum supplementation of vit E either with selenium or vit A as antioxidants, it was not adequate to improve the herds reproductive performance.

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Treatment of placental retention with ecbolic drugs and its effect on subsequent fertility in crossbred cows

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ABSTRACT

This study was conducted to examine the fertility response in cows with retained fetal membranes (RFM) following treatment with ecbolic drugs. Dairy cows (n=32) that had RFM more than 12 hr after parturition were randomly assigned to 1 of 4 treatment groups; group 1,daily intrauterine infusion of 4 oriprim bolus until membranes were expelled and i.m. injections of 30 IU synotocinon, 15 mg luprostiol plus 10mg estradiol valerate; group 2, oriprim as similar to group 1 and oxytetracycline injections; group 3, oriprim as in groups 1 and 2 plus a herbal drug, 20 g metrali daily for 5 days and group 4 served as untreated controls. Cows were monitored daily. Subsequent disease conditions were recorded. The blood samples were collected from all cows for determination of biochemical parameters and hormonal profile. The results revealed that the interval from parturition to expulsion of fetal membranes was unaffected by treatment regimen. Reproductive efficiency, as determined on the basis of the interval from parturition to conception (136.8 ± 26.3 day) and number of services per conception (3.0± 0.50) was significantly better in group 1 than that of control cows. The study also showed that globulins and immunoglobulins levels were higher in RFM cows. Significant increase in serum alkaline phosphatase and lactic dehydrogenase levels in RFM cows suggested that estimation of these biochemical parameters would have prognostic value in postpartum complications. It may be concluded that the administration of ecbolic drugs in cows with RFM may hasten the resumption of normal reproductive activity.

Key words: Cows, Ecbolic drugs, Fertility, Placental retention

Retention of fetal membranes (RFM) for longer than 12hr after parturition is considered pathologic in cattle and is attributable to failure of separation of the microvilli of cotyledons from the crypts of the caruncles (Roberts 1986). RFM have been associated with an increased incidence of postpartum metritis (Halpern *et al.* 1985), reduced subsequent fertility (Martin *et al.* 1986), and increased culling rates (Joosten *et al.* 1988).

In cows with RFM tissue samples of cotylédons and caruncles contain lower concentrations of prostaglandin F2 alpha (PGF2α) than tissue samples taken from cows that expel their fetal membranes (Leidl *et al.* 1980) suggesting a beneficial effect may be possible from PGF2α administration. Furthermore, the biochemical aspects of etiology and pathogenesis of RFM after parturition is incompletely understood. The objective of this study was to compare the effects of various treatments in cows with RFM on the duration of retention of RFM, severity of subsequent disease and reproductive efficiency during the subsequent breeding season. In addition the study was undertaken to assess the levels of different biochemical parameters and hormone profile in the affected and control cows.

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MATERIALS AND METHODS

Cows

Friesian × Sahiwal crossbred cows with RFM (n=32) and non RFM (n=8) maintained under uniform feeding and housing conditions at Military Dairy Farm, Bareilly, were used in the study. Cows in RFM group were selected on the basis of having retention of fetal membranes for 12 hr or longer after parturition.

Treatment protocol

Cows with RFM were assigned to 1of the 4 treatment groups at the time of inclusion in the study. Group 1 (n=11) cows were treated daily with 4 oriprim bolus administered by means of intrauterine infusion until membranes were expelled and also received 30 IU syntocinon, 15mg luprostiol plus 10mg estradiol valerate administered by i.m. injections; group 2 (n=11) cows were infused with oriprim in a manner identical to group1 and also given daily 30 ml oxytetracycline i.m., group 3 (n=6) cows treated with oriprim in a manner

identical to group 1 or 2 and fed with 20 g metrali for 5 days and group 4 (n=4) cows served as untreated controls. All cows were monitored daily until membranes were expelled. Rectal temperatures were recorded daily. Non RFM cows (n=8) were taken to compare the normal biochemical and hormonal profile.

Biochemical parameters

Blood was collected prior to treatment in both the RFM groups (n=32) and after the placental expulsion in the non-RFM groups (n=8). The serum levels of various biochemical estimates viz. cholesterol, glucose, protein, albumin, globulin, calcium, phosphorus, and enzymatic parameters like lactic dehydrogenase (LDH) alkaline phosphatase (AKP) were estimated using diagnostic reagent kits. Serum immunoglobulins (Ig) were estimated as per Mc Ewan *et al.* (1970) with some modification.

The serum samples collected from RFM and non-RFM cases were also subjected for estimation of estrogen and progesterone concentrations by RIA technique.

Clinical observations

During puerperium all treated animals were examined repeatedly per vaginum and per rectum with special reference to disturbances such as incomplete uterine involution with open or partially open cervix and disappearance of lochia. The uterus of RFM cows were examined by rectal palpation at 4-5 days interval till involution of the uterus was complete. Involution of uterus was considered as complete when size and position of uterine horns were comparable to that of non-gravid state and the difference in the diameter between the 2 horns at the bifurcation was less than 30 mm (Olson *et al.* 1986). Further the breeding records were maintained on all cows until pregnancy was confirmed by palpation per rectum or the cow was removed from the study. The fertility status in all treated groups were compared by use of selected fertility index such as days to first postpartum estrus, calving to conception interval and number of services required per conception. The data were statistically analyzed as per Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The fertility parameters in crossbred cows with RFM following different treatment regimens (Table1) indicated that interval from parturition to expulsion of fetal membranes and uterine involution period were unaffected by the treatment protocol. However, in group 1 the period of onset of first postpartum estrus, calving to conception interval and number of services per conception were significantly ($P < 0.05$) lower than that of other treated groups and control cows. Similar observations with other PGF2α analogues was reported by Stevens *et al.* (1995). A local deficit of PGF2α in the placenta of RFM cows (Heuwieser *et al.* 1993) and its uterotonic effect with other ecbolic drugs are the reasons, which lend support to this therapy in RFM cows. Though the role of PGF2α in uterine involution is not well understood. There is a massive postpartum release of PGF2α, which is related to the degree, and rate of uterine involution and it can be accelerated by exogenous PGF2α injections (Kindahl *et al.* 1984, Madej *et al.* 1984).

The primary detrimental effect of RFM is that it is a predisposing factor in the development of acute or chronic postpartum metritis (Sandals *et al.* 1979, deBois 1982). The present results (Table 2) are in agreement with these reports. Moreover, the disease RFM is innocuous but there is a very close association between RFM and development of metritis by which the condition affects fertility as also reviewed by Laven and Peters (1996).

The serum globulin was significantly higher ($P<0.05$) in RFM cows than those in non-RFM cows (Table 3). These findings are similar to observations of Nakhashi *et al.* (1999)

Table 1. Fertility parameters in crossbred cows with or without RFM following different treatment regimens

Treatment groups	Expulsion of FM (hours)	disappearance of lochia (days)	Uterine involution period (days)	Ist postpartum estrus (days)	Calving to conception interval (days)	Services per conception	Animals which did not conceive (no.)
Gr.1	85±32.5	14.1±4.2	29.8±2.7	80.1±7.8	136.8±26.3	3.0±0.50	Nil
(n=11)	(48-120)	(11-23)	(26.35)	(36-150)	(36-171)	(1-5)	
Gr.2	85.7±12.0	14.5±2.9	30 6±3.1	93.3±10.6	198.7±44.7	3.8±1.2	2
(n=11)	(48-120)	(10-18)	(21-36)	(42-187)	(66-270)	(2-10)	(18.18%)
Gr.3	96.0±16.0	15.8±2.7	29.8±1.8	106.2±16.5	177.5±16.0	4.0±1.5	2
(n=6)	(72-120)	(12-19)	(28-32)	(41-195)	(116-255)	(2-10)	(33.3%)
Gr.4	96.0±24	17.5±0.7.5	33.5±2.5	100±15.0	195±15.5	4.5±0.5	2
(n=4)	(72-120)	(16-18)	(30-36)	(95-115)	(180-210)	(4-5)	(50.0%)
Non-RFM (nontreated, n=8)	8.67±3.33 (3-12)	-	-	62.1±18.0 (48-72)	122.8±16.8 (72-135)	2.5±0.50 (1-3)	Nil

Values are given as mean± SD; figures in parenthesis indicate range; values in a column with different superscripts are significantly ($P<0.05$) different.

Table 2. Postpartum disease development in RFM crossbred cows

Treatment group	No.	Disease/condition			Infertile
		Fever	metritis	mastitis	
Gr 1	11	6	11	Nil	Nil
Gr 2	11	5	11	1	1
Gr 3	6	5	6	1	1
Gr 4	4	4	4	-	1

in buffaloes with RFM. There was no significant change observed in other serum biochemical estimates in cows with RFM and normal calving. However, a significantly higher level of alkaline phosphatase (11.44 ± 0.31 vs 10.17± 0.38 KA units per 100ml, $P < 0.01$) and lactic dehydrogenase (529.90 ± 18.5 vs 401.50 ± 11.70 IU per lit, $P < 0.01$, Table 3) were observed in cows with RFM as compared to cows without RFM. This indicates an increased enzymatic activity associated with inflammatory changes or trauma to the placental tissue. These results are similar to earlier findings of Derashri *et al.* (1983) who reported higher levels of these enzymes in the buffaloes with RFM. The increased levels of enzymes in the serum may result from leakage out of necrotic and damaged cells (Woothon 1964). Immunoglobulins in serum (Table3) of RFM cows were higher ($P< 0.05$) than that of normal cows indicating some bacterial infections on the day of RFM in cows.

Table 3. Serum biochemical estimates, enzymes, immunoglobulins and hormonal levels (mean ± SD) in RFM and non-RFM crossbred cows

Parameters	RFM cows (n=32)	Non FRM cows (n=8)
Cholesterol (mg %)	122.25 ± 32.5	95.03 ± 14.3
Glucose (mg %)	43.27 ± 11.67	42.28 ± 4.66
Protein (g %)	6.45 ± 0.65	6.08 ± 0.27
Albumin (A, g %)	3.68 ± 0.32	4.27± 0.28
Globulin (G, g %)	2.77 ± 0.33 *	1.81 ± 0.27
A/G ratio	1: 1.33	1: 2.36 **
Calcium (Ca, mg %)	9.90 ± 0.80	9.62 ± 0.35
Inorganic phosphorus (p, mg %)	4.48 ± 0.56	4.69 ± 0.29
Ca/ p ratio	1: 2.20	1: 2.05
Lactic dehydrogenase (IU/ litre)	529.90 ± 18.5**	401.50 ± 11.70
Alkaline phosphatase (KA units/ 100 ml)	11.4±0.31**	10.17±0.38
Total immunoglobulin (mg/100ml)	16.54±1.18*	13.70±0.78
Progesterone (ng/ml)	3.25±0.57*	1.61±0.49
Estradiol (pg/ml)	670.0±87.5	808.0±130.7

*P<0.05, **P<0.01.

In addition there was no difference in circulatory levels of estradiol in both the groups (Table 3), but the serum progesterone levels were significantly higher (3.25± 0.57 ng/ml) in cows with RFM as compared to non-RFM (1.61± 0.49 ng/ml) cows. This observation supports findings of Leidl *et al.* (1980). However, Peter and Bosu (1987) did not find any difference in peripartum serum progesterone levels in RFM and non-RFM cows.

From this study it can be concluded that (i) the administration of ecbolic drugs in cows with RFM may hasten the resumption of the normal reproductive activity, and (ii) biochemical diagnostics will be of great value in RFM cases because this will help in the management of therapeutic and preventive measures of this condition, though further studies on estimation of AKP and LDH are needed to find its usefulness as predictive marker of RFM.

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Postnatal histological changes in epididymis of Madras Red ram lambs maintained under grazing and feed lot systems*

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ABSTRACT

The present study was made on histology in which epididymal tubules revealed columnar type of epithelium up to 4 months of age and subsequently in all the 3 regions of the epididymis pseudostratified columnar was noticed. By seventh months of age epididymal tubules of lambs under feedlot system showed sperms, whereas sperms were noticed in the tubules only by 8 month of age in lambs under grazing system. There were significant differences in the dia of the epididymal tubules between the regions of caput, corpus and cauda and also between age groups. At all ages, the dia of the epididymal tubules increased from caput, through corpus to cauda epididymis.

Key words: Epididymis, Grazing, Feedlot systems,Lambs, Postnatal development

The scientific reports on postnatal development of epididymis in ram lambs were scanty. Hence, present study was done on postnatal histological changes in epididymis of Madras Red Ram lambs maintained under grazing and feedlot systems.

MATERIALS AND METHODS

Healthy Madras Red ram lambs (26), born at Livestock Research Station, Kattupakkam, Tamil Nadu, were utilized for this study. Six ram lambs i e 2 ram lambs at birth, 1 month and 3 months of age were slaughtered and the epididymis were collected for the present study. The remaining 20 ram lambs were weaned at 3 months (90 days) of age. At the weaning, the ram lambs were equally and randomly divided into 2 groups of different feeding systems viz. grazing and feedlot systems. Ram lambs under grazing system were sent for grazing daily. Concentrate feed @ 100g/lamb from 3 to 5 months of age and 200g/lamb from 5 to 8 months of age was fed in bulk for all the ram lambs as a supplement to grazing. Under feedlot system, each ram lamb was kept in separate wooden partitioned pens. They were fed individually *ad lib.* with pellet feed comprising of 50% concentrate and 50% lucerne-meal from 3 to 8 months of age. Every month, 2 ram lambs from grazing and 2 ram lambs from feedlot systems were slaughtered from 4 to 8 months of age and epididymis were collected for the present study.

Immediately after slaughter small tissue pieces of epididymis were cut and fixed in Bouin's solution for 24 hr. Adopting the standard methods described by Luna (1968), the tissues were dehydrated in ascending grades of alcohol, cleared in xylol and embedded in paraffin. Paraffin sections of 5-6mm thickness were cut with microtome. The sections were mounted on clean slides and stained with Hematoxylin and Eosin (Humason 1972) and Periodic-Acid-Schiff reagent (Drury *et al.* 1967) for histological studies. The data were statistically analyzed as per Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

At birth the epididymal tubular epithelium appeared to be simple columnar type at caput (Fig.1), corpus and cauda (Fig.2). The cytoplasm of the cells showed vacuoles with oval or spherical nucleus located in the middle of the cell. By 3 months, the epididymal tubules are lined by columnar epithelium with elongated nucleus pushed towards the basement membrane. At 4 months the histological features of the epididymis was more or less similar to the ram lambs of the 3 months except an increase in size of the tubules. The epithelium in caput (Fig.3) and corpus was lined by pseudostratified columnar epithelium with in district cilia whereas cauda was lined by pseudostratified columnar epithelium with distinct cilia at 5 month (Fig.4). Ciliated

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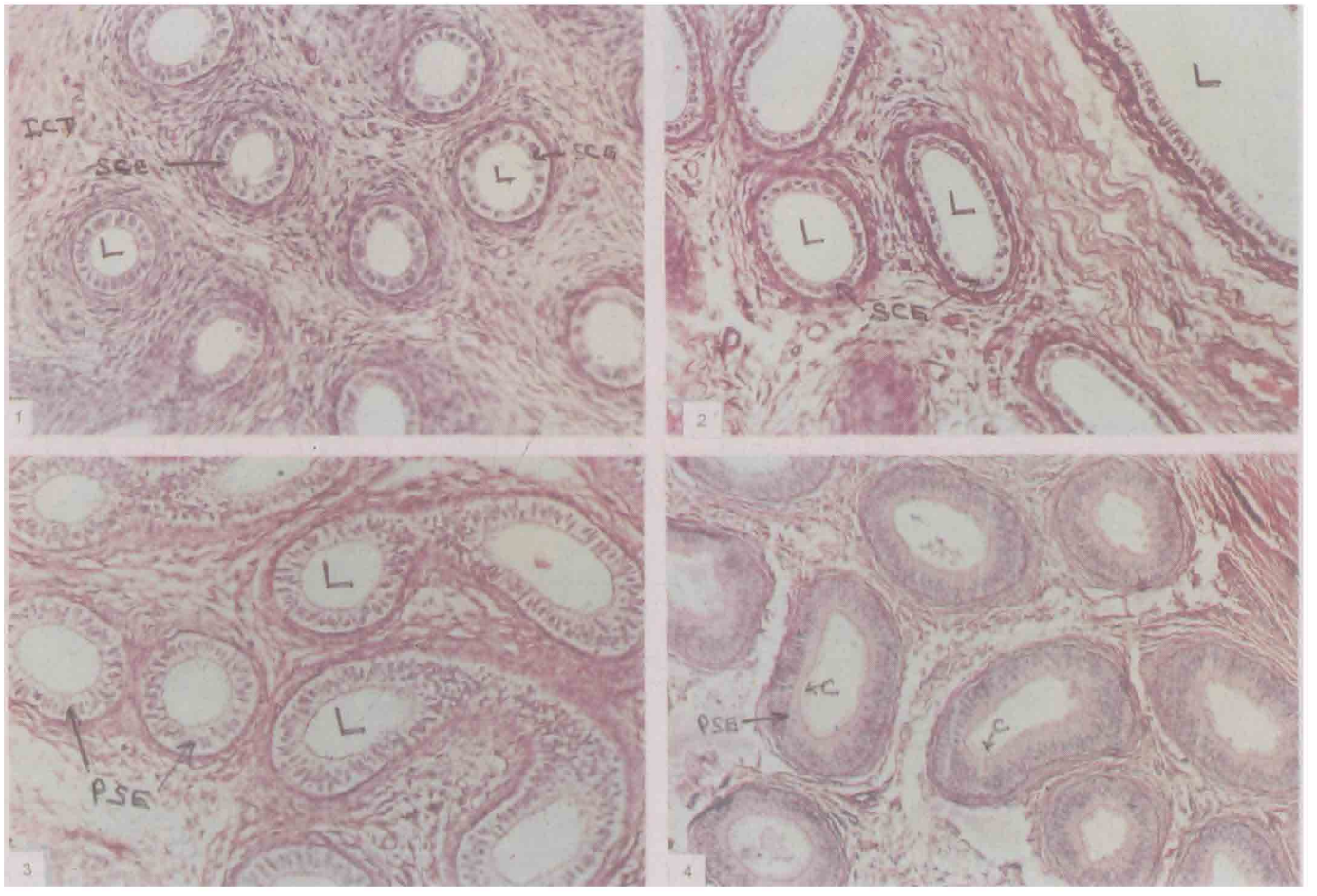


Figs 1-4. **1**. Photomicrograph of caput epididymis - at birth. Note the tubules lined by simple columnar epithelium with vacuoles (SCE) and dense inter-tubular connective tissue (ICT) (H&E 160). **2**. Photomicrograph of cauda epididymis-at birth. Note the tubules lined by simple columnar with vacuoles (SCE) and large lumen (L) than in caput. (H&E 160). **3**. Photomicrograph of caput epididymis-5 months. Tubules lined by pseudostratified columnar epithelium (PSE) (H&E 160). **4**. Photomicrograph of cauda epididymis-5 months. Tubules lined by pseudostratified epithelium (PSE) with distinct cilia (C) (H&E 160).

pseudostratified epithelium indicated that the functional status of epididymal tubules has already started at 5 month of age. The pseudostratified epithelium consisted of columnar cells and small polygonal basal cells. The histological features of the epididymal tubules at 6 month of age was same as that of the epididymal tubules at 5 month except the cilia were distinct in all the 3 regions. The epididymal tubules were empty up to 6 months. In 7 and 8 months of age also the epididymal tubules were lined by pseudostratified columnar ciliated epithelium in caput and corpus whereas in the cauda the pseudostratified epithelium contained indistinct cilia. At 7 months of age in ram lambs of feedlot system, the tubules in all the 3 regions contained a few sperms contrary to empty tubules in lambs of grazing system. This indicated that intensive management and individual feeding like feedlot system have stimulated early sperm production and functional status of reproductive organs including epididymis. At 8 month of age, the lambs under grazing system contained sparse sperms only in a few tubules (Figs 5 and 7) whereas lambs under feedlot system showed densely packed sperms in majority of tubules (Figs 6 and 8). This indicates that the ram lambs under feedlot system contrary to ram lambs under grazing reached to active sperm production stage even by 7 months of age. The histological changes noticed in the epididymis during postnatal period in Madras Red Ram lambs agree with the reports of Carmon and Green (1952) in Hampshire lambs and Ali *et al.* (1989) in Deccani rams. In the present study, PAS positive reaction was noticed in cilia, luminal border, basement membrane, intertubular connective tissue of the epididymis. This was in agreement with the observations of Sharma *et al.* (1986) in goats.

The dia of epididymal tubules increased from birth to 8 months in caput (77.26 to 330.19 μm), corpus (87.93 to 352.45 μm) and cauda (112.37 to 555.30 μm) (Table 1). There was significant difference in the dia of epididymal tubules between 3 regions (caput, corpus and cauda) and between age groups,

Table 1. Postnatal changes in the diameter (mm) of epididymal tubles of Madras Red ram lambs

Pre-weaning group (mean)

Age (months)	Caput	Corpus	Cauda
0	77.26	87.93	112.37
1	80.69	89.69	120.61
3	85.64	92.34	125.47

Post-weaning group (mean±SE)

Age (months)	Caput			Corpus			Cauda		
	Grazing	Feedlot	Average	Grazing	Feedlot	Average	Grazing	Feedlot	Average
4	103.19 ±0.20	126.63 ±5.45	114.91^{a} ±4.96	110.62 ±0.40	146.07 ±3.36	128.34^{a} ±6.49	160.34 ±0.12	273.33 ±2.62	216.83 ±20.01
5	134.72 ±2.52	157.32 ±4.84	146.20^{a} ±4.62	145.89 ±1.60	170.77 ±4.17	158.33^{b} ±4.93	277.45 ±1.80	375.78 ±1.77	326.62^{b} ±17.43
6	151.65 ±1.12	237.25 ±0.89	194.45 ±15.15	168.12 ±1.29	260.03 ±1.30	217.08^{c} ±17.33	315.00 ±3.32	410.58 ±1.62	362.79^{c} ±16.94
7	261.81 ±18.42	325.17 ±1.51	293.49^{c} ±14.52	296.28 ±8.66	361.10 ±2.49	328.69^{d} ±12.31	408.24 ±4.44	526.43 ±1.37	467.33^{d} ±21.02
8	288.60 ±19.29	371.79 ±0.86	330.19^{d} ±17.59	317.06 ±16.80	387.84 ±2.77	352.45^{d} ±15.13	501.12 ±21.87	609.49 ±4.07	555.30^{e} ±22.15
Mean	187.99^{a} ±17.26	243.70^{b} ±21.06	-	207.59$_{a}$ ±18.99	266.36^{b} ±21.80	-	332.43^{a} ±26.30	439.12$_{b}$ ±26.28	-

P<0.01 Means bearing different superscripts differ significantly.

Table 2. Postnatal changes in the epithelial hight (μm) of epididymal tubules of Madras Red ram lambs

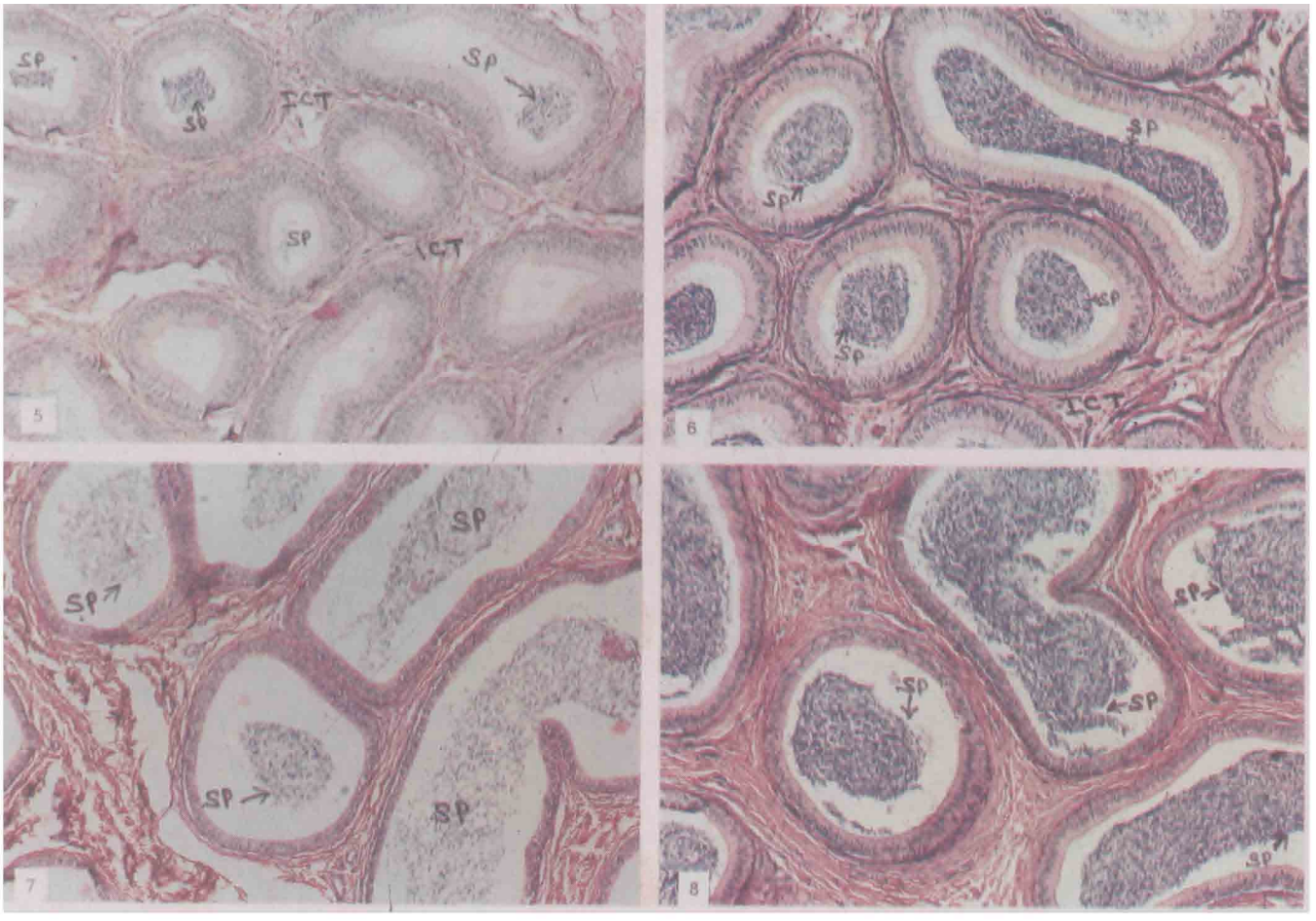
Pre-weaning group (mean)

Age (months)	Caput	Corpus	Cauda
0	6.20	7.10	8.67
1	7.16	8.72	10.65
3	10.31	12.08	17.12

Post-weaning group (mean±SE)

Age (months)	Caput			Corpus			Cauda		
	Grazing	Feedlot	Average	Grazing	Feedlot	Average	Grazing	Feedlot	Average
4	11.47 ±0.28	17.38 ±0.27	14.43^{a} ±1.06	15.14 ±0.25	21.65 ±0.60	18.40^{a} ±1.20	20.66 ±0.21	45.15 ±0.13	32.91^{a} ±4.33
5	19.24 ±0.56	23.64 ±0.41	21.44^{b} ±0.85	22.60 ±0.39	26.18 ±0.68	24.39^{b} ±0.75	48.43 ±0.76	59.07 ±0.24	53.76^{b} ±1.93
6	21.61 ±0.37	36.40 ±0.26	29.00^{c} ±2.62	26.14 ±0.23	40.70 ±0.27	33.42^{c} ±2.58	51.75 ±0.12	54.38 ±0.38	53.07^{b} ±0.51
7	40.54 ±2.07	55.46 ±0.23	48.00^{d} ±2.89	41.19 ±0.64	45.33 ±0.19	43.26^{d} ±0.80	38.43 ±5.15	22.47 ±0.13	30.45^{a} ±3.82
8	49.55 ±1.29	58.13 ±0.51	53.84^{e} ±1.67	47.14 ±1.10	52.38 ±0.25	49.76^{e} ±1.08	26.61 ±0.73	20.49 ±0.17	23.55^{a} ±1.15
Mean	28.48^{a} ±4.55	38.20^{b} ±5.12		30.44^{a} ±2.70	37.25^{b} ±2.60		37.18 ±2.89	40.32 ±3.59	

P<0.01 Means bearing different superscripts differ significantly.



Figs 5-8. **5.** Photomicrograph of caput epididymis-8 months. Grazing system. Note the sparsely distributed sperms (SP) in few tubules and less amount of inter-tubular connective tissue (ICT) (H&E 63). **6.** Photomicrograph of caput epididymis-8 months-Feedlot system. Note the densely packed sperms (SP) in majority of tubules (H&E 63). **7.** Photomicrograph of cauda epididymis-8 months - Grazing system. Tubules with sperms (SP) (H&E 63). **8**. Photomicrograph of cauda epididymis-8 months - Feedlot system. Note the densely packed sperms (SP) in tubules (H&E 63).

but there was no significant difference between the left and right epididymis. At all ages the dia of the epididymal tubules increased from caput through corpus to cauda epididymis. This observation was in agreement with the observations of Raja and Rao (1983) in exotic bulls. John (1985) observed differential growth rate of epididymal tubule dia in buffaloes.

In the present investigation, the epithelial height of the epididymal tubule was recorded at birth as 6.20 and 7.10 µm in caput and corpus regions which increased to 53.84 and 49.76 µm at 8 months of age respectively (Table 2). The epithelial height of the epididymal tubules at cauda region was 8.67 µm at birth, which increased to 53.07 µm at 6 months of age then declined at 23.55 µm at 8 months of age. There was significant difference in the epithelial height of epididymides between ages. The epithelial height was observed to be the highest in cauda and lowest in caput up to 6 months of age, then the epithelial height showed reverse pattern i e the epithelial height was highest in caput and lowest in cauda. Similar variation in the epithelial height in different ages of the epididymis was reported in Holstein bulls (Macmillan and Halfs 1969) in Murrah buffalo bulls (John 1985) and in goats (Gupta and Singh 1988).

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Effect of bovine somatotropin on blood serum minerals, thyroid hormones and reproductive performance of lactating crossbred cows

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ABSTRACT

Apparently healthy multiparous lactating crossbred cows (24), on the basis of milk production and parity were randomly allocated to 4 treatment groups. These were control and 3 dose rates of 250 mg, 350 mg and 500 mg sometribove zinc suspension in a sustained release preparation. Cows in control group received placebo injections of normal saline. Treatments were initiated at 55 to 92 days postpartum and continued for 140 days. The injections were given subcutaneously on left or right side, at every 14 days for 10 injection cycles. Blood samples were collected on second day of sometribove injection. Blood samples were collected from all the cows in separate test-tubes, via jugular venipuncture during each injection cycle. Administration of bovine somatotropin did not affect the blood serum calcium, phosphorus, magnesium, sodium, potassium and chloride concentrations and remained within the normal range expected in lactating dairy cows. The serum triiodothyronine and thyroxine concentrations did not differ and remained almost similar in treated and control cows. The percentages of cows remaining pregnant and calving rate were almost comparable in bovine somatotropin treated and control cows. Services per conception were lower in 250 and 500 mg groups when compared with controls, suggesting that services per conception were not affected due to bST treatment. Bovine somatotropin treatment had no discernible effect on gestation length.

Key words: Bovine somatotropin, Blood serum minerals, Lactating cows,Reproductive performance, Somatotropin, Thyroid hormones

Administration of bST to lactating ruminants elicits a number of changes in nutrient partitioning and utilization, the end result is an improved supply of metabolites to the mammary gland for milk synthesis (McDowell 1991). Among the blood key metabolites, the role of minerals in nutrient partitioning is not adequately investigated. Further, the effects of bST on thyroid status of lactating cows are not clear because very few studies have been conducted on this aspect of research. The paper presents the possible effects on reproductive performance, thyroid hormone status and concentration of minerals in blood of crossbred cows treated with recombinant bovine somatotropin.

MATERIALS AND METHODS

Apparently healthy multiparous lactating crossbred cows (24) in the second to sixth lactation (Gir × Holstein Friesian and Gir × Jersey with 50 to 75% exotic inheritance) from Cow Unit Scheme, Unit No. 5, Aarey Milk Colony, Goregaon, Mumbai, were used for the present study. All the animals were maintained under identical managemental conditions throughout the study. The feeding practices were followed as per the standard norms. Based on milk production and parity, the experimental animals were randomly allocated to 4 treatment groups, viz. control, and 3 dose rates of 250 mg, 350 mg and 500 mg sometribove zinc suspension in a sustained release preparation. The cows in the control group received placebo injections of normal saline, equivalent to the 500 mg bovine somatotropin treatment. Sometribove is a sterile prolonged release injectable formulation of a recombinant DNA derived bovine somatotropin analogue in single dose syringes, each containing 500 mg of sometribove zinc suspension. The injections were administered subcutaneously at every 14 days, in the area between the pins and the tailhead (ischiorectal fossa), alternately on left or right side, between 9: 00 AM and 10: 00 AM of treatment days. Doses lower than 500 mg were given by fitting plastic sleeves on the syringe's barrel (250 mg = white, 350 mg = red). This limited the distance the plunger of the syringe could travel and thus a fixed amount of sometribove could be administered.

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In this experiment therefore, one syringe was used to inject 2 animals in the 250 mg group, whereas, 150 mg sometribove remaining after injection of 350 mg had to be discarded. The experimental treatments commenced at 55 to 92 days postpartum and continued for 140 days i. e. for 10 injection cycles of 14 days each. Before the commencement of the experiment, the milk yield of the experimental animals were recorded for 7 consecutive days, which averaged 7.22 ± 0.46/ animal a day.

Blood samples (15 to 20 ml) were collected from all the cows in separate test-tubes, via jugular venipuncture on the second day of each somatotropin injection, during each injection cycle. They were allowed to clot and clear serum separated by centrifugation. The serum samples after addition of preservative (0.1 ml of 1: 1250 solution of merthiolate to each 4 ml of blood serum) were stored at -20°C until used for biochemical analysis.

The estimation of calcium, phosphorus, magnesium and chloride were carried out on the auto analyzer by using the biochemistry kits. The serum sodium and potassium were estimated by flame photometry (Wootton 1964). The serum concentrations of triiodothyronine (T3) and thyroxine (T4) were measured by using radio immunoassay kits supplied by the Bhabha Atomic Research Center, Mumbai. The indices of reproductive performance, viz. services per conception, pregnancy rate and calving rate were recorded (Jainudeen and Hafez 1993) for 6 months from the commencement of experiment i.e. 5th May 1995 to 31st October 1995. Gestation length was recorded.

Analysis of variance of data was done according to Snedecor and Cochran (1967) by using randomized block design. Differences in means were tested using critical difference (CD) test.

RESULTS AND DISCUSSION

The effects of bovine somatotropin on concentrations of blood serum minerals and thyroid hormones are presented in Table 1, and effects on reproductive performance in Table 2. Administration of bovine somatotropin did not affect the serum concentrations of calcium, phosphorous, magnesium, sodium, potassium and chloride, and remained within the range expected in lactating dairy cows. These observations are in agreement with the findings of McGuffy *et al.* (1990) and Singh *et al.* (1994). Bauman and Collier (1985) speculated that animals probably adapt absorption of minerals from the alimentary tract or rate of accretion and mobilization from reserves. Regardless, it is evident that the changes in mineral partitioning are well coordinated. However, apparently the serum phosphorous concentrations were on the higher side of range in bST-treated cows. Whitaker *et al.* (1989) also reported higher phosphate concentrations in bST-treated cows and heifers, although these increases were all within the normal ranges, but they were consistent and the treated heifers had higher concentrations than the cows.

Administration of bovine somatotropin did not significantly affect the serum triiodothyronine concentrations, which remained almost similar in treated and control cows. The response in serum triiodothyronine is in accordance with Cisse *et al.* (1991). Administration of bovine somatotropin did not alter the serum thyroxine concentrations in treated and control cows. This response in serum thyroxine is consistent with report of West *et al.* (1990). Cows exposed to heat stress had reduced serum T_3 and T_4 (Magdub *et. al.* 1982). Mohammed and Johnson (1985) reported lower T3 and a slight reduction in T_4 when cows went from thermoneutral to heat stress conditions, but administration of pituitary derived bST (pbST) during heat stress resulted in no further alteration of the T_3 and T_4 contents. Eisemann *et al.* (1986) found no change in plasma T_3 and T_4 when pbST was administered to Hereford heifers. Oldenbrock *et al.* (1993), however, reported increased thyroxine concentrations due to bST treatment. They used Jersey, Dutch Red and White and Friesian cows, which were subcutaneously injected with 640 mg of recombinant bST at 28 days interval for 4 consecutive lactations.

The pregnancy rate was 4 of 6 cows (66.66%) in control and 350 mg groups, and 5 of 6 cows (83.33%) in 250 mg and 500 mg groups. The percentage of cows becoming pregnant was almost comparable in control and bovine somatotropin treated cows. Hemken *et al.* (1991) reported 88% pregnant cows in control compared with 62% receiving bST. Similarly,

Table 1.Effect of bovine somatotropin on concentration of serum minerals and thyroid hormones in experimental animals

Parameter	Control	Bovine somatotropin treatment		
		250 mg	350 mg	500 mg
Calcium (mg/dl)	10.16 ± 1.23	8.96 ± 1.93	9.51 ± 0.70	9.23 ± 0.65
Phosphorus (mg/dl)	6.98± 0.55	7.07 ± 0.38	7.37 ± 0.28	7.45 ± 0.30
Magnesium (mg/dl)	3.02 ± 0.28	3.04 ± 0.32	2.91 ± 0.32	2.99 ± 0.32
Sodium (mEq/L)	124.18± 2.45	123.88± 2.37	122.90± 2.19	118.97± 2.00
Potassium (mEq/L)	4.73 ± 0.13	4.64 ± 0.09	4.79± 0.14	4.70 ± 0.22
Chloride (mEq/L)	99.94 ± 0.75	99.52± 1.31	98.21± 2.06	96.66± 0.22
Triiodothyronine (ng/ml)	0.86 ± 0.15	1.23± 0.07	0.91 ± 0.05	0.72 ± 0.06
Thyroxine (ng/ml)	32.99 ± 2.42	33.79± 1.30	35.71± 1.47	32.79± 2.71

Table 2. Effect of bovine somatotropin on reproductive performance of experimental animals

Parameter	Control	Bovine somatotropin treatment		
		250 mg	350 mg	500 mg
Number of cows	6	6	6	6
Pregnancy rate	4/6 (66.66%)	5/6 (83.33%)	4/6 (66.66%)	5/6(83.33%)
Calving rate	4/6 (66.66%)	5/6 (83.33%)	4/6 (66.66%)	4/6(66.66%)
Services per conception	3.25	1.20	4.25	1.40
Gestation length (days)	272	280	279	281

Figures in parenthesis indicate percentage.

Cole *et al.* (1992) in cows administered high doses of bST during 2 consecutive lactations reported that bST- treated animals had reduced pregnancy rates in year 1, but not in year 2 of the treatment. Chalupa *et al.* (1987) also reported lower pregnancy rate in bST-treated cows. Morbeck *et al.* (1991) reported that conception rate at first and second lactations and overall was not affected among bST-treated cows.

Calving rate was 66.66% in control, 350 mg and 500 mg groups, and 83.33% in 250 mg group. The calving percentage was almost comparable in bST-treated and control cows. Services per conception were 3.25, 1.20, 4.25 and 1.40 for the control, 250 mg, 350 mg 500 mg groups respectively. Services per conception were lower in 250 and 500 mg groups when compared with control. In 350 mg group, however, the services per conception were higher than that in control. These findings showed that bST treatment did not affect services per conception. Our observations are in agreement with Morbeck *et al.* (1991). Eppard *et al.* (1987) reported that cows receiving somatotropin averaged 96% conception rate and 2 services per conception, which were comparable to the non treated controls. The overall gestation length averaged 278 days with 272 days in control, 280 days in 250 mg, 279 days in 350 mg and 281 days in 500 mg groups. Bovine somatotropin treatment had no discernible effect on gestation length. The observation is in accord with Eppard *et al.* (1987). In conclusion, administration of bovine somatotropin did not affect the blood metabolites studied. The percentages of cows remaining pregnant, calving rate and services per conception were not affected due to bST treatment. Further, the treatment had no discernible effect on gestation length.

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Effect of exogenous bovine somatotropin on the blood serum protein profile in lactating crossbred cows*

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ABSTRACT

Apparently healthy multiparous crossbred cows (24), based on milk production and parity were randomly allocated to 4 treatment groups to study the effect of exogenous bovine somatotropin on the serum protein profile in lactating crossbred cows. The treatments were control and 3 dose rates of 250, 350 and 500 mg sometribove zinc suspension in a sustained release preparation. Sometribove is a sterile prolonged release injectable formulation of a recombinant DNA derived bovine somatotropin. Cows in the control group received placebo injections of normal saline. Treatments were initiated at 55 to 92 days postpartum and continued for 140 days. The injections were administered subcutaneously in the area between the pins and tail head (ischiorectal fossa), alternately on the left/right side, at every 14 days interval, for 10 injection cycles. The blood samples were collected on the second day of sometribove injection.

Administration of bovine somatotropin did not affect the serum total proteins, albumin and blood urea nitrogen concentrations, which remained almost similar in treated and control groups. The serum creatinine and total immunoglobulins concentrations were not affected except in the group treated with 250 mg sometribove for creatinine, and in the group treated with 350 mg sometribove for total immunoglobulins, which in these groups were higher. The serum globulin concentration was not affected except in the group treated with 250 mg sometribove which in this group was lower.

Key words: Blood serum protein profile, Exogenous bovine somatotropin, Lactating cows, Somatotropin

The effect of bovine somatotropin (bST) in lactating cows is currently receiving considerable research interest, as its administration causes a substantial increase in the milk production. Industrial production of bovine somatotropin made by the recombinant DNA technology has made possible the manipulation of bovine lactation physiology. It seems that bST produces an increase in IGF - 1 and that this agent increases the milk synthesis. Administration of bST to lactating ruminants elicits a number of changes in the nutrient partitioning and utilization, the end result is an improved supply of metabolites to the mammary gland for milk synthesis (McDowell 1991). Somatotropins are not lipophilic and cannot be stored in the adipose tissue. Any exogenously administered somatotropin can be expected to be degraded by the lysosomal enzymes in blood or at the target organs and degradation products such as amino acids recycled to make new proteins in the same way as any other endogenous protein (Peel and Bauman 1987). The information on the effects of bovine somatotropin on the concentration of blood metabolites either in indigenous or in crossbred cows is not available. Further, it is important to determine the effects of exogenous bovine somatotropin on bovine immune responses. In this study, the effects of bovine somatotropin on serum proteins, NPN metabolites and on the circulating concentrations of total immunoglobulins is investigated, on which no studies have been reported from India.

MATERIALS AND METHODS

Apparently healthy multiparous lactating crossbred cows (24) in the second to sixth lactation (Gir× Holstein Friesian and Gir × Jersey with 50 to 75% exotic inheritance) from Cow Unit Scheme, Unit No. 5, Aarey Milk Colony, Goregaon, Mumbai, were used for the present study. All the animals were maintained under identical managemental conditions throughout the study. The feeding practices were followed as per the standard norms. Based on milk production and parity, the experimental animals were randomly allocated to 4 treatment groups, viz. control, and 3 dose rates of 250 mg. 350 mg and 500 mg sometribove zinc suspension in a sustained release preparation. The cows in the control group received placebo injections of normal saline, equivalent to

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the 500 mg bovine somatotropin treatment. Sometribove is a sterile prolonged release injectable formulation of a recombinant DNA derived bovine somatotropin analogue in single dose syringes, each containing 500 mg of sometribove zinc suspension. The injections were administered subcutaneously at every 14 days, in the area between the pins and the tailhead (ischiorectal fossa), alternately on left or right side, between 9:00 AM and 10:00 AM of treatment days. Doses lower than 500 mg were given by fitting plastic sleeves on the syringe's barrel (250 mg = white, 350 mg = red). This limited the distance the plunger of the syringe could travel and thus a fixed amount of sometribove could be administered. In this experiment therefore, one syringe was used to inject 2 animals in the 250 mg group, whereas, 150 mg sometribove remaining after injection of 350 mg had to be discarded. The experimental treatments commenced at 55 to 92 days postpartum and continued for 140 days i. e. for 10 injection cycles of 14 days each. Before the commencement of the experiment, the milk yield of the experimental animals was recorded for 7 consecutive days, which averaged 7.22 ± 0.46 / animal a day.

Blood samples (15 to 20 ml) were collected from all the cows in separate test-tubes, via jugular venipuncture on the second day of each somatotropin injection, during each injection cycle. They were allowed to clot and clear serum separated by centrifugation. The serum samples after addition of preservative (0.1 ml of 1:1250 solution of merthiolate to each 4 ml of blood serum) were stored at -20°C until used for biochemical analysis.

The biochemical estimations of blood serum total proteins, albumin, blood urea nitrogen and creatinine were carried out on the auto analyzer by using the biochemistry kits. The globulin values were procured by subtracting albumin values from the total protein values. For albumin: globulin ratio, the albumin values were divided by globulin values. Estimation of serum total immunoglobulins was done by zinc sulphate turbidity test (McEwan *et al.* 1970).

The analyses of variance of the data were done according to Snedecor and Cochran (1994) by using randomized block design. Differences in means were tested using critical difference (CD) test.

RESULTS AND DISCUSSION

The results of the effects of bovine somatotropin on total proteins, albumin, globulin, albumin: globulin ratio, blood urea nitrogen, creatinine and total immunoglobulin are presented in Table 1.

Table 1. Effect of bovine somatotropin on protein parameters in experimental animals

Parameter	Control	Bovine somatotropin treatment		
		250 mg	350 mg	500 mg
Total proteins (g/dl)	7.98^{ab}	7.73^{b}	8.17^{a}	8.06^{a}
Albumin (g/dl)	3.45	3.64	3.68	3.59
Globulin (g/dl)	4.36^{a}	4.12^{b}	4.43^{a}	4.47^{a}
Albumin: globulin ratio	0.74^{a}	0.89^{b}	0.84^{b}	0.81^{ab}
Blood urea nitrogen (mg/dl)	35.93	38.66	37.32	36.65
Creatinine (mg/dl)	1.33^{a}	1.42^{b}	1.26^{a}	1.32^{a}
Total immunoglobulins (mg/ml)	40.61^{a}	41.07^{a}	47.34^{b}	43.05^{ab}

Means with different superscripts in the rows differ (P<0.05).

Total proteins

Administration of bovine somatotropin did not change the serum total protein concentrations. This observation is in agreement with the findings of Gallo and Block (1990) and Windisch and Kirchgessner (1992). Among the bovine somatotropin treated groups, the serum total protein concentrations were significantly lower in the 250 mg group over the 350 mg (P<0.01) and 500 mg (P<0.05) groups. The concentrations, however, did not differ in 350 mg and 500 mg groups.

Albumin

Administration of bovine somatotropin did not alter the serum albumin concentrations compared to control cows. This response in serum albumin is consistent with reports of Whitaker *et al.* (1989). Contrary to this observation, Gallo and Block (1990) and McGuffey *et al.* (1990) reported a decrease in the serum albumin concentrations in lactating Holstein cows.

Globulin

Administration of bovine somatotropin did not change the serum globulin concentrations in 350 mg and 500 mg groups over the controls. The concentration, however, was significantly lower in the 250 mg than that in control. Whitaker *et al.* (1989) reported that the serum globulin concentration was not affected by 500 mg recombinant bovine somatotropin treatment in dairy cows.

Albumin: globulin ratio

Administration of bovine somatotropin increased the albumin: globulin ratio in the 250 mg and 350 mg groups over the controls. The difference in the albumin: globulin ratio of 500 mg and control groups did not approach statistical significance. McGuffey *et al.* (1990) recorded reduced serum albumin: globulin ratio in high producing Holstein cows treated with recombinant bovine somatotropin, which were fed low CP rations.

Blood urea nitrogen

Administration of bovine somatotropin did not affect the blood urea nitrogen concentrations. McDowell *et al.* (1987) reported that in cows at peak lactation, treatment with pituitary derived growth hormone did not affect the plasma concen-

trations of urea, but in the same cows later in lactation, plasma concentrations of urea decreased in response to pituitary derived growth hormone. Irreversible losses of urea in these cows increased at peak lactation and tended to decrease during mid lactation. They opined that these changes in plasma concentrations and irreversible losses of urea might reflect the use of amino acids as glucogenic substrates at peak lactation when the cows were in substantial energy deficit and increased protein synthesis in mid lactation when the cows were in positive energy balance. Annexstad *et al.* (1990) reported higher levels of blood urea nitrogen concentration in lactating Holstein cows treated with bovine somatotropin.

Creatinine

Administration of bovine somatotropin did not alter the serum creatinine concentration in 350 mg and 500 mg groups over the controls. The concentration, however, was significantly higher ($P<0.01$) in 250 mg than that in the control group. Among the bovine somatotropin treated groups, the serum creatinine concentration was significantly higher ($P<0.01$) in 250 mg over 350 mg and 500 mg groups. The concentration, however, did not differ in 350 mg and 500 mg bovine somatotropin treated groups. Eisemann *et al.* (1989) in their study on growing beef steers reported that plasma concentrations of creatinine were unaltered due to bovine somatotropin treatment.

Total immunoglobulins

Administration of bovine somatotropin did not change the serum total immunoglobulin concentration in 250 mg and 500 mg groups over the controls. The concentration, however, was significantly higher ($P<0.05$) in 350 mg than that in the control group. Among the bovine somatotropin treated groups, the serum immunoglobulin concentration was significantly higher ($P<0.05$) in 350 mg over 250 mg group. The concentration, however, did not differ in 350 mg and 500 mg and 250 mg and 500 mg groups. Burton *et al.* (1991) in their study on purebred Holsteins concluded that bST injected at doses that effectively increased milk yield had no apparent detrimental effects on the bovine humoral immunity as measured by the blood immunoglobulin profiles throughout lactation.

In conclusion, administration of bovine somatotropin did not affect the serum total proteins, albumin and blood urea nitrogen concentrations. The serum creatinine and total immunoglobulins concentrations were not affected, except in the group treated with 250 mg bST for creatinine and in the group treated with 350 mg bST for total immunoglobulins, which in these groups were higher. The administration of bovine somatotropin lowered the serum globulin in the group treated with 250 mg bST over the controls.

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Effect of alliums on total cholesterol content of liver and muscle of Japanese quail (*Coturnix coturnix japonica*)

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ABSTRACT

Effect of onion, garlic, age and sex on total cholesterol in liver and muscle of quails was studied. No significant effect of onion or garlic on the total cholesterol content of liver and muscle was observed. This effect was not significant even when the birds were fed for 3 and 6 weeks duration. However, the results on the basis of pooled data obtained as per statistical analysis revealed a significant decrease in the total cholesterol content in liver and muscle. The total cholesterol contents in the liver and muscle in both sexes at 3 physiological stages of growth, viz. 3-week old (immature), 6-week old (onset of sexual maturity) and 9-week old (mature) birds were determined. Inconsistent results were obtained when observed between age within a sex or between sex within an age group.

Key words: Alliums, Cholesterol, Liver, Muscle, Quail

Hypercholesterolemia, the cause for coronary heart diseases (CHD), is difficult and costly to be controlled. The most practical and least expensive way in overcoming CHD is resorting to non-pharmacological procedures (Gyarfas 1992). Onion and garlic are dietary hypolipidemic agents (Augusti 1990). Consumption of poultry products which are comparatively rich in cholesterol is increasing. Further information on variations in the total cholesterol content in the tissues because of age and/or sex have not been established for the Japanese quail (*Coturnix coturnix japonica*). In view of the significance of onset of CHD and the tendency of change in the food habits towards an increase in consumption of poultry products, the present study on the effect of onion, garlic, age and sex on tissue cholesterol in Japanese quail was made.

MATERIALS AND METHODS

Healthy, 3-week old Japanese quails (310) were selected from the Kerala Agricultural University Poultry farm, for the study.

On the first day of the experiment, birds (15 females and 15 males) which formed the control group were slaughtered. Liver and breast muscle samples were collected from these birds for assay of tissue total cholesterol. The remaining 3-week old birds (180) were distributed equally under 3 groups (T_0, T_1 and T_2) with equal numbers of either sex and as uniformly as possible with respect to body weight. T_0 was the treatment control group on basal diet. T_1 group was fed on the onion supplemented diet and T_2 group on the garlic supplemented diet. All the birds were maintained under standard farm conditions. All the 3 groups of birds were reared on grower mash for 3 weeks and on layer mash thereafter till the end of the experiment (6 weeks). Mixing the feed with mashed onion or garlic, each @ 1% of the total feed was carried out daily before feeding. The feed and water were provided *ad lib*. The birds were maintained on 18 hr photoperiod for the first 3 weeks of the experiment and 12 hr photoperiod thereafter.

Chicks (15 males and 15 females) were slaughtered at the end of 3 weeks from each of the 3 groups and the remaining at the end of the experiment (6 weeks) for collection of liver and breast muscle. All the tissue samples collected were labelled and stored at -70°C until analysed. Lipid from known weight of all the tissues was extracted by the procedure of Bligh and Dyer (1959) and stored in labelled vials at -70°C for the assay of total cholesterol in liver and muscle. The total cholesterol in the aliquot of the lipid extracts of liver and muscle was determined (Searcy and Berquist 1960). With this the number of liver and muscle samples analysed for total cholesterol content under each of the 3 dietary treatments were 30 (15 males and 15 females) each at sixth and ninth week of age. The results obtained were analysed statistically by following the analysis of variance method (Snedecor and

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Cochran 1973).

RESULTS AND DISCUSSION

The result of the total cholesterol content in the liver and muscle analysed for interaction effect due to dietary treatments, age and sex is shown in Table 1.

The influence of dietary onion or garlic on total cholesterol in liver and muscle was not significant in male and female birds of both age groups. Labate and Dam (1980) observed no decrease in the liver cholesterol when the Japanese quails were fed 7an inhibitor of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) enzyme. Keshavarz (1976) reported that the liver cholesterol of WLH birds did not change due to dietary supplementation of curcumin, a compound that increases excretion of cholesterol. This compound in rats decreased the liver cholesterol (Rao *et al.* 1970). Qureshi *et al.* (1983a) and Qureshi *et.al.* (1983b) observed that feeding of ginseng and solvent extracts of garlic, respectively, to WLH birds decreased liver HMG-CoA enzyme. Likewise, lovastatin and colestipol, which are potent inhibitors of HMG-CoA had no influence on the liver cholesterol of WLH and NH cross birds respectively (Luhman *et al.* 1990). Sklan *et al.* (1992) reported a decrease in the liver cholesterol in birds consequent to garlic feeding but without any effect on onion feeding, Sebastian *et al.* (1979) and Zacharias *et al.* (1980) also observed no change in liver cholesterol content because of onion and garlic feeding in rabbits. Further, lovastatin and colestipol had no influence on muscle cholesterol of WLH birds and liver cholesterol of WLH and NH cross birds respectively (Luhman *et al.* 1990). Ajuyah *et al.* (1991) also reported no change in the muscle cholesterol level when Hubbard birds were fed on linolenic acid rich canola or flax seeds.

Although, the influence of dietary onion or garlic on total cholesterol in liver and muscle was not significant in the male and female birds of both age groups, the liver and muscle cholesterol of the pooled T_1 and T_2-groups was lower than that of the pooled T_0-group (the data obtained for the pooled group is as per the statistical pooling, hence the same is referred to as pooled data in the Table). The liver and the muscle cholesterol of the pooled T_1 and T_2-groups was similar. This indicates that there could be a decrease in the synthesis of cholesterol in the liver and/or muscle of pooled T_1 and T_2-groups due to decrease in the activity of HMG-CoA enzyme because of feeding on onion and garlic.

In the control group (group that had 3-week old birds, 6-week old birds of T_0-group and 9-week old birds of T_0-group of both sexes), there was an increased liver cholesterol level in both male and female birds as age advanced, which could be because of stimulatory effect of sex steroids (Leclercq 1984). However, the liver cholesterol was similar in the 6 and 9-week old T_0-female birds, which probably could be because of decreased oestrogen released by the ovary, once the laying has started (Noble and Cocchi 1990) or it could be due to similar rate of incorporation of acetate into nonsaponifiable lipids in the liver (Furuse *et al.* 1991).

In the control group, the liver cholesterol in both sexes of 3-week old birds was similar indicating negligible influence of gonadal hormones on cholesterologenesis. The liver cholesterol in the 6 and 9-week old female birds was higher than that in the male birds of similar age. Oestrogen timulates increased incorporation of acetate and glucose into nonsaponifiable lipids in the liver (Sugano and Wada 1967), hence, an increase in liver cholesterol in the female birds.

In the control group, there was an increased muscle cholesterol level in both male and female birds as age advanced, The turnover, of plasma cholesterol reduced in thyroidectomised WLH birds (Sugano and Wada 1967). Leenstra *et al.* (1991) observed an increase in concentration of plasma thyroxine in female broiler chicken than that in the male birds. The plasma thyroxine increased with age in both the sexes. The source for the muscle cholesterol is either plasma cholesterol or that which is synthesised in the muscle. Hence, an increase in the muscle cholesterol level with age in both sex could be due to influence of circulating thyroxine which increases with age (Leenstra *et al.* 1991).

Table 1. Total cholesterol content (mg%) of liver and muscles in Japanese quail

Tissue	Treatment	Female birds			Male birds			Pooled data[4]
		3-week old	6-week old	9-week old	3-week old	6-week old	9-week old	
Liver	T0	625.50±11.94^{A1}	1213.75±67.23^{B2}	1315.83±52.95^{AB1}	458.40±9.27^{A1}	745.24±10.10^{B1}	1023.00±82.55^{C1}	1074.26±92.96^{b}
	T1	-	962.17±72.93	1086.76±53.08	-	531.11±11.48	808.58±81.28	847.15±98.28^{a}
	T2	-	882.31±65.84	1036.34±56.58	-	475.86±11.28	756.52±86.57	787.61±101.28^{a}
Muscle	TO	123.70±7.48^{A2*}	179.54±5.87^{B2}	229.42±7.32^{C1}	91.52±5.12^{A1*}	150.45±6.33^{B1}	227.25±8.61^{C1}	196.67±7.33^{b}
	T1	-	176.49±5.77	215.18±8.91	-	125.17±6.39	210.94±9.19	181.94±8.01^{a}
	T2	-	165.48±5.87	212.49±7.79	-	115.97±6.41	218.65±7.99	176.15±6.01^{a}

[1]Superscript bearing different capital letter within a row and sex differs significantly; [2]superscript bearing different number within a row between similar age of different sex differs significantly; [3]superscript bearing different small letter within a column differs significantly; [4]all values are significantly different at $P \leq 0.01$, except '*' indicating significantly different at $P \leq 0.05$;S: Refers to mean value of treatment irrespective of age and sex.

In the control group, comparison of the results of total cholesterol in muscle between sex of similar age revealed an increase in the muscle cholesterol content in the 3 and 6-week old female birds compared to 3 and 6-week old male birds which could be attributed to level of thyroxine as suggested by Leenstra *et al.* (1991). Further, the muscle cholesterol in the 9-week old female was not higher than that in the 9-week old male birds which could be due to reduced plasma cholesterol in the female birds as much of the plasma cholesterol is diverted towards yolk formation in the laying birds as the first egg laid in the present study was on 43rd day of age.

In conclusion, no significant effect of onion or garlic on either liver or muscle cholesterol was found. Likewise, the results of the total cholesterol in liver or muscle were found to be inconsistent when observed between age within a sex or between sex within an age group.

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Isolation of *Ehrlichia canis* in cell culture

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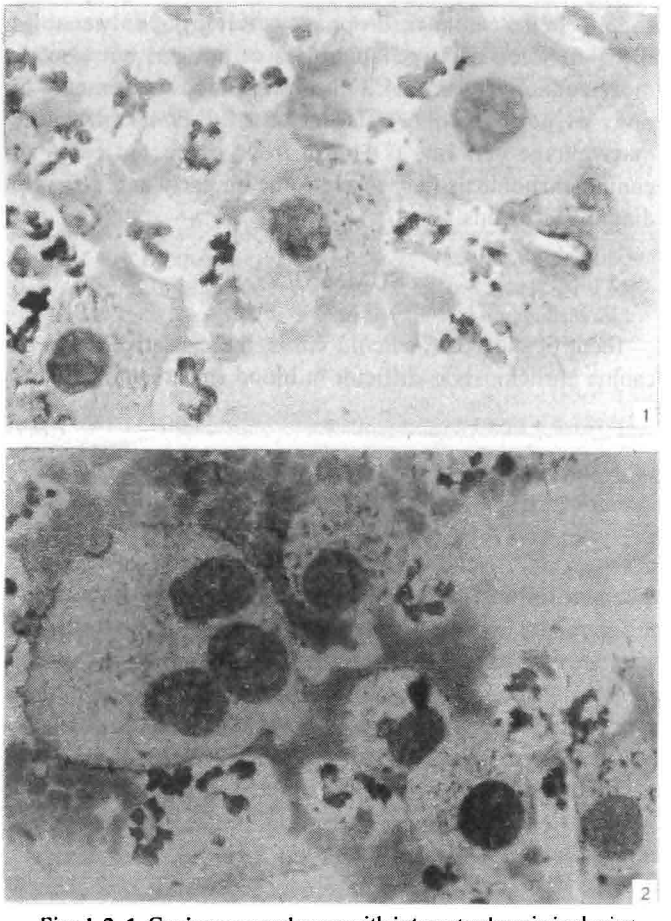
Canine ehrlichiosis, a tick-borne disease of dogs caused by the rickettsia *Ehrlichia canis* was recorded in 1944 for the first time by Mudaliar but there is no isolation of the rickettsia in cell culture in India, to date. Therefore, present study was made on the isolation of *E.canis* in a canine macrophage cell line (DH 82).

Whole heparinized blood was drawn from a dog that had a history of tick infestation with pyrexia and generalized lymphadenopathy at a private clinic in Chennai with clinical signs of ehrlichiosis. Erythematous pustules in the abdomen and oedema of the jaw and hind limbs were also observed. Peripheral blood smears of the dog revealed the presence of *E.canis* morulae in the monocytes.

DH 82 continuous canine macrophage cell line, established from, progenitor cells of a canine malignant histiocytosis, was obtained from Department of Infectious Diseases and Physiology, College of Veterinary Medicine, Stillwater, Oklahoma. The cells were grown and maintained in 25 cm and 75 cm culture flasks and the buffy coat cells separated from the heparinized blood were overlaid on a semiconfluent monolayer of canine macrophage cells (DH 82) in Minimum Essential Medium (MEM) containing 10% foetal bovine serum and 2mM glutamine as described (Dawson *et al.* 1991). The cells were cultured in 5% CO_2 at 37°C and after 4 to 6 hr of incubation, the old medium was removed and replaced with fresh medium. After the third or fourth day the culture was examined for the presence of ehrlichial inclusions.

The presence of small intracytoplasmic inclusions within 72 hr of inoculation were found in cell culture monolayers. Many of the infected cells were 3-4 times their original size and contained many intracytoplasmic inclusion (Fig.1). Some of the infected cells had 2 to 3 nuclei (Fig.2), and a few of these appeared to be in the process of mitosis. Serial propagation of *E.canis* in canine macrophage cells could not be carried out after the second split owing to contamination of the culture but the present study showed that *E.canis* can be isolated from dogs exhibiting clinical signs of ehrlichiosis in a canine macrophage cell line.

An *in vitro* cultivation system for *E.canis* was first developed from peripheral blood monocytes of infected dogs (Nyindo *et al.* 1971) and later serial propagation was



Figs 1-2. **1.** Canine macrophages with intracytoplasmic inclusion of *Ehrlichia canis*. **2.** Multinucleated canine macrophages with intracytoplasmic inclusion of *E. canis*.

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established in primary cultures of canine blood monocytes (Hemelt *et al.*1980). Both of these techniques, however, require weekly bleeding of normal dogs for collection of monocytes which is time consuming and costly and hence continuous cell lines were utilized. Canine peritoneal macrophages (Stephenson and Osterman, 1977), murine macrophage cell line (Cole *et al.*1985) and canine macrophage cell line (Dawson *et al.*1991) were employed for massive propagation of *E.canis*. Buffy coat cells separated from the blood were layered directly onto DH 82 cell line, whereas Dawson *et al.* (1991) first cultured *E.canis* in primary cultures of canine blood monocytes and later transferred the infective culture supernatants to DH 82 cells. Inoculation of peripheral blood leukocytes directly onto a semiconfluent monolayer of DH 82 cells consumes less time and is simple. Hence, may be used in substantiating clinical evidence of a rickettsial disease by isolation of the causative agent in a short time. The cell culture test is more reliable than the detection of morulae on stained peripheral blood smears for diagnosis of subclinical canine ehrlichiosis.

The primary monocyte cell culture, although effective, is expensive and time consuming. The adaptation of *E.canis* to the canine macrophage cell line not only enables the generation of large quantities of antigen but also, is more economical in that it eliminates the requirements of dogs as normal donors. Isolation of *E.canis* in canine macrophage cell line (DH 82) from suspected cases of canine ehrlichiosis may prove to be an early and accurate diagnostic means.

SUMMARY

Identification of *Ehrlichia canis*, the causative agent of canine ehrlichiosis is difficult in blood smears and the successful isolation of the rickettsia *Ehrlichia canis* in a continuous cell line is described. *E.canis* was isolated in a canine macrophage cell line DH 82 from blood collected from a dog that was presented with clinical signs of ehrlichiosis. Intracytoplasmic inclusion bodies were detected within 3 days of inoculation and many of the infected cells were 3-4 times their original size.

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Arginase activity of buffalo polymorphonuclear cells exposed to *Pasteurella multocida* lipopolysaccharide and L-arginine

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Key words: Arginase, L-arginine, Lipopolysaccharide, *Pasteurella multocida*, Polymorphonuclear cells

Arginase plays a major role in urea synthesis in liver. Its activity is also reported in blood cells of cattle, sheep and horses. It is also detected in buffalo red cells (More 1986), lymphocytes and macrophages (Schneider and Dy 1985) and polymorphonuclear cells (Sahoo *et al.* 1998). However, biological significance of arginase presence in such cells is speculative. Present work attempts to examine arginase activity in buffalo phagocytic polymorphonuclear cells exposed to its activator *Pasteurella multocida* lipopolysaccharide and additional arginine, the common substrate for the arginase and nitric oxide synthease.

Three healthy male Murrah buffaloes (*Bubalus bubalis*) of 2 year of age were maintained under identical conditions. Peripheral blood was collected from the jugular vein of buffaloes using disodium salt of ethylene diamine tetraacetic acid (1 mg/ml) as an anticoagulant. The polymorphonuclear cells were isolated from blood (Birnboim and Kanabus-Kaminska 1985) and washed in phosphate buffered saline (PBS) before counting and resuspended in the same buffer to a concentration of 2×10^7 cells/ml. This procedure generally yielded PMNs with 90% viability and 92% purity. A pure culture of *Pasteurella multocida* serotype B: 2 strain P52 was obtained from the culture collection unit of the Standardization Division of the Institute. The lipopolysaccharide (LPS) was extracted (Westphal and Jann 1965) and quantitated gravimetrically. PMNs (2×10^7) were incubated in tubes with 100 mg of *P. multocida* LPS alone or with 4 mM L-arginine for 30 min at 37°C. Following incubation the tubes were then centrifuged at 6000 g for 10 min.

The pellet obtained was solubilized with 1% Triton x-100 (in PBS) and nmoles of L-ornithine produced/min (Bergmyer 1984). Statistical analysis was done by student's 2 tailed 't' test (Snedecor and Cochran 1980).

The arginase activity of *P. multocida* LPS activated PMNs increased significantly ($P<0.01$). and the enzyme activity further increased ($P<0.05$) in presence of 4 mM L-arginine together with *P. multocida* LPS (Table 1). Buffalo PMN cells on activation with *P. multocida* produced nitric oxide and hydrogen peroxide (*Roy et al.* 1996). The arginase activity in our experiment was studied with reference to the observations of Schneider and Dy (1985) who reported that lymphocytes and macrophages on activation *in vitro* contained or released arginase. The increase in arginase activity is expected to decrease the concentration of L-arginine which is also required in the synthesis of polyamines, regulatory role of arginase *in vivo* is directed against the propagation.

Table 1. Effect of *P. multocida* LPS and L-arginine on arginase activity of buffalo PMNs

Cells $2 10^7$	*P. multocida* LPS (μg)	L-arginine (mM)	nmoles of L-ornithine produced $2 10^7$ PMN_s homogenate/min
PMN	—	—	3.86 ± 0.23(3)
PMN	180	—	9.10 ± 0.26**(3)
PMN	180	4	14.57 ± 0.78**(3)

*P<0.05 compared to control PMN cells; ** P<0.01 compared to control PMN cells. (figures in the parentheses indicate the number of animals taken).

Various functions centered around metabolic processes involving L-arginine and L-ornithine, were attributed to modulation of immune defense mechanisms by altering local arginine concentrations (Currie *et al.* 1979; Olds *et al.* 1980 and Albina *et al.* 1990). An arginase activity was induced in murine macrophages exposed to LPS and suggested that arginase II (AII) form induction could diminish NO (nitric oxide) production by limiting the availability of L-arginine, the common substrate for both A II and nitric oxide synthase (NOS) and influenced one another. An induction of A II diminished NO" production by decreasisng L-arginine availability in murine macrophages (Wang *et al.* 1995). The pathophysiological consequence of induced AII arginase activity in macrophages exposed to LPS lowered the

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intracellular levels of L-arginine in macrophages. To an extent the L-arginine is necessary for the expression of macrophage-mediated cytotoxicity (Hibbs *et al.* 1987), because it is the substrate for NOS catalysed production of cytotoxic levels on NO (Hibbs *et al.* 1988). Similar observations were recorded in buffalo PMN cells by Roy *et al.* (1996). It is concluded that an increase in arginase activity of buffalo PMN cells activated by *P. multocida* together with L-arginine could be attributed to induction of arginase-II, which needed further characterization.

SUMMARY

Biological significance of arginase presence in phagocytes is specutative and present work was attempted to examine the enzyme activity as influenced by *P. multocida* LPS and level of L-arginine. The arginase activity of *P. multocida* LPS activated neutrophils increased significantly ($P<0.01$) and the enzyme activity further increased ($P<0.05$) in the presence of 4 nm L-arginine. This suggested the role of arginase in granulocyte dependent immunodefense.

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Aeromonas sobria mastitis in a dairy cow

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Key words: Aeromonas, Bovine mastitis, Drug resistance

The Jersey cow was found affected with peracute mastitic condition, viz. both hind quarters were enlarged, swollen, firm and warmer on palpation, at a private dairy farm in Bareilly. The milk secretion from these quarters was somewhat watery with fibrin clots. The Jersey cow was treated with intra-mammary infusion of ampicillin 75 mg, cloxacillin 200mg as single dose along with intravenous injection of ampicillin. However, the animal did not respond to this treatment. The milk from the cow was referred for bacteriological investigation where the *Aeromonas sobria* was isolated and was characterized for its virulence properties.

Microbiological analysis

The milk samples from both the affected hind quarters were collected and cultured among others on ampicillin dextrin agar (Havelaar *et al.* 1987) at 37°C for 24 hr. Typical oxidase positives colonies suggestive of *Aeromonas* spp. were further characterized biochemically (Lee and Donovan 1985). Antibiotic resistance pattern was determined by disc diffusion method (Mastsen and Barry 1974).

To characterize the isolate for virulence factors, the organism was grown as overnight shake culture in trypticase soya broth, supplemented with 0.6% (w/v) yeast extract and 3% (w/v) cassamino acid (Gray *et al.* 1990). The cells were pelletted out by centrifugation at 3 000 g for 15 min. The supernatant was filtered and tested for cytotoxicity in baby hamester kidney-21 (BHK-21) cells, vascular permeability reaction in rabbit skin (Sandefur and Peterson 1976) and haemolysin titeration in microtitre plates (Pin *et al.* 1995). Haemolytic activity was also assessed by spotting the organism onto trypticase soya agar containing 5% rabbit blood. Congo-red bind binding assay was performed as per Ishiguro *et al.* (1985).

Milk samples from both the quarters yielded *Aeromonas* in pure culture, which was biochemically characterized as *A. sobria*. There was no evidence of involvement of other pathogens.

Antibiogram revealed the organism to be sensitive to only ciprofloxacin whereas it was resistant to ampicillin, colistin, co-trimoxazole, gentamicin, nitrofurantion, streptomycin, cephotaxime, cephalexin, chloramphenicol, nalidixic acid, furazolidone, norfloxacin and oxytetracyclin. The animal responded to intramammary infusion of ciprofloxacin and showed remarkable recovery within 5 days, however, the treatment was continued for 7 days. Agarwal and Kapoor (1998) reported the resistance of aeromonads to antibiotics like ampicillin, cephalexin, cephazolin, colistin, furazolidone and oxytetracyclin. But, the isolate in this study was resistant to a large number of routinely used antibiotics which is a matter of serious concern for veterinarians as well as public health authorities.

Investigations into virulence factors revealed the cytotoxic, necrotic and haemolytic nature of the isolate. The organism also demonstrated ability to bind congo-red dye within 24 hr at 37°C. The culture free toxin preparation induced shrinkage and sloughing of BHK-21 cells. There was loss of cytoplasmic extension and disorganisation of cell sheet. The toxin caused dermonecrotic changes in rabbit skin, when tested for vascular permeability reaction.

The isolate produced ß-haemolysis on blood agar. The culture free supernatant haemolysed rabbit erythrocytes up to a titre of 1: 32 in broth assay. Haemolysin is considered to be chief virulence factor of *Aeromonas* spp. responsible for variety of clinical manifestations in both human beings and animals (Vadivelu *et al.* 1992, Baloda *et al.* 1995). The cytotoxic, dermonecrotic and haemolytic factor(s) were responsible the mastitis by damaging the udder. Furthermore, positive congo-red binding test shows invasive ability of the isolate (Ishiguro *et al.* 1995).

Since, the aeromonads have been reported to cause gastroenteritis, meningitis, osteomyelitis, septic arthritis, endocarditis, peritonitis, eye and urinary tract infections (Khardori and Fainstein 1988, Ascencio *et al.* 1995), shading of multiple drug resistant *A. sobria* in the milk can pose a threat to human health.

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SUMMARY

Aeromonas sobria was isolated from the milk of peracute mastitic cow. The organism was found to be haemolytic, cytotoxic, necrotic to rabbit skin and having ability to bind congo-red dye. It showed multidrug resistance and was sensitive to only ciprofloxacin among the 14 antimicrobials tested. Presence of pathogenic, multidrug resistant *A. sobria* in milk can pose risk to human health.

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Identification of immunodominant polypeptides common to *Babesia bigemina* and *Theileria annulata*

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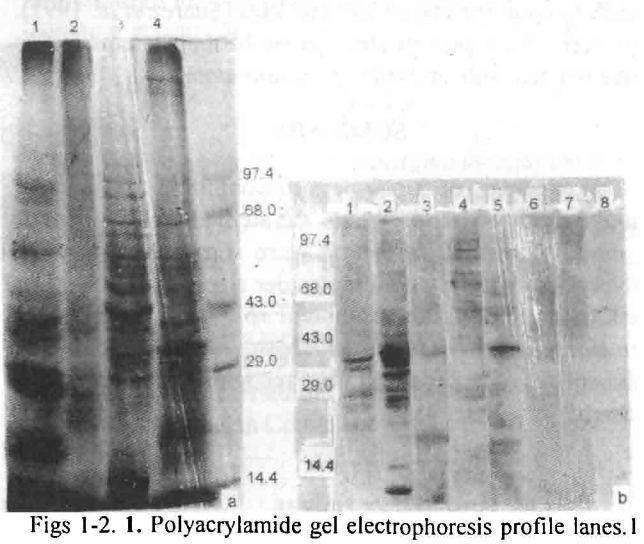
Key words: *Babesia bigemina*, Immunodominant, Polypeptide, *Theileria annulata*

The infection of cattle with tick-borne haemoprotozoa *Theileria annulata* and *Babesia bigemina* are co-enzootic in India. These 2 species belong to the same sub phylum apicomplexa, and share common antigens and cross-reaction between the species that occur in serological assays (FAO 1984). However, the specific antigens responsible for this cross-reactivity have not been identified. In this paper, the existence of 3 polypeptides common to *T.annulata* and *B. bigemina* has been reported.

The schizonts of *T. annulata* were obtained by multiplication of infected bovine lymphoblastoid cells in tissue culture (Subramanian *et al.* 1986). Suspensions of the infected cells in PBS (1×10^7 cells.ml^{-1}) were distributed in 0.5 ml aliquots and stored freeze-dried. The piroplasms of *T. annulata* were separated from blood of experimentally infected bovine calf (Ray *et al.* 1998) at the height of erythrocytic parasitaemia (>35%) and preserved at -20° C in PBS. The merozoites of *B. bigemina* were separated by differential lysis of uninfected bovine erythrocytes with ammonium chloride (0.83%) from bovine blood infected with *B. bigemina* (parasitaemia 8%) (Tewari *et al.* 1995). Sera were obtained from cattle recovered from experimental infection with either *T. annulata* or *B. bigemina*. Sera were collected when antibody titres against intact homologous organisms exceeded 640^{-1} as measured using indirect fluorescent antibody test (Ray and Subramanian 1986, Reddy *et al.* 1997).

The bovine lymphoblasts infected with schizonts of *T. annulata* piroplasms of *T. annulata* and merozoites of *B. bigemina* were separately prepared for electrophoresis by lysis in sample buffer (125 mM Tris HCl, pH 6.8, 10% glycerol 4% SDS and 10% b- mercaptoethanol).It was followed by incubation at 95°C for 4 min (Laemmli 1970). About 50 µl of each of the antigens were loaded per lane of 5-15% gradient polyacrylamide gel and electrophoresed at 30 mA constant current. The separated proteins were electro-transferred following standard Western blotting technique (Towbin *et al.* 1970) to nitrocellulose membranes using Bio-Rad transblot apparatus. The transfer was performed at 30 mA constant current for 5 hr at (25°C) in an electrode solution consisting of 192 mM glycine and 25 mM Tris mixed with 20% methanol (pH 8.3). Free protein binding sites on the nitrocellulose were blocked with 6% (w/v) bovine serum albumin (BSA) in PBS. The antigens were detected by incubating the blots in bovine reference antisera diluted 1: 100 with PBS containing 1% BSA followed by horse radish peroxidase linked anti-bovine immunoglobulin (Sigma) and development with 3-3 - diaminobenzidine (0.5 mg/ml) in PBS and 0.06% hydrogen peroxide.

The antigens of *T. annulata* and *B. bigemina* were separated into 9-15 polypeptides with apparent molecular



Figs 1-2. **1.** Polyacrylamide gel electrophoresis profile lanes.1 Merozoite antigen of *B. bigemina*, 2. Normal bovine RBC, 3. Schizont antigen of *T. annulata*. Piroplasm antigen of *T. annulata* **2.** Immunoblot - reaction of bovine antiserum (*T. annulata*) with lanes, 1. Piroplasm and 2 Schizont antigen of *T. annulata*, 3. *B. bigemina*. Reaction of bovine antiserum (*B. bigemina*) with lanes, 4. *B. bigemina*, 5. Schizont antigen of *T.annulata* and, 6. Piroplasm antigen of *T. annulata*. Reaction of normal bovine serum with *T. annulata* in lane 7 and *B. bigemina* in lane 8.

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sizes of 12-97.4 kDa (Fig. 1). In immunoblot, the serum antibodies of cattle recovered from *T. annulata* infection showed predominant high response to *T. annulata* antigens in the Mr of 28-32 kDa. Few polypeptides in Mr of 14 to 20 KDa were also recognized in the schizont antigen (Fig. 2 lanes 1,2). When reacted against the heterologous species, antibodies from *T. annulata* infected cattle bound primarily *B. bigemina* polypeptides of 32, 19 and 50 kDa (Fig. 2 Lane 3). Serum from cattle infected with *B. bigemina* bound to multiple homologous polypeptides in apparent molecular sizes of 14 to 97 kDa (Fig. 2, lane 4). Against the heterologous *T. annulata* polypeptides, the dominant responses were only against schizont antigens of 32,19 and 50 kDa. (Fig.2, lane 5). No reactivity was obtained with the piroplasm of *T. annulata* (Fig. 2, lane 6).

The polypeptides of 50 kDa, 32 kDa and 19 kDa were shown to be antigens common to schizonts of *T. annulata* and merozoite of *B. bigemina*. Earlier, the 32 kDa polypeptide has been shown to be a common immunodominant protein in different parasitic stages of *T. annulata* (Kachani *et al.* 1992, Sengupta *et al.* 1997) and *T. sergenti* (Oligitani *et al.*1987.).

The presence of more dominant polypeptides in schizont antigen in comparison to piroplasms of *T.annulata* against the homologous antiserum, and lack of reactivity of the piroplasms to heterologous antibodies suggested more prominent role of the schizonts in immunological cross-reactivity. Several polypeptides of *B. bigemina* in the Mw 55 to 80 kDa were recognized by homologous in antisera. The apical complex polypeptides were detected in *B. bigemina* and *B. bovis* in the Mw of 58 to 60 kDa (Suarez *et al.* 1991). However, in the present study, none of the proteins in this range reacted with antibodies to *T. annulata.*

SUMMARY

The existence of 3 polypeptides common to *Theileria annulata* and *Babesia bigemina* was studied. The antigens of *T. annulata* and *B. bigemina* were separated into 9-15 polypeptides with apparent molecular sizes of 12-97.4 kDa. Results revealed more prominent role of schizont of *T. annulata* in immunological cross-reactivity. Several polypeptides of *B. bigemina* were recognized by homologus antisera.

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Sudden death of piglets during immature schistosome infection

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In comparison to nasal schistosomosis, little attention is paid on hepatic schistosomosis in domestic animals albeit recording some outbreaks. It is not surprising, then, to have ignored death of animals during pre-patency of schistosomosis. We have attempted to draw attention on this aspect through this communication.

In the present work, 23 piglets of local breed, 2-3 months of age, 2-3 kg weight and of either sex were procured from local piggries prior their scavenging. Two piglets were kept as uninfected control, while remaining were infected each with 1500 cercariae of *Schistosoma incognitum* by polythene tail method (Shames *et al.* 2000).

From the infected piglets, two piglets died within 24 hr of the infection without any apparent symptom. Afterwards, 4 more piglets died- 1 on 12th DPI, other on 18th and 2 on 21st DPI. All these 4 piglets, prior to death, showed severing, weakness of hind legs, subnormal temperature, cold extremities, dehydration and loss of eye reflexes. These piglets were perfused and immature flukes were recovered. It may be emphasized that the 2 uninfected controls and remaining 15 infected piglets (including untreated infected) survived for rest of the experiment, which lasted upto 52 DPI.

No gross and histopathological changes were observed in the liver and intestine of the piglets died within 24 hr and on 12th DPI but emphysema was seen on gross and microscopic examination of the lungs. The piglets, died on 18th and 21st DPI, also showed emphysematous changes which were more severe. The intestines were without histopathological alterations while apparently normal liver showed cellular swelling and mild fatty changes.

There are some findings, which reflect probability of *S. incognitum* being responsible for death of these piglets. First, the uninfected controls remained alive, whereas infected died with yielding immature blood flukes. Secondly, the symptoms exhibited by 4 piglets prior to death and emphysema of lungs were alike to symptoms and lesions of anaphylactic shock which might have occurred due to *S.incognitum* infection. The one likely hood for happening of the anaphylactic shock is by transferring circulating schistosome antigens by their infected mothers (which are inhabiting in an endemic area), to their fetus which on getting second dose of schistosome cercariae reacted acutely. In human schistosomosis, transfer of circulating schistosome antigens by infected mothers to their uninfected children and prenatal sensitization have been demonstrated (Camus *et al.* 1976). Same might be occurring in animal schistosomosis.

Our observations are not the first as previous workers have also recorded death of the animals during pre patency of schistosomosis but without paying serious attention on the subject (Dutt 1962, Sadun *et al.* 1966, Dutt 1967, Agrawal 1978). It is important to investigate biochemical, pathological, immunological, pharmacological and other changes in immature schistosomosis to find out cause of death of the animals.

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SUMMARY

Six piglets infected with *Schistosoma incognitum* died on 24 hr to 21 day of the infection with the symptoms and lesions of anaphylactic shock. Such eventuality is suggested to happen because of prenatal sensitization of the offsprings by their infected mothers.

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Development and distribution of lymphocyte subsets in jejunal and ileal Peyer's patches of pigs

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The gut associated lymphoid tissues (GALT), lamina propria (LP) and intraepithelial lymphoytes (IE) form the major lymphocyte pool of the gut (Pabst 1987). In pigs, the distribution of B-and T-cell was studied in lymphoid and non lymphoid areas (Rothkotter and Pabst 1989, Barman *et al.* 1997). The Peyer's patches (PP) perform a central role of antigen uptake and induction of immune responses. In sheep, pigs and dogs, jejunal and ileal Peyer's patches differ in their structure and function. Pigs as the omnivorous animals can provide basic information for production and function of intestinal lymphoid tissues for their type of digestion and luminal antigens. However, little is known about the development of lymphocyte subsets in different compartments of the gut of Dum variety of pigs maintained in free grazing system. The present study was an attempt to elucidate how B- and T- cell subsets are distributed in PP, LP, IE area of small intestine in the postnatal life along with changes of luminal antigens.

Four litters of piglets (Dum variety) were used. The animals were reared in conventional managemental condition. The piglets were grouped as day-old (n=5), 1-month-old (n=5), 3-month-old and adult (n=5). The animals were euthanized and the abdomen was opened to separate small intestines. Tissue pieces containing PP from proximal, middle and terminal jejunum and ileum were collected respectively. The 0.5 cm long tissues were snap frozen and preserved in liquid nitrogen (-196°C) until processed.

A standard alkaline phosphatase and antialkaline technique was used for the demonstration of B- and T-lymphocyte subsets (Rothkotter *et al.* 1991). Briefly 5 µm, cryostat sections were incubated with properly diluted primary monoclonal antibody (Table 1) in a humid chamber. In subsequent steps the sections were incubated with rabbit anti-mouse immunoglobulin followed by alkaline phosphatase and antialkaline phosphatase complex. The colour reaction was carried out with a fast red solution. Finally, sections were counterstained with Harris haematoxylene and mounted in glycerol. The distribution of B- and T-lymphocytes was examined under Zeiss microscope and evaluation was done by semi quantitative way. In PP, entire follicle covered with stained cells was termed as very frequent (+++), most of follicular cells that stained were called frequent (++), only a few cell stained recorded as less frequent (+) and lymphoid cells that stained scatteredly were termed as occasional (±). The unstained follicular cells were recorded as negative (-). The cellular distribution in the dome area and interfolicular area were evaluated as it was done in follicles. In absorptive epithelium the occular grid was moved in villi or crypt and epithelium covering 8 divisions and the staining cells were counted in each field. Evaluation of B-lymhocytes was done

Table 1. Monoclonal antibodies to pig lymphocytes used in the study

Antibodies Preferential	Surface molecules	Preferential cell type	Dilution used	Reference
MAC-80	CD2	T-cell	1:30	Lunney and Pescovitz (1988)
74-12-4	CD4	T-helper cell	1:500	
76-2-11	CD8	T-cytotoxic cell	1:40	
8/1	Resting whole T-cell	T-cellsubsets	1:300	Saalmuller *et al.* (1987)
CVI-SWIgA-27.9	IgA	IgA$^+$cell	1:500	Van Zaane and Hulst (1987)
CVI-SWIgM-28.4	IgM	IgM$^+$cell	1:500	
CVI-SWIgG-34.32	IgG	IgG$^+$cell	1:500	

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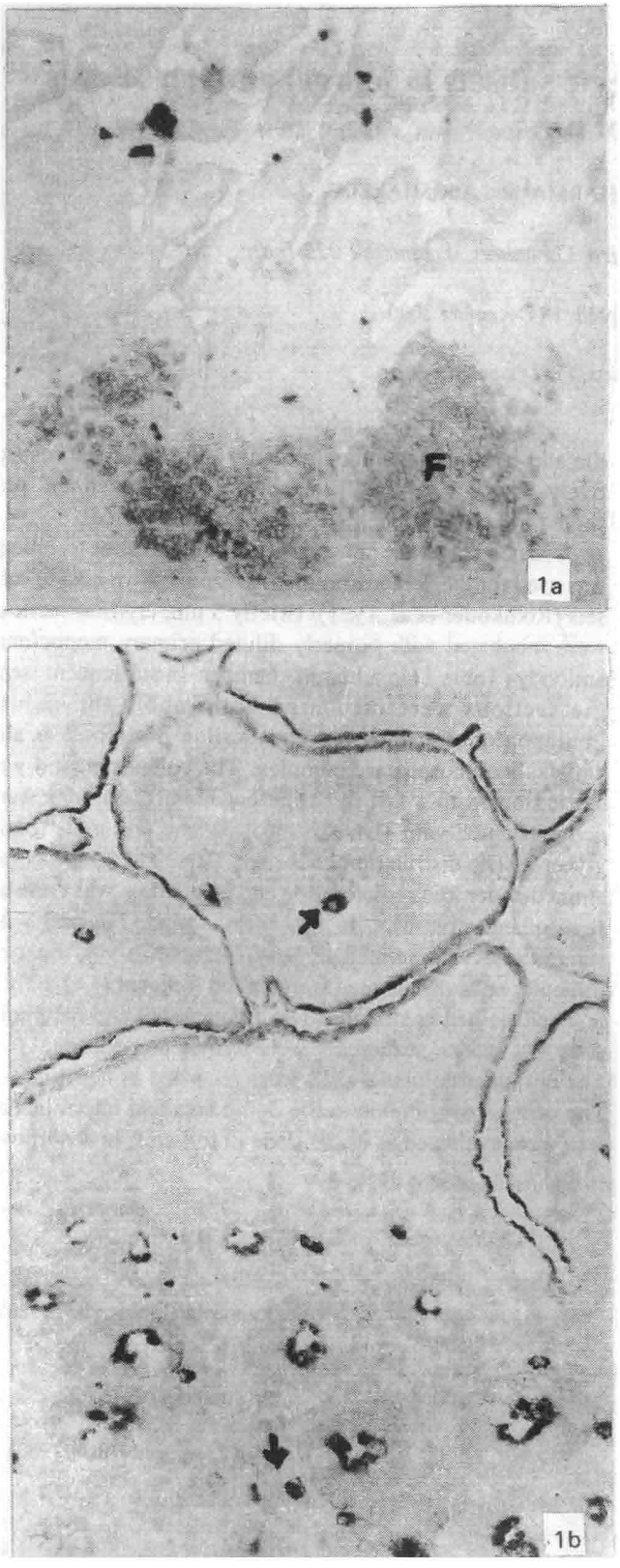

Fig. 1. Distribution of B-cell (a, b) subsets in PP of pigs. $SIgM^+$ cells are present in the follicle of day-old piglets (a). In the LP of Jej-PP in adult pigs contains $cLgA^+$ cells and more frequent in the crypt (b). Magnification (a) × 90; (b) × 180.

by counting the stained cells containing cytoplasmic immunoglobulins per high power field (HPF).

The B-lymphocytes were distributed in follicle, dome, IFA and in lamina popria. The follicles of jej-PP and il -PP of day-old piglet were almost covered by surface IgM positive (sIM^+) cells (Fig. 1a). However, $sIgM^+$ cells were scatteredly distributed in the one-month-old piglet and absent in adult pig. In post-weaned piglets follicles were stained intercellularly. The $sIgM^+$ acts as antigen receptor in unprimed animals. On contact with the antigen these $sIgM^+$ cells form immunocomplexes (Barman *et al.* 1997). The $sIgM^+$ cells were also found in the FAE of day-old piglet. The $sIgM^+$ cells located in FAE along with 'M' cells play a role of antigen sampling of the follicle of PP. The present observation on low $sIgM^+$ cells in 1-month-old pig and onward suggested different stages of the development of B- cell. Again, the types of luminal antigens determine the fate of the development, as the germ free pigs of the comperable age group retain higher $sIgM^+$ cells (Barman *et al.* 1997).

The cytoplasm containing IgM ($cIgM^+$) cells started appearing in the dome, IFA, villus and crypt lamina propria of 1-month-old piglet, highest numbers (3-4/HPF) were recorded in 3-month-old piglet. In all age groups of pigs cytoplasm containing IgG ($cIgG^+$) cells were scanty (1-2/HPF). The number of $cIgA^+$ cells were increased lately and maximum numbers (10-14/HPF) were recorded in adult pig (Fig. 1b). The numbers of $cIgA^+$ cells were found more in crypt lamina propria than in villus lamina propria. Among the Ig isotypes, $cIgM^+$ was predominant isotype in 1-month-old piglet but in older groups $cIgA^+$ cells were frequently present (Pabst *et al.* 1988, Barman *et al.* 1997). Predominance of IgA at mucosal site in older pigs suggested increased antigenic mass in the intestine; which created an environment for switching IgM lymphocytes to IgA isotype (Husband and Dunkley 1990). Again, preferential location of cytoplasmic Ig (cIg) in the crypt region might be because of the fact that secretory component (SC) is essential to attain the proteolytic resistance property.

The T-lymphocytes were present in follicle, dome, IFA and in LP and IE area. The $CD2^+$ cells were mostly located in IFA and intensity was highest in adult (+++). The $8/1^+$ T-cells which recognize resting T- cells, were predominantly found in day-old piglet and absent in adult. The numbers of $CD4^+$ and $CD8^+$ cells increased with age and highest numbers were recorded in adult. In non-lymphoid areas T- cells were also found in IE and LP area. The $CD2^+$ cells in LP of jejunum of adult pig contained 3-4 cells/0.04 mm area and in crypt 5-6 cells/0.04 mm area were found. The $8/1^+$ T- cells were also found in LP and IE area at day-old and 1-month-old piglet and disappeared from 3-month-old and older ones. The $CD4^+$ cells were present in LP and absent in IE area. The $CD8^+$ cells were absent in LP and IE area at day-old piglet and highest number was recorded in adult pig (5-6 cells/0.04 mm area in villus and 7-8 cells/0.04 mm area in crypt). The

distribution of T- cell subsets in different lymphoid compartments and in absorptive tissues of different age groups was recorded by Saalmuller *et al.* (1987) and Barman *et at.* (1997). In this study, the increased frequency of $CD8^+$ cells in IE area of the adult animals clearly indicated the antigen dependent growth of this population of T- cell subset (Saalmuller *et al.* Barman *et al.* 1987). In the present study $8/1^+$ T- cells were identified in all lymphoid and non-lymphoid compartments of day old piglets but not found in adult pigs. The present investigation further confirmed that MoAb 8/1 can differentiate between resting T- cells and activated T- cells (Saalmuller *et al.* 1987).

SUMMARY

Development and distribution of lymphocyte subsets in jejunal and Peyer's patches of pigs were studied.

Lymphcyte subsets in jej-PP and il PP were distributed in different histotopographic areas. However, in animals with frequent contact with gut antigens the frequency of B- and T- cells was increased. Most particularly $CD8^+$ T- cells appeared in IE area. This basic information can be interpolated to study the alteration of lymphocyte subsets in viral and bacterial infection.

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Clinico-haematological, biochemical and urinary changes of induced chronic cadmium toxicity in goats

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Cadmium (Cd) acts as organ poison to induce severe pathogenic effects in man, animals and birds. Farm animals are commonly exposed to Cd poisoning through gestation of agricultural waste products, grazing on contaminated grasslands and also administration of anthelmintic (Bartik and Piskac 1981). Information is limited on clinico-pathological features of Cd-toxicity in small ruminant (Ping *et al.* 1996). This study presents a detailed report on clinico-pathological changes of induced Cd-toxicity in goat.

Clinically healthy female Black Bengal goats (16), 12-13 months of age, and weighting 12.5-13.5 kg, were divided into 2 equal groups, viz. 1 and 2. They were fed on 0.5 kg goat ration; 1 kg fresh leguminous hay, supplemented with feed additives twice a day, and were given deionized water *ad lib.* Animals were housed in the groups in concrete-floored pens of laboratory animals sheds. Reagent grade crystallized Cadmium chloride, was dissolved in deionized water to give 100 µg of Cd ml^{-1} and fed in a feeding bottle to each goat of group 2 daily for 90 days. The deionized water as the sole source of drinking water was availabe *ad lib.* The remaining 8 goats received the deionized water containing sucrose, serving as control (group1). All goats were examined daily and progressive development of clinical signs was recorded.

The ruminal motility, respiration and heart rates and body weight gain were recorded on 0 day and thereafter at 30 day intervals. Blood and urine samples were collected at different intervals. EDTA admixed blood (1 mg ml^{-1}) samples were used for haemograms. Blood samples containing sodium fluoride (10 mg ml^{-1}) were utilized for glucose estimation (Nelson and Somogyi 1979). Serum was used for the estimation of total protein and albumin: globulin (A:G) ratio (biuret method), urea nitrogen (UN) (pheny-hypochloride method) as described by Varley (1969). The routine examination of urine for pH, specific gravity, glucose, protein and casts (Benjamin 1985), and biochemical analysis for urinary urea nitrogen (UN) and creatinine (Cr) (Frankel *et al.* 1970) was also performed. The difference between the groups in the mean values of all parameters were analysed using Student's 't'-test (Snedecor and Cochran 1968).

All Cd-treated goats began to show signs of toxicity from 60 day, consisting of partial loss appetite, diarrhoea, bullness and depression. Subsequently, brown coloured faces with mucous, dehydration, emaciation, unthriftiness, dry scaly skin, rough body coat with alopecia and polyuria were observed. On day 80 onwards, the animals had inability to get up, oliguria and salivation without any mortality. A significat ($P<0.05, 0.01$) increase in heart (78.87±0.27 and 83.26±0.29 min^{-1} and respiratory (27.50±0.25 and 32.75±0.29 min^{-1}) rates and decrease in ruminal motility (3.87±0.12 and 3.37±3.37±0.17 min^{-1}) and body weight gain (12.275±0.21 and 10487±0.15g) were observed on day 60 and day 90 respectively. None of the control animals showed any clinical signs of toxicity. Clinical signs of polyuria followed by oliguria suggested the possibility of tubular damage. Ping *et al.* (1997) also reported nephrotoxic effect of Cd in sheep. Dullness and depression with unthriftiness might be due to gradual loss of appetite, retention of non-protein nitrogenous substances and proteinuria resulting from nephrotoxicity. Appearance of dry and scaly skin with alopecia in Cd treated animals suggested zinc starvation as Cd is a zinc-antagonistic (Bartik and Piskac 1981). The onset of gastrointestinal signs might be because of entry of Cd into GI tract through mucous membrance. Ruminal motility decrease was possibly due to necrosis and sloughing of lining mucosal epithelium as in sheep (Ping *et al.* 1997). The progressive loss of body weight resulted from gradual onset of anorexia, as reported in calves (Lynch *et al.* 1976). The Cd-treated animals showed significant ($P<0.05, 0.01$) hypohaemoglobinemia, low MCHC and PCV, and erythrocytopenia from day 60 onwards, suggesting anaemia which confirmed the reports in sheep (Ping et al. 1996). Anaemia is possibly resulted from toxic depression of bone marrow activity (Kessels *et al.* 1990). Ch-treated animals also had significant ($P<0.05, 0.01$)

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Table 1. Haematological and blood biochemical profiles in control and Cd-treated goats (n=8:mean±SE)

Parameters	Groups	Days** of observations 30	60	90
Hb (g%)	C	10.48±0.20	10.53±0.16	10.56±0.15
	I	10.32±0.21	8.78±0.17*	6.67±0.15**
PCV (%)	C	30.42±0.50	32.50±0.49	32.50±0.46
	I	32.77±0.48	29.75±0.53	24.68±0.45**
TEC ($\times 10^6$µl)	C	12.50±0.12	12.68±0.18	12.63±0.17
	I	12.41±0.07	10.48±0.11*	8.52±0.16**
TLC ($\times 10^3$µl)	C	8.70±0.12	8.69±0.12	8.69±0.13
	I	8.84±0.15	9.24±0.14*	10.65±0.12**
MCHC (%)	C	31.69±0.88	31.76±0.79	31.81±0.67
	I	31.69±0.88	29.63±0.90*	27.12±0.87*
Blood glucose (mg%)	C	52.78±0.45	52.71±0.30	52.81±0.34
	I	52.86±0.40	45.59±0.50**	40.93±0.51**
Total protein (g%)	C	7.84±0.04	7.89±0.04	7.91±0.04
	I	7.79±0.03	6.32±0.05*	5.62±0.10*
A:G ratio	C	1.28±0.01	1.28±0.01	1.28±0.01
	I	1.28±0.01	1.19±0.02*	1.16±0.02*
Serum UN (mg%)	C	18.99±0.13	18.94±0.10	18.81±0.14
	I	26.59±0.27**	53.80±0.29**	96.00±0.34**
Serum Cr (mg%)	C	1.33±0.02	1.32±0.02	1.31±0.02
	I	2.68±0.03*	5.19±0.05**	7.31±0.03**

n=Number of animals; **P<0.01; *P<0.05; C, control; I, induced.

leucocytosis on day 60 and 90 (Table 1) because of increase of neutrophils which are attributed to stress conditions (Kuhl 1976).

Blood biochemical changes of Cd-intoxicated goats (Table 1) showed a significant (P<0.05, 0.01) decrease in blood glucose, total serum protein and AG ratio from day 60 onwards. In Cd-treated animals hypoglycaemia could be due to either gradual loss of appetite or by excretion of glucose through urine due to tubular damage. The transient hypoproteinaemia in Cd-treated goats, as also recorded in sheep (Ping *et al.* 1996), possibly resulted from proteinuria caused by failure of renalreabsorption due to marked tubular damage (Lauwerys *et al.* 1984). Cd-treated animals also showed significant (P<0.01) increase in serum UN and Cr from day 30 onwards, which confirmed the reports of Ping *et al.* (1996). It is fact that UN and Cr are mainly excreted through the kidneys and damage to the glomeruli and/or tubules could result in their retention in the blood giving rise to azotaemia. Significant increase in serum UN and Cr suggested extensive damage to renal tissues, signifying severe nephrotoxicity.

Urine analysis revealed marked decrease in urinary pH (6.77±0.04 and 6.50±0.03), specific gravity (1.022±0.0002 and 1.015±0.0006) and increase in glucose (2 to 3+ and 4+), protein (3+ to 4+), presence of pus cells (7+10 to 12+) and granular/epithelial casts (4+ and 5+) on day 60 and day 90 of Cd-treated goats. These findings clearly suggested massive damage to kidneys. The lowered urinary pH is attributed to metabolic acidosis resulting from uremia. A marked decrease in specific gravity indicated lack of ability of the renal tubules to concentrate urine. The observations of renal glycosuria and proteinuria in Cd-treated goats are in conformity with the reports of Glasser *et al.* (1978) in sheep. Appearance of glucose in urine suggested marked destruction of nephrons which impaired the tubular reabsorption. Nephrosis caused by heavy metals usually results into proteinuria due to increased permeability of glomerular filtration (Lanwerys *et al.* 1984) Significant presence of granular/epithelial casts and pus cell was possible indication of massive tubular damage.

Cd-treated goats further showed significantly (P<0.01) decrease in urinary UN(970.70±3.40, 790.49±2.67 and 525.76±2.62 mg dl^{-1}) and Cr (79.03±0.51, 66.45±0.79 and 50.20±0.52 mg dl^{-1}) on day 30, 60 and 90 respectively. A significant decrease of these values in urine of the nephrotoxic animals was anticipated in view of increase in their concerned blood values.

Results clearly suggested that Cd produces gastrointestinal and renal toxicity in animals. Since this heavy metal is rapidly becoming an unavoidable environmental pollutant, the clinico-pathological profiles reported herein, may by utilized and as one of the diagnostic tools to detect cadmium toxicity in farm animals under field conditions.

SUMMARY

The clinico-pathological changes of induced cd-toxicity in goats were studied. Blood glucose, total serum protein, and A : G ratio decreased from day 60 onward. Urinary pH, specific gravity decreased and glucose, protein, pus cells increased. Results clearly revealed that cd-produces gastrointestinal and renal toxicity.

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Biochemical effects of centbucridine spinal analgesia in goats*

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The studies related to biochemical effects of administration of centbucridine either alone or along with xylazine or pentazocine on blood and CSF have not been studied. Therefore the present experiment was conducted to assess the influence of centbucridine either alone or along with xylazine or pentazocine in biochemical attributes of blood and CSF with their use as subarachnoid analgesics in goats.

Healthy male goats (6), weighing 20 to 30 kg and of 12 to 18 months age were used. A polyethylene catheter was introduced at the lumbosacral space through 15 gauze needle to fix in the subarachnoid space for collection of CSF and retained in the place with the help of adhesive tape. Four pre-experimental blood and CSF samples were collected from each animal for recording of normal biochemical attributes. Biochemical parameters included glucose, chloride, total protein, magnesium, sodium, phosphorus and potassium. The animals were kept off feed for 24 hr prior to each treatment. The study was conducted with 3 treatments and each animal was subjected to the treatments at an interval of 8 days in the following manner.

Treatments 1, 2 and 3 included centbucridine hydrochloride @ 1mg/kg, xylazine @ 0.05 mg/kg followed by centbucridine @ 1mg/kg and pentazocine lactate @ 2mg/kg followed by centbucridine hydrochloride @ 1mg/kg body weight respectively. In all the groups injections were made in subarachnoid space.

The blood samples were collected at 0, 6, 24, 48, 72, 96 and 120 hr and CSF samples at 0, 6, 24, 48 and 72 hr post treatments for the estimation of biochemical parameters, viz. glucose, total protein, chloride, magnesium, phosphorus, sodium, potassium. All the estimations were done on biochem autoanalyser and using diagnostic kits. The data were analyzed for analysis of variance by using hierarchial classification (Snedecor and Cochran 1980).

The significant variations in the biochemical attributes of blood and the CSF after treatments 1, 2 and 3 are given in Table1. The blood glucose level remained significantly ($P<0.05$) high in treatments 1 and 3 between 24 to 48 hr and in treatment 2 between 24 to 72 hr. CSF glucose also showed a significant ($P<0.05$) rise at 48 hr in treatment 1 and at 24 hr in treatments 2 and 3. The present findings corroborate with the result of epidural administration of bupivacaine (Tiwari *et al.* 1989). Strawitz *et al.* (1961) reported that following stress, cortisone induced gluconeogenesis plays an important role in enhancing the blood glucose levels. Feldberg and Symonds (1986) stated that the xylazine as $\alpha 2$ adrenergic inhibitor suppresses the release of insulin from pancreatic β-cells and there is an increased production of glucose in the liver. Kaneko (1980) reported that with an increase in the blood glucose, CSF glucose also undergoes a corresponding change. Cousins and Mather (1984) suggested that because of slow absorption of narcotic opioids from the CSF, increase in glucose level could be maintained over a longer interval. Therefore increase in blood glucose levels may be attributed to one or combined effects of these factors. Significant ($P<0.05$) increase in the serum total proteins were recorded between 6 to 24 hr in treatments 1, 2 and 3 and CSF total protein in treatments 1 and 2 between 6 to 48 hr and between 6 to 24 hr in treatment 3. These findings are in agreement with the observations recorded by Tiwari *et al.* (1989) after subarachnoid use of bupivacaine alone or with analgesics. Baltch *et al.* (1969) experimentally produced aseptic meningitis in dog and reported significant ($P<0.05$) increase in the CSF total protein at 48 hr. The use of local and systemic analgesic and implantation of catheter might have caused short-lived inflammation of meninges leading to increase in total protein levels in serum and CSF.

Significant ($P<0.05$) decrease in serum and CSF chloride levels were observed in all the 3 treatments. The serum chloride level showed significant ($P<0.05$) drop between 6 to 72 hr in treatments 1 and 3 and between 6 to 96 hr in treatment 2. CSF chloride level decreased significantly between 6 to 48 hr in all the 3 treatments (Table 1). Tiwari *et al.* (1989, 1998) reported decrease in serum and CSF chloride levels after subarachnoid and epidural administration

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Table 1. Significant variations (mean±SE) in biochemical attributes of blood and cerebrospinal fluid at different time intervals following spinal centbucridine in goats

Time (hr)	Treatment 1	Treatment 2	Treatment 3
	Blood glucose (mg/dl)		
0	47.13±1.80	44.46±1.36	49.25±1.71
6	59.96±1.59	62.50±2.00	59.10±2.57
24	68.88±2.30*	69.61±2.18*	67.26±3.83*
48	78.81±2.68*	76.16±2.0*	73.60±2.98*
72	59.66±3.40	84.51±1.20*	64.55±5.03
96	56.61±3.19	65.38±4.46	59.50±1.84
120	58.83±3.27	59.90±3.18	59.55±1.90
	CSF glucose		
0	45.15±1.02	45.08±0.77	45.88±0.71
6	55.26±1.63	57.65±1.52	55.36±2.05
24	62.60±3.00	70.53±0.64*	67.95±2.5*
48	63.01±2.86*	62.96±0.93	59.26±1.48
72	59.51±2.17	54.71±0.99	57.81±1.67
	Total protein in blood (md/dl)		
0	7.78±0.31	6.26±0.21	6.50±0.25
6	8.91±0.19*	7.66±0.36*	7.48±0.31*
24	0.96±0.31*	8.36±0.43*	8.21±0.22*
48	7.80±0.21	8.03±0.54	8.08±0.18
72	6.70±0.25	7.38±0.39	6.88±0.18
96	6.48±0.18	6.96±0.19	6.40±0.19
120	6.53±0.20	6.71±0.06	6.65±0.19
	Total protein in CSF		
0	13.33±0.88	11.28±0.45	13.15±0.80
6	18.83±0.54*	19.50±0.42*	18.15±0.30*
24	23.66±0.88*	23.50±0.42*	20.50±0.42*
48	18.66±0.76*	18.66±0.76*	16.33±0.21
72	15.50±0.56	15.50±0.56	14.33±0.42
	Chloride in blood (meq/litre)		
0	107.60±1.86	104.16±1.19	100.16±1.74
6	96.00±0.96*	95.00±1.09*	93.83±0.98*
24	95.66±0.71*	89.16±0.91*	96.00±0.51*
48	93.00±1.37*	84.33±0.88*	95.50±0.99*
72	87.50±1.28*	79.16±0.30*	94.50±0.99*
96	98.50±1.34	90.66±0.42*	99.33±1.05
120	103.66±1.48	97.831±0.42	102.83±0.94
	Chloride in CSF		
0	124.33±0.92	124.16±0.54	124.50±0.62
6	118.83±0.60*	118.33±0.76*	118.50±0.62*
24	115.83±0.65*	113.66±0.42*	111.66±0.92*
48	119.66±0.49*	118.50±0.42*	118.66±0.42*
72	122.66±0.66	122.83±0.30	123.±0.68
	Phosphorus in blood (meq/litre)		
0	2.66±0.15	2.43±0.10	3.40±0.38
6	2.15±0.17	1.86±0.12	2.68±0.18
24	1.63±0.19*	1.28±0.17*	2.46±0.27*
48	1.25±0.16*	1.25±0.21*	2.10±0.36*
72	1.31±0.20*	1.18±0.21*	2.05±0.25
96	2.18±0.15	2.06±0.12	2.55±0.16
120	2.40±0.17	2.45±0.17	2.93±0.36

Treatment 1, Centbucridine hydrochloride @ 1mg/kg body weight, treatment 2, xylazine @0.05 mg/kg body weight + centbucridine hydrochloride @ 1mg/kg body weight, treatment 3, pentazocine lactate @ 2mg/kg body weight + centbucridine hydrochloride @ 1mg/kg body weight.* (P<0.05).

of bupivacaine and lignocaine alone and along with buprenorphine, pethidine, pentazocine, xylazine and detomidine. Raidurg *et al.* (1995) reported similar changes in serum chloride after epidural use of xylazine and attributed it to metabolic acidosis. Similar is the probability in the present experiment as well. There was a significant (P<0.05) decrease in the serum phosphorus levels between 24 to 72 hr in treatments 1 and 2 and between 24 to 48 hr in treatment 3. The CSF phosphorus level remains unaffected. Tiwari *et al.* (1989) reported similar finding after subarachnoid use of bupivacaine and systemic analgesics in goats. Sodium potassium and magnesium concentrations in serum and CSF did not reveal any significant change in any of the 3 treatments and ranged within the normal levels.

SUMMARY

Spinal administration of centbucridine either alone or along with xylazine and pentazocine caused significant increase in blood and CSF glucose and total proteins, and significant decrease in blood and CSF chloride and blood phosphorus. However, addition of analgesics prolonged decrease or increase in these parameters.

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Detomidine as a sedative in yaks

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Key words: Detomidine, Sedation, Yaks

Sporadic reports are available on anaesthesia in yaks (Jalanka 1989). The present paper deals with evaluation and efficacy of detomidine hydrochloride as sedative in yaks (*Bos grunniens*).

The present experiments were conducted on 7 yaks of either sex. The animals were kept off feed and water for 24 and 36 hr prior to the experimentation. Detomidine was administered @ 0.025 mg/kg IM. The efficacy of the drug was determined by observing various clinico- physiological and haemato-biological parameters. Clinico-physiological parameters included corneal, palpebral and pedal reflexes. Onset time (time lapse between the time of injection and development of incoordination), down time (time lapse between the time of injection and coming of animal in sitting and/ or lateral position) and recovery time (time lapse between the time of injection and coming of animal back to standing ataxia and normal gait), rectal temperature, heart rate, respiratory rate and ECG using bipolar base apex lead system were recorded at 0 hr (prior to injection) and 5, 15, 30, 45 and 60 min after injection.

The haemato-biochemical parameters included estimation of haemoglobin, packed cell volume, TEC, TLC, sodium, potassium, chloride, blood urea nitrogen, inorganic phosphorus and total proteins. Statistical analysis of data was done using student 't' test at 1% and 5% level of significance (Snedecor and Cochran 1980).

Detomidine hydrochloride is a non-opioid potent sedative and analgesic agent (Clark and Taylor 1986; Alitalo *et al.*1988). The dose rate computed for yak (0.025 mg/kg) is significantly lower as used in horses (Clarke and Taylor 1986). The dose rate was computed after pilot trials based on the subjective analysis. Detomidine produced dose dependent sedation in the present study. The average weak time, down time and recovery time were 71.67 ± 15.776 sec, 7.56 ± 1.9 min (sitting) and 12.14 ± 2.93 min (lateral) and 47.8 ± 5.42 min (standing ataxia) and 61.33 ± 6.00 min (normal gait) respectively. Higher doses caused good sedation and moderate analgesia up to 74 min on trunk and up to 45 min on limbs in infant calves (Peshin *et al.* 1991). In this study the sedation was observed for 55-60 min but analgesia was present for only 10-15 min. Nociceptive reflexes as tested with forceps were suppressed.

Urination was a constant feature in this study, which was most probably due to alpha-2 adrenoceptors agonism in kidneys. The diuretic effect of detomidine was also reported in horses (Short *et al.* 1986).

No significant changes were observed in temperature and haematological values. Highly significant bradycardia ($P< 0.01$) was observed for 15 min after injection of detomidine hydrochloride, which remained up to 30 min (41.4±3.0 and 46.7±2.23 in comparison to base value of 66.6±4.58). Thereafter heart rate increased. In yak the bradycardia could be due to alpha-2 agonistic activity of detomidine on central component of adrenoceptors. Electrocardiographic changes revealed an increase in T- wave amplitude after 5 min interval in all the animals. Sino-atrial block was observed only in 2 animals at 5 and 15 min intervals respectively. One animal revealed atrio-ventricular block at 15 min interval and bifid P-wave was seen in another yak. The primary T-wave changes without any change in QRS complex are the common observations in ECG recording with various anaesthetics in most of the species of animals. In this study the minor ECG changes such as SA block or AV block could be due to myocardial hypoxia because of significant oligopnea. Highly significant ($P<0.01$) decrease in respiratory rate was seen at 15 min interval that remained as such up to 60 min. The decrease in respiratory rate seen in yaks is comparable to horses (Lowe 1985) and cow calves (Sharma *et al.* 1991). The detomidine did not produce any change in the rectal temperature. There were no changes of clinical importance in blood and plasma concentration of different components, which remained within normal range when, compared to base values. Therefore, possibility of any systemic toxicity of detomidine is excluded. It was concluded that detomidine hydrochloride, @ 0.025 mg/kg IM, can safely be used for effective sedation in yaks.

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SUMMARY

Detomidine hydrochloride, @ 0.025 mg/kg IM, was used to study clinico-physiological and haemato-biological effects in 7 yaks of either sex. Sedation was produced for 55-60 min with analgesia remaining for only 10-15 min. Urination was a constant feature. Highly significant bradycardia and decrease in respiration rate were noticed. Rectal temperature and blood and plasma concentrations of different components remained within normal range.

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Effect of dinitroaniline herbicide on certain blood enzyme activities in broiler chicks

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Herbicides of dinitroaniline group are being extensively used in large number of important crops. Though considered as moderately toxic, its widespread and continuous use may cause deleterious effects in animals. Limited data on the toxicological studies of this herbicide indicated its deleterious effects on various metabolic processes including hyperexitability and tremors (Gupta *et al.* 1983), stimulation of hepatic microsomal system in rats and tremors of legs and whole body, loss of balance and tonic spasm particularly of hind legs in sheep (IVRI 1981). Alterations in locomotor activity alongwith inhibition of blood esterases notably cholinesterase and carboxylesterase are characteristic of organophosphate compound toxicity (Jorthner and Ehrich 1987, Capodicasa *et al.* 1991) but no such information is available for basalin. The present study was therefore, undertaken to know existance of any such correlation between use of dinitroaniline herbicide and animals to consider this correlation as a possible indicator of basalin-induced toxicity.

Day-old broiler chicks (32) were procured from the College of Animal Science, and were kept in thermostatically controlled batteries for 1 week. These were then randomly divided into 4 groups each containing 8 chicks and were transferred into eages. The birds of groups 1, 2 and 3 were given feed containing 500, 1000 or 1500 ppm dinitroaniline herbicide, respectively, for 8 weeks in a controlled manner so that each bird received its average dose @ 50 mg/kg in group 1, 100 mg/kg in group 2 and 150 mg/kg in group 3. The control group birds were given equivalent amount of normal feed. The doses used were ¼, ½, ¾ of initial dose which produced apparent sign of toxicity as observed in our own laboratory that dinitroaniline herbicide @ 200 to 500 mg/kg given orally to chicken produced alteration of gaits followed by loss of balance and respiratory distress within 10-15 min, which was reversible after 2-10 hr (unpublished data). Blood samples, collected at 2-week-intervals were used to estimate enzyme activities, viz. plasma acetylcholinesterase (Correll and Ehrich 1991), plasma carboxylesterase (Levine and Murphy 1977), plasma acid phosphatase (Abou-Donia 1978), serum alkaline phosphatase (Frankel and Reitman 1963) and serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) (Yetzidis 1960). For histopathological examinations, paraffin embedded sections of liver of 5-6 µm thickness from all the 32 chicks after 8 weeks were cut and stained with haematoxylin and eosin following the procedure of Lillie (1965) and Parsons *et al.* (1984). The data were subjected to 1-way analysis of variance for statistical analysis (Snedecor and Cochran 1967) and $P<0.05$ was taken as statistically significant.

The various plasma and serum enzyme activities measured at 2-week-intervals, are presented in the Table 1. The acetylcholinesterase and carboxylesterase activities were inhibited in a dose dependent manner. Significant reduction of acetylcholinesterase activity was observed after 8 weeks in group 2 and from 2 weeks onwards in group 3. Enzyme activity for plasma carboxylesterase showed significant reduction in all the 3 groups at 8 weeks. Inhibition of plasma acetylcholinesterase and carboxylesterase by various pesticides were reported by Jortner and Ehrich (1987), Capodicasa *et al.* (1991) and Rishi and Garg (1997). The increase in plasma acid phosphatase activity is an indicator of lability of lysosomal membrance of hepatic tissues or of nerves and neurolgia (Ntiforo and Stein 1967). It seems therefore that dinitroaniline herbicide at levels 1000 or 1500 ppm in feed could affect lysosome labilization. Increase in acid phosphatase activity alongwith reduced plasma cholinesterase activity was reported by Abou-Donia (1978) in leptophos treated hens who suggested this observation as warning index for over exposure to teptophos. Release of acid phosphatase in liver damage causing increased activity of enzyme in plasma was also reported for teptophos-, triorthocresyl phosphate- and malathion-treated birds (Ntiforo and Stein 1967). The serum alkaline phosphaase activities significantly increased only in group 3 chicks receiving 1500 ppm dinitroaniline containing feed. However, the activities of serum alkaline aminotransferase (ATL) and serum aspartate

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Table 1. Effect of dinitroaniline herbicide on blood enzyme activities in broiler chicks

Weeks	Groups	Dinitroaniline herbicide in feed (ppm)	Enzyme activities			
			Esterases		Phosphatases	
			Acetylcholinesterase (ACHE)+	Carboxylesterase (CE)+	Alkaline phosphatase (ALP)++	Acid phosphatase (ACP)+
2	C	0	0.217±0.017	0.093±0.006	32.99±1.41	0.125±0.003
	1	500	0.212±0.085 (97.23)	0.080±0.006 (94.62)	30.47±1.22 (92.36)	0.188±0.004 (94.40)
	2	1000	0.206±0.029 (94.93)	0.084±0.006 (90.32)	30.80±0.006 (93.36)	0.124±0.005 (99.20)
	3	1500	0.197±0.032 (90.88)	0.079±0.006 (84.95)	38.67±0.83 (117.21)*	0.126±0.004 (100.80)
4	C	0	0.228±0.063	0.123±0.006	30.32±1.97	0.128±0.003
	1	500	0.216±0.065 (96.94)	0.155±0.006 (93.49)	30.93±0.093 (102.01)	0.125±0.005 (97.65)
	2	1000	0.208±0.080 (93.71)	0.108±0.006 (90.38)	28.42±0.006 (93.73)	0.130±0.004 (101.56)
	3	1500	0.201±0.037 (90.35)	0.104±0.008 (84.55)	42.08±1.81 (138.78)*	0.139±0.005 (108.59)
6	C	0	0.219±0.079	0.110±0.006	32.80±1.15	0.138±0.004
	1	500	0.215±0.078 (97.86)	0.086±0.006 (86.00)	31.48±1.62 (95.97)	0.126±0.004 (91.30)
	2	1000	0.205±0.031 (93.49)	0.078±0.008 (78.00)	32.86±0.008 (100.80)	0.150±0.004 (108.69)
	3	1500	0.187±0.027 (84.48)*	0.068±0.006 (68.00)*	42.36±1.34 (129.14)*	0.168±0.004 (121.73)*
8	C	0	0.241±0.049	0.113±0.006	32.60±1.00	0.130±0.004
	1	500	0.229±0.052 (94.83)	0.080±0.006 (74.34)*	31.77±0.094 (97.45)	0.129±0.004 (99.23)
	2	1000	0.219±0.027 (90.82)*	0.070±0.006 (61.95)*	34.93±0.006 (107.14)	0.152±0.004 (161.92)*
	3	1500	0.185±0.072 (76.68)*	0.062±0.006 (54.87)*	46.60±1.35 (142.94)*	0.223±0.005 (171.53)

+Mean value±SE (n=8) represents optical density values; ++ mean value±SE (n=8) represents IU/litre serum; values in parenthesis indicate per cent of control activity; *values differ significantly from control within column.

aminotransferase (AST) were not altered in any group except that mild alteration of ALT occurred in group 3 after 6 and 8 weeks (mean values 23.84±1.01 and 30.75±1.05 IU/litre serum against control values of 19.87±1.08 and 19.67±0.72 IU/litre serum respectively). The histopathological examination of liver slices of all the 32 chicks after 8 weeks treatment indicated mild congestion and few focal areas in only group 3 chicks.

Thus increase in alkaline phosphatase activity alongwith increase in acid phosphatase and decrease in acetylcholinesterase activity in chicks receiving feed containing 1500 ppm dinitroaniline herbicide daily for 8 weeks indicated mild hepatotoxic effects which were further supported by histopathological studies of liver in these chicks.

SUMMARY

A dinitroaniline herbicide, in feed at 500, 1000 and 1500 ppm level, when given to broiler chicks for 8 weeks caused marked inhibition of plasma acetylcholinesterase, plasma carboxylesterase and elevation of plasma acid phosphatase and serum alkaline phosphatase activities in a dose dependent manner. Histopathological examination of liver in chicks receiving feed containing 1500 ppm herbicide examination mild morphological changes after 8 weeks exposure, thus indicating mild hepatotoxic effect.

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Effect of varying levels of rumen ammonia concentration on certain blood constituents

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Ammonia concentration in rumen fluid is the most important constraint governing microbial growth. The manipulation of the rumen to enhance protein availability to ruminant is particularly important since bypass protein sources are not available generally and they are expensive. Therefore supplementation must provide adequate levels of ammonia in the rumen for more microbial protein synthesis. Availability of this protein reflects change in certain blood metabolites. Keeping in view this fact the present study was carried out.

Three healthy fistulated male bufalo calves of 1 to 2 years of age were fed ration comprising of concentrate and wheat straw in 35.65 ratio once daily for 3 weeks. The quantity of the ration fed to each animal was computed according to the standards recommended by NRC (1978). After the expiry of 3 weeks adaptation period, blood samples were collected from individual animals before feeding (oh) and at 2, 4 and 6 hr post feeding for 2 consecutive days. These animals served as the control group. The same animals were given intra ruminal infusion of 3 different levels of ammonium bicarbonate i.e. 50g treatment 1, 100g treatment 2,and 150g treatment 3. The scheduled quantity of ammonium bicarbonate in various treatments was dissolved in distilled water to make a volume of 2 litres and was infused intra ruminally at the rate of 350 ml/hr with the help of I.V. set for 6 hr for 10 days. Thereafter blood samples were collected on the expiry of each treatment period at similar intervals as in control group.

The blood samples were analyzed for blood glucose, blood urea nitrogen BUN, total blood protein, serum glutamate oxaloacetate transaminase SGOT and serum glutamate pyruvate transaminase (SGPT) with the help of semi auto analyzer using the reagent rit.

The overall mean concentrations of blood glucose were 64.23 mg/dl for control and 58.91, 56.76 and 42. 72 mg/dl for treatment 1, 2 and 3 respectively (Table).

The data revealed a gradual decrease in blood glucose concentration with increasing levels of ammonium bicarbonate infusion. Similar observations have been reported by Leonard (1977). However, blood glucose concentration in various groups studied fell in the normal range (45 to 65 mg/dl) as reported in buffaloes (Bapat 1981, Garg and Nangia 1985). Since the major source of glucose in ruminants is the production and absorption of rumen propionate (Cridland 1984), the difference in blood glucose may be attributed to variation in the concentration of rumen propionate in different groups.

The overall mean concentrations of BUN were 10.37 mg/dl for control and 12.59, 16.87 and 18.45 mg/dl for treatments 1, 2 and 3, respectively, (Table 1) and fall within safe limit without hampering physiological function (Dabadghao *et al.* 1991). Results showed that blood urea nitrogen increased linearly with the higher level of ammonium bicarbonate infusion. The BUN level depends on absorption of the ruminal ammonia which is affected by rumen pH. Ammonia exists as free ammonia at high pH and as ammonium ions at lower pH. As tissue membranes are permeable to the lipid soluble ammonia form but impermeable to the charged ammonium form, absorption of ammonia will be greater at higher pH than at a low pH. Therefore a small change in rumen pH through its effect on unionized ammonia concentration can have a marked effect on ammonia absorption from the rumen. Since ammonium bicarbonate was infused intraruminally more ammonia may have been absorbed in treatment groups due to higher rumen pH in these animals as compared to control animals.

The overall mean values of total blood protein were 9.64 g/dl for control and 7.08, 8.22 and 8.61 g/dl for treatments 1, 2 and 3, respectively, and were lower in treatment groups as compared to those of control. However, the blood protein values recorded in the present study were in agreement with the values reported by Paul and Vadlamndi (1974) and Sharma *et al.* (1994) in ruminant species.

The concentration of SGOT were 117.53 U/L for control and 175.70, 175.66 and 209.5 U/L for treatments 1, 2 and 3 respectively and that of SGPT were 28.52 U/L for control and 32.47, 35.61 and 40.83 UL for treatments 1, 2 and 3 respectively. The values in respect of both SGOT and SGPT

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Table 1. Influence of ammonium bicarbonate infusion on blood glucose, blood urea nitrogen blood protein, serum glutamate oxaloacetate transaminase and serum glutamate pyruvate transaminase

Paramenters	Treatment	Pre-feeding (mean±SE)	Post-feeding hr (mean±SE)			Overall r (mean±SE)
		0	2	4	6	
Blood glucose (mg/dl)	Control	56.48^{a}±2.52	65.21^{a}±5.02	68.60^{a}±3.75	66.60^{a}±2.70	64.23^{a}±1.95
	Treatment 1	57.06^{a}±3.21	63.53^{a}±3.18	60.76^{b}±2.20	54.28^{b}±5.70	58.91^{b}±1.92
	Treatment 2	57.01^{a}±2.66	55.68^{b}±3.09	57.08^{b}±4.66	57.21^{b}±0.74	56.76^{b}±1.46
	Treatment 3	43.23^{b}±5.03	38.15^{c}±3.34	44.81^{c}±1.89	44.71^{c}±1.71	42.72^{c}±1.63
Blood urea nitrogen (mg/dl)	Control	11.28^{a}±0.58	11.00^{a}±1.09	09.70^{a}±0.40	09.53^{a}±0.71	10.37^{a}±0.37
	Treatment 1	13.30^{b}±0.70	13.70^{b}±1.72	10.80^{b}±0.72	12.58^{b}±1.25	12.59^{b}±0.59
	Treatment 2	15.93^{c}±0.91	16.11^{c}±1.02	18.66^{c}±1.05	16.80^{c}±0.49	16.87^{c}±0.47
	Treatment 3	16.80^{c}±0.34	16.65^{c}±0.61	20.76^{d}±0.71	19.58^{d}±0.55	18.45^{d}±0.45
Total blood protein (G%)	Control	09.77^{a}±0.91	10.61^{a}±0.98	10.95^{a}±1.10	07.21^{a}±0.67	09.64^{a}±0.53
	Treatment 1	07.82^{b}±0.87	06.09^{b}±0.33	08.92^{b}±1.48	05.47^{b}±0.55	07.08^{b}±0.51
	Treatment 2	10.23^{ac}±1.70	07.21^{b}±1.00	08.92^{b}±1.98	06.51^{a}±0.43	08.22^{ab}±0.73
	Treatment 3	08.09^{b}±0.89	08.07^{bc}±1.17	10.02^{a}±1.13	08.26^{ac}±0.72	08.61^{ac}±0.47
Serum glutamate oxaloacetate transaminase (U/L)	Control	115.30^{a}±4.80	125.30^{a}±3.54	107.00^{a}±4.74	121.70^{a}±3.81	117.53^{a}±2.46
	Treatment 1	183.50^{b}±19.60	165.80^{b}±17.40	176.20^{b}±19.90	177.50^{b}±25.50	175.70^{b}±9.80
	Treatment 2	181.80^{b}±26.60	189.30^{b}±18.80	154.30^{b}±16.40	177.20^{b}±21.20	175.66^{b}±10.20
	Treatment 3	216.70^{c}±21.20	208.30^{bc}±32.20	226.80^{c}±25.50	186.30^{b}±19.70	209.54^{c}±12.10
Serum glutamate pyruvate transaminase (U/L)	Control	26.33^{a}±3.16	30.40^{a}±3.00	30.41^{a}±2.10	26.91^{a}±4.10	28.52^{a}±1.54
	Treatment 1	33.23^{b}±1.73	37.90^{b}±2.04	31.88^{a}±2.29	26.86^{a}±4.28	32.47^{b}±1.53
	Treatment 2	37.25^{b}±1.30	33.90^{b}±2.04	37.85^{b}±3.01	34.31^{b}±2.46	35.61^{ab}±1.23
	Treatment 3	36.78^{b}±4.46	39.58^{bc}±3.27	44.76^{b}±1.69	42.20^{c}±2.02	40.83^{c}±1.55

Values with common superscript do not differ significantly between treatments. Treatments 1, 2 and 3 represent ammonium bicarbonate infusion at 50g, 100g, and 150g levels per day respectively.

were significantly higher in treatment groups as compared to control. Variation in numinal ammonia nitrogen concentration due to infusion of ammonium bicarbonate did exert its effect on the concentration of both the enzymes which seemed to be positively related to ruminal ammonia nitrogen concentration.

SUMMARY

Effect of varying levels of rumen ammonia concentration on certain blood metabolites was studied. Blood glucose level decreased with increasing level of ammonium bicarbonate. Blood urea nitrogen increased with increase in ammonium bicarbonate. Total blood protein was lower in treated group compared to control group. SGOT and SGPT were significantly higher in treated groups.

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Effect of supplemental ammonium chloride on biochemical changes and amelioration of experimental urolithiasis in goats*

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ABSTRACT

Effect of supplemental ammonium chloride on the biochemical changes and amelioration of induced urolithiasis was studied on 12 male Malabari goats of 9 to 12 months age, during the experiment of 84 days. Goats were divided into 2 groups (1 and 2) of 6 animals each and were fed individually at maintenance level. Calculogenic ration A contained 1.194% calcium, 0.0578% phosphorus and 1.202% magnesium. In addition, ammonium chloride was supplemented to the extent of 1% in ration B. Supplemental ammonium chloride did not have any significant influence on body weight gain, feed and protein efficiency and haematological parameters. Serum calcium and phosphorus concentrations were higher, whereas, concentration of serum magnesium was lower. Urine calcium and phosphorus levels significantly decreased whereas urine magnesium and nitrogen levels remained unaffected. Apparent availability of calcium, phosphorus, magnesium and nitrogen were not influenced significantly. Supplemental ammonium chloride causes significant increase in per cent retention of calcium and phosphorus. Presence of visible sand like calculi and micro-calculi in the kidney with other gross histopathological lesions in the kidney and bladder as in group 1 were not observed in group 2, which confirmed the possible anticalculogenic effect of ration B. It is concluded that supplemental ammonium chloride was effective in the prevention of urolithiasis in goats when fed with high magnesium calculogenic ration possibly due to increased excretion of chloride ions in the urine.

Key words: Amelioration, Ammonium chloride, Goats, Urolithiasis

Among the causative factors known to cause urolithiasis, mineral imbalance particularly calcium, phosphorus and magnesium are the most important for farm animals especially ruminants (Bellenger *et al.* 1981). High dietary magnesium causes urolithiasis in goats, sheep and calves (Rice and McMurray 1981). Anticalculogenic activity of some of the chemical agents prevents urolithiasis in some farm animals possibly by reducing the urine pH (Bushman *et al.* 1967). Crookshank (1970) reported that among the several ammonium salts compared, ammonium chloride had better anticalculogenic activity with significantly higher feed efficiency in lambs. Hence the investigation was taken up to study the biochemical changes and to ascertain the anticalculogenic effect of ammonium chloride while supplemented with high magnesium calculogenic ration in goats.

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MATERIALS AND METHODS

Male Malabari goats (12) of 9-12 months age were selected and divided into 2 groups (1 and 2) of 6 animals in each. A basal concentrate mixture was prepared by using wheat, Bengal gram, gingelly oil-cake, black gram husk, salt and mineral mixture. Hybrid Napier grass with 34.93% DM was chopped and fed to animals as roughage part of the ration. The per cent chemical compositions of the compounded feed and grass are presented in Table 1. Basal calculogenic

Table 1. Chemical compositions of compound feed and grass (% of dry matter)

Nutrients	Compound feed	Hybrid Napier
Crude protein	22.94	9.75
Crude fibre	10.01	43.69
Ether extract	4.12	3.07
Nitrogen free extract	51.66	39.64
Total ash	11.27	3.85
Calcium	0.84	0.14
Phosphorus	0.51	0.18
Magnesium	0.31	0.23

ration (ration A) was prepared by using compound feed (concentrate) fortified with calcium oxide, phosphorus pentoxide and magnesium oxide to have the estimated levels of 1.194, 0.578 and 1.202% of calcium, phosphorus and magnesium respectively. Ration B was constituted by using calculogenic ration supplemented with ammonium chloride to the extent of 1%. Animals in groups 1 and 2 were fed with rations A and B respectively. All the animals were fed individually at maintenance level with weighed quantities of concentrate and roughage in the ratio of 4: 1. Drinking water was provided *ad lib.* throughout the experimental period of 84 days. All the animals were subjected to balance trials for 5 days, initially before the feeding of experimental ration and later at the end of feeding trial to assess the balance of calcium, phosphorus, magnesium and nitrogen. Blood samples were collected from all the animals at monthly intervals through jugular puncture for the estimation of total erythrocyte count (TEC), total leukocyte count (TLC), haemoglobin, plasma protein, serum calcium, phosphorus and magnesium. Calcium and magnesium levels were measured in feed, faeces, urine and serum by atomic absorption spectrophotometer (AOAC 1990). Feed and faecal phosphorus levels were determined colorimetrically (AOAC 1990). Urine and serum phosphorus was determined by spectrophotometer using commercial phosphorus kit. Statistical analyses of data were carried out by using student t-test (Snedecor and Cochran 1980).

During the experimental period, all the animals were closely observed for any clinical signs of obstructive urolithiasis. Fresh urine samples were collected at monthly intervals to determine the pH and examined microscopically for the presence of crystals. The crystals were collected and subjected to qualitative chemical analysis (Winer 1959). All the animals were slaughtered at the termination of experiment and the urinary organs were collected and subjected to detailed postmortem examination for the presence of gross calculi and other gross lesions. The calculi materials were collected and subjected to spot test (Winer 1959) for identification of its chemical composition. The specimens from kidney and bladder were collected and preserved in neutral buffered formalin (10%) for histopathological study with routine paraffin embedding method and sections were stained with haematoxylin and eosin.

Table 2. Effect of supplementation on body weight gain and feed intake of goats

Parameters	Group 1	Group 2
Initial body weight (kg)	22.39±0.93	22.53±1.24
Final body weight (kg)	27.59±4.50	27.82±1.70
Cumulative weight gain (kg)	5.20±0.42	5.29±0.44^{NS}
Average daily gain (g)	61.90±0.004	62.98±0.002^{NS}
Average daily dry matter consumption (g)	928.57	917.98b^{NS}
Dry matter consumption per 100 kg body weight	3.71	3.64
Average cumulative feed efficiency	15.02±0.141	14.58±0.109^{NS}
Average cumulative protein efficiency	2.69±0.022	2.6±0.20^{NS}

NS, Not significant.

RESULTS AND DISCUSSION

Average daily gain, dry matter intake (DMI), feed efficiency and protein efficiency values (Table 2) of goats in group 1 fed with calculogenic ration (ration A) and group 2 fed with calculogenic ration supplemented with ammonium chloride to the extent of 1% (ration B), revealed no significant differences between them. Crookshank (1970) observed that supplemental ammonium chloride along with calculogenic ration significantly increased the feed efficiency in lambs. A significant effect in this regard was not observed in this investigation, perhaps because of higher protein level in the basal ration. Data on TEC, TLC, haemoglobin and plasma protein values (Table 3) were within the normal range in all the animals irrespective of the rations, indicating that supplemental ammonium chloride did not have any significant influence on these parameters.

Both the groups had gradual decrease in the serum calcium level (Table 3) during the experiment. But the rate of

Table 3. Effect of supplementation on important blood parameters in goats fed calculogenic ration

Parameters	Group 1		Group 2	
	Initial	Final	Initial	Final
TEC (10^6/mm^3)	12.41±0.72	12.70±0.63 a	12.45±0.63	13.34±0.66 a
TLC (10^3/mm^3)	11.93±0.51	11.08±0.69 a	12.16±0.72	11.86±0.59 a
Haemoglobin (g/dl)	8.37±0.26	8.27±0.22 a	8.57±0.27	8.40±0.23 a
Plasma protein (g/dl)	7.77±0.29	8.09±0.36 a	7.38±0.62	7.43±0.48 a
Serum calcium (mg/dl)	10.41±0.18	7.08±0.23 a	10.32±0.21	8.63±0.20 b
Serum phosphorus (mg/dl)	5.35±0.11	7.67±0.07 a	5.31±0.16	6.10±0.10 b
Serum magnesium (mg/dl)	2.60±0.12	6.08±0.15 a	2.53±0.20	4.70±0.14 b

a, b, Means bearing different superscripts differ significantly ($P<0.01$).

decrease in serum calcium was significantly (P<0.01) pronounced in group 1, when compared to group 2. Serum phosphorus and magnesium concentration (mg/dl) were significantly (P<0.01) higher in group 1, when compared to that of group 2. These results tend to suggest that high magnesium calculogenic ration had a tendency to decrease the absorption of dietary calcium and increased the absorption of dietary phosphorus and magnesium. Decrease in serum calcium, and increase in serum phosphorus and magnesium because of higher dietary magnesium were reported by James (1973) and Chicco *et al.* (1973). The results also tend to suggest that supplemental ammonium chloride significantly (P<0.01) suppressed the effect of high dietary magnesium on serum calcium, phosphorus and magnesium and maintained their levels within the normal range.

Total urine calcium, phosphorus and magnesium (g/day) and urine concentrations (mg/dl) of these elements (Table 4) were gradually increased during the progress of experiment in both groups. Goats maintained under group 1 excreted significantly higher calcium (P<0.05) and phosphorus (P<0.01), when compared to that of group 2, whereas no significant difference could be noticed between the groups with regard to magnesium excretion. These results suggested that supplemental ammonium chloride increased utilization of calcium and phosphorus and did not have any significant influence over the utilization of magnesium. Higher urinary excretion of calcium, phosphorus and magnesium because of high magnesium in the diet were observed by James (1968, 1973) and Chicco *et al.* (1973). Urine nitrogen excretion increased gradually in both the groups towards the termination of experiment and no significant difference existed between the groups, suggesting that supplemental ammonium chloride did not have any influence over the utilization of nitrogen. Apparent availability of calcium, phosphorus, magnesium and nitrogen (Table 4) were also unaffected due to supplemental ammonium chloride.

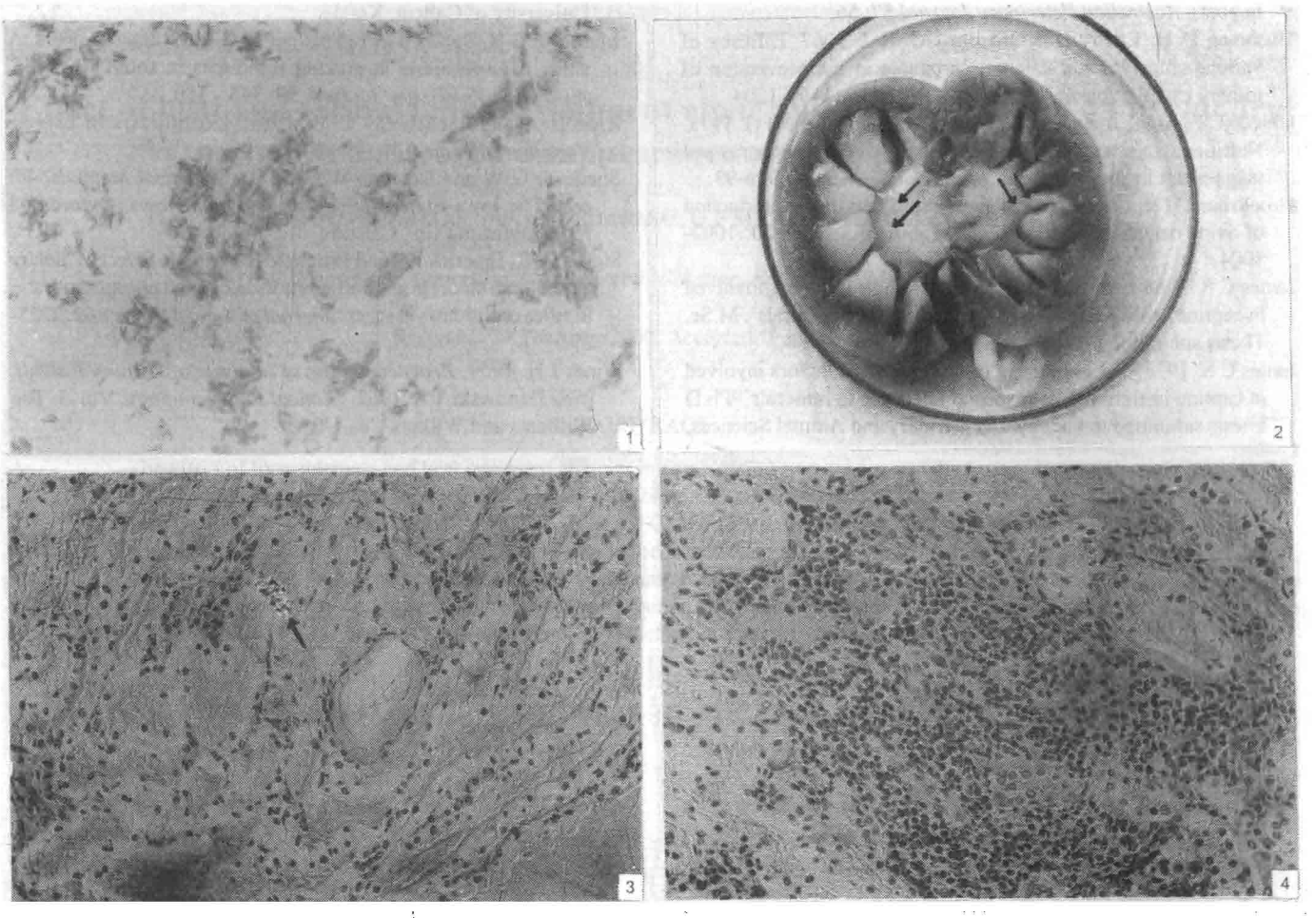
Group 2 had significantly high calcium (P<0.01) and phosphorus (P<0.05) retention when compared to group 1, indicated that supplemental ammonium chloride significantly counteracted the influence of magnesium over calcium and phosphorus retention and thereby increased the per cent retention of calcium and phosphorus. Per cent retention of magnesium and nitrogen were increased in both the groups towards the termination of experiment and no significant differences could be observed between the groups. Higher the dietary magnesium in the ration, higher will be the retention of magnesium but had no influence over the per cent retention of nitrogen (James 1968, 1973). Supplemental ammonium chloride did not have any significant influence over per cent retention of magnesium and nitrogen.

Microscopical examination of urine samples collected from goats in group 1 showed severe crystalluria having crystals with the shape of prismatic "coffin lid", which is a typical characteristic of struvite crystals (Fig. 1), whereas crystalluria was absent in group 2. Chemical analysis of the crystals confirmed the presence of ammonium, magnesium and phosphate. Bushman *et al.* (1967) observed that supplemental ammonium chloride causes a significant shift in urine pH from alkaline to acidic, which prevented the crystal growth and calculi formation. Such a finding was not observed during this study, however, it seems that goats fed ration containing supplemental ammonium chloride had a slight reduction (8.4 to 8) in urine pH along with appreciable increase in urine volume (1860.63 ml/day) than animals maintained under group 1 (1412.9 ml/day). These results tend to suggest that reduction of urine pH may be attributed to the excess chloride ions supplied by the supplemental ammonium chloride as observed by Bushman *et al.* (1967) or may be due to the diuretic effect (Stewart *et al.* 1991), which prevented supersaturation of urine by the crystalloids and consequent crystal growth. Severe crystalluria and consequent development of urinary calculi exhibited by group 1 may be explained in such a way that when high magnesium were fed, the urine concentration of magnesium and phosphorus will be increased and also the urinary urea which is easily decomposed to ammonium ions resulting in high level of ammonium ions in the

Table 4. Effect of supplementation on mineral and nitrogen balance in goats

Period	Parameters	Calcium		Phosphorus		Magnesium		Nitrogen	
	Group	1	2	1	2	1	2	1	2
Final (day 84)	Intake	13.25	13.35	6.593	6.626	13.51	13.57	39.54	41.06
	Outgo								
	Faecal (g/day)	5.115^{a}	4.304^{a}	3.74^{a}	3.53^{a}	6.101^{a}	6.503^{a}	11.61^{a}	12^{a}
	Urine (g/day)	0.191^{c}	0.128^{d}	0.53^{a}	0.231^{b}	1.617^{a}	1.58^{b}	11.45^{a}	10.95^{a}
	(mg/dl)	12.86	5.589	35.69	10.08	108.9	68.97	-	-
	Total (g/day)	5.305	4.432	4.27	3.761	7.718	8.084	23.06	22.95
	Balance (g/day)	7.939	8.917	2.232	2.865	5.793	5.432	16.49	18.12
	Retention (%)	59.85^{a}	66.55^{b}	35.24^{c}	43.24^{d}	42.49^{a}	40.01^{b}	41.3^{a}	43.5^{b}

Means bearing different superscripts differ significantly; a,b (P<0.01) and c,d (P<0.05).



Figs 1-4. **1.** Urine crystals (fresh mount) ×100. **2.** Visible calculi in the kidney. **3.** Tubular destruction, developing microcalculi, hyalinized pink stained proteinaceous mass. H&E × 450. **4.** Inflammation with infiltration lymphocytes and macrophages. H&E × 450.

urine, a condition optimal for precipitation of highly insoluble magnesium ammonium phosphate crystals as suggested by McIntosh *et al.* (1974). From both the experimental groups none of the animals exhibited any clinical signs of obstructive urolithiasis during the experimental period.

Postmortem examination of goats under group 1 had moderate enlargement of kidney with white patches and focal haemorrhages over the cortex, numerous visible sand like rudimentary calculi in the pelvis (Fig. 2) and thickened mucosal layer with focal haemorrhages and erosions in the bladder. Absence of obstructive urolithiasis (James 1968, 1973) in goats when maintained on identical calculogenic ration was not fully observed in the present investigation, perhaps due to shorter experimental period (84 days). Histopathological examination of kidneys showed severe destruction of tubular epithelium, dilated tubules with proteinaceous casts and microcalculi of varying stages of development (Fig. 3) dilated blood vessels with infiltration of inflammatory cells in the interstitial area. Mucosal cells of the urinary bladder were engorged and sloughed off in some places. Urinary organs collected from goats in group 2 had slight enlargement of the kidney. No visible calculi in urinary tract or any other gross lesions in the urinary organs of goats could be observed. Tubular epithelium of kidneys had hyalinization with pink stained proteinaceous casts in some tubules and focal areas of inflammation and infiltration of inflammatory cells (Fig. 4) in between the tubules. No microcalculi could be detected in the tubules. These gross and histopathological observations confirmed the possible beneficial effect of ammonium chloride in the prevention of urinary calculi in goats.

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Effect of heat and formaldehyde treatment alone and in combination on *in situ* dry matter and nitrogen disappearance of some protein sources

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ABSTRACT

The effect of formaldehyde and heat treatment alone or in combination on nitrogen and dry matter disappearance was studied using 3 fistulated adult male goats maintained on basal diet of moth chara. The experimental feeds used were guar-meal (GM), soybean-meal (SBM), sunflower-cake (SFC) and fish-meal (FM); control consisted of untreated feeds (T_1), while to protect the protein from microbial degradation, feeds were treated with formaldehyde @ 1.5 g/100 g CP (T_2), heated at 150°C for 2 hr (T_3) and treated by combination of above (T_4). The extent of dry matter and nitrogen disappearance was determined by *in situ* polyester bag technique. The individual feeds were incubated in rumen for 2, 4, 6, 8, 10, 12, and 24 hr. T_4 groups showed maximum reduction in nitrogen, disappearance followed by T_2, T_3 and T_1 respectively.

Key words: Formaldehyde, Fistulated buck,Heat, Nitrogen disappearance

Ruminal degradation of quality protein generally results in high ammonia production at a rate much more than that of the microorganisms can incorporate into their own body, leading to a wasteful process either being extracted in the urine or ventilated through lungs. So, for economy, ammonia in the rumen fluid should be present in the optimum concentration. Various techniques have been evolved to protect dietary protein from excessive microbial degradation, viz. physical, chemical or their combination. Degradability of dietary protein in the rumen depends on the dietary protein source and their processing treatments. There is need to screen various protein sources and corresponding treatment to evaluate their efficiency of protein protection. Therefore in this study, effect of formaldehyde and heat treatment alone and in combination, on DM and N disappearance of some protein sources was studied.

MATERIALS AND METHODS

Adult male goats (3) were fitted with permanent rumen fistula of 25 mm diameter. Animals were tied individually and kept on basal feed i.e. moth chara (*Phaseolus aconitifolius*) and water was made available all the time throughout the experimental period. After the fistulation 15 days acclimatization was allowed prior to *in situ* studies. Experimental feeds used were guar- meal (GM), soybean-meal (SBM) sunflower- cake (SFC) and fish- meal (FM). Each feed was grounded to obtain 2 mm particle size and stored in plastic airtight container. The native protein of all the 4 experimental sources being 39.40, 43.20, 42.00 and 65.50% for GM, SBM, SFC, FM, respectively, were protected from microbial degradation in rumen by following treatments, viz. T_1 being control, untreated group, T_2 being, treated with formaldehyde @ 1.5g/100 g CP, T_3 being, heating in hot air oven at 1500 C° for 2 hr and T_4 being combination of T_2 and T_3 treating first with formaldehyde @ 1.5 g/100 g followed by heating in hot air oven at 1500 for 2 hr. Feed sample (5g)was kept in a polyester bag, measuring 10cm x 12 cm with 30 µm mesh size. Each bag, containing sample was incubated on consecutive days in rumen of each animal. The bags were withdrawn after 2,4,6,8,10,12 and 24 hr of incubation. After withdrawal the bags were rinsed with water (Mehrez and Orskov 1977) and were dried in hot air oven at 700°C for 48 hr. The dried residue was weighed together with bags and DM disappearance was calculated and dried residue from bags was removed and analyzed for nitrogen by microkjeldahl method (AOAC1975) and nitrogen disappearance was calculated by the following formula:

$$\frac{\text{Total N incubated - N in residue}}{\text{Total N incubated}} \times 100$$

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Table 1. Effect of formaldehyde, heat and combined treatment on per cent DM and N disappearance*

Feed		Incubation hours						
		2	4	6	8	10	12	24
GM								
T1	DM	42.60	48.21	58.89	65.43	74.28	86.00	97.03
	N	33.75	38.75	50.00	54.79	65.00	70.50	95.00
T2	DM	30.57	39.43	40.99	46.50	58.72	65.44	71.35
	N	12.50	22.50	30.00	36.08	42.50	56.25	76.25
T3	DM	38.45	44.30	52.80	55.82	65.31	76.74	87.30
	N	23.75	27.50	36.25	38.75	46.25	58.83	77.50
T4	DM	26.39	36.36	37.63	45.64	54.46	63.80	91.30
N	9.79	20.00	27.50	30.00	38.50	50.00	71.25	
SBM								
T1	DM	45.00	55.19	58.78	65.90	71.80	78.01	91.30
	N	34.63	42.27	45.00	50.00	56.87	67.50	85.20
T2	DM	32.57	44.33	41.68	46.64	55.37	59.35	66.70
	N	16.45	25.90	28.75	33.75	37.50	42.50	68.75
T3	DM	40.99	49.82	53.71	56.00	63.11	69.61	81.63
	N	23.75	30.00	31.25	32.50	40.00	47.58	73.00
T4	DM	29.87	38.19	41.18	45.92	51.33	56.94	62.58
	N	13.12	24.58	26.25	31.25	28.54	40.00	58.75
SFC								
T1	DM	45.09	55.44	68.75	72.19	77.28	80.88	90.46
	N	43.75	61.66	75.00	76.25	80.00	83.75	96.04
T2	DM	31.50	39.41	51.28	58.72	63.33	68.20	79.19
	N	18.75	29.86	38.75	42.47	46.25	50.00	63.75
T3	DM	36.12	44.30	56.91	62.02	67.93	74.22	84.70
	N	24.99	36.25	47.41	52.50	57.80	65.00	78.91
T4	DM	28.04	35.10	46.23	53.19	62.33	67.89	78.50
	N	16.25	25.00	37.50	38.95	42.50	46.25	62.50
FM								
T1	DM	47.00	52.01	56.08	57.23	58.72	59.13	64.28
	N	54.80	58.07	61.87	63.75	64.79	66.95	71.45
T2	DM	28.20	32.20	35.90	40.06	44.04	48.48	56.56
	N	31.01	35.00	37.50	40.00	41.25	46.25	54.37
T3	DM	30.46	35.84	40.39	44.06	46.38	49.66	54.12
	N	36.66	41.25	43.75	46.87	47.50	51.25	60.00
T4	DM	25.82	30.65	34.78	38.34	42.86	46.12	55.35
	N	26.25	31.25	36.45	37.50	38.75	43.75	51.25

*Each value is mean of six observations

RESULTS AND DISCUSSION

The nitrogen disappearance of untreated and treated protein sources (Table1), showed an increasing trend from 2 to 24 hr of incubation irrespective of feed or treatment.

Formaldehyde reduced nitrogen disappearance as compared to T_1, in all the feeds at similar hours of incubation. In T_3 group i.e. heat treated also showed the reduction in nitrogen disappearance in all the feeds at similar time intervals as compared to control but it was less effective than the formaldehyde treatment in reducing nitrogen disappearance. At 2 hr incubation disappearance was maximum for FM i.e. 54.80%, while at 24 hr minimum disappearance was in FM i.e. 71.45%. In (T4) i.e. combination of formaldehyde and heat treatment maximum reduction in nitrogen disappearance took place as compared to T_1, T_2 and T_3. At 2 and 4 hr incubation minimum disappearance was observed in GM i.e. 9.79 and 20%, respectively, while at 24 hr disappearance was minimum for fish-meal i.e., 51.25%.

The results of DM disappearance of different feeds, untreated and treated are presented in Table 2. The observed trend of DM disappearance was more or less similar to nitrogen disappearance, however, the rate of DM disappearance for vegetable protein sources used were comparatively higher than the rate of nitrogen disappearance of that particular feed at similar time of incubation period. In animal protein i.e. fish-meal, the rate of DM disappearance was lowest compared to nitrogen disappearance at similar

hour of incubation.

The values of nitrogen disappearance of untreated feeds were in agreement with Lohan (1986) and Boer *et al.* (1987). The disappearance of nitrogen from polyester bag was higher in FM as compared to GM and SB since cellulose material in vegetable protein affords some protein protection, (Ganev *et al.* 1979). Formaldehyde, heat and combination of both reduced nitrogen disappearance. Weakley *et al.* (1983), Tiwari and Yadav (1988) formaldehyde treatment and Sampath and Sivaraman (1987) and Schroder *et al.* (1989) reported heat treatment as the effective means, to reduce nitrogen disappearance from rumen.

It may be concluded that formaldehyde plus heat treatment were the most effective in the order followed by formaldehyde and heat, individually, for reducing DM and N disappearance from the rumen of goat.

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Optimization of sample size for chromosomal analysis

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Key words: Cattle, Length ratio, Repeatability, Sample size, Y chromosome

Chromosome preparations at metaphase stage vary in length from one preparation to another because of differences in contraction and differences in response to treatments etc. Consequently, even the members of a pair of homologous chromosome in the same cell can show some differences (Ford 1973). It is necessary therefore to measure the chromosomes in several cells to obtain accurate estimation of chromosomal parameters. Since the availability of sufficient number of metaphase fields for estimating the length variation of chromosome is always a constraint and as it involves a number of steps which requires a lot of time, chemicals, manpower etc., so, an attempt was made to optimize and determine the minimum sample size for cytogenetic study. Various observations on length of Y chromosome in crossbred bulls were used as model parameters for chromosomal analysis.

Blood samples from 20 Karan Fries (Holstein Freisian and Tharparkar crossbred) bulls, maintained at the Artificial Breeding Complex of National Dairy Research Institute, Karnal, were collected at least 3 times to get maximum material for lymphocyte culture. Metaphase plates (35) from each bull were taken to study the length variation. Short-term lymphocyte culture with suitable modifications was carried out for this study.

Quantification of different portions of Y chromosome i.e., short arm of Y chromosome (Yp), long arm of Y chromosome (Yq) and total length of Y chromosome (Yp+q) were carried out from enlarged photographs of metaphase plates, screened under light microscope, by using Dial Calipers, having an accuracy of 0.05 mm. Variations of length of Y chromosome, expressed in terms of length ratio, were estimated using formula as described by Eldridge *et al.* (1983). To determine the minimum number of metaphase plates required for measuring the length ratio of Y chromosome, average length ratios of Y chromosome from each bull were calculated using different number of observation, viz. first 5, first 10, first 15, . . . up to 35 observations, and correlation coefficient were estimated among the mean length ratios at different number of observations. Significance among correlation coefficient was estimated by using 'Z' transformation (Amble 1975).

The repeatability of estimate of average length ratio of Y chromosome at different sample sizes are given in Table 1. Correlation coefficients (r) between the length ratios of first 5, 10, 15, 20, 25 and 30 observation with 35 observations were 0.353, 0.813, 0.780, 0.852, 0.910 and 0.958 respectively. The highest 'r' value (0.958) obtained between 30 and 35 observation was significantly different from the 'r' values obtained by considering 5, 10 and 15 observations with 35 observations whereas it differed nonsignificantly from 'r' values obtained by considering 20, 25 and 30 observation with 35 observations. The increase in number of observations

Table 1. Repeatability of estimate of average length ratio (LR) of Y chromosome at different sample sizes

	AF5LR	AF10LR	AF15LR	AF20LR	AF25LR	AF30LR	AF35LR
AF5LR	1.000	-	-	-	-	-	-
AF10LR	0.5297	1.000	-	-	-	-	-
AF15LR	0.2246	0.8417	1.000	-	-	-	-
AF20LR	0.2855	0.8672	0.9382	1.000	-	-	-
AF25LR	0.3063	0.8052	0.8119	0.8990	1.000	-	-
AF30LR	0.3379	0.8253	0.8138	0.8652	0.9665	1.000	-
AF35LR	0.3528^{a}	0.8128^{b}	0.7801^{b}	0.8518^{bc}	0.9104^{bc}	0.9577^{c}	1.000

Correlation coefficients with similar superscripts do not differ significantly from each other. AF, Average of first.

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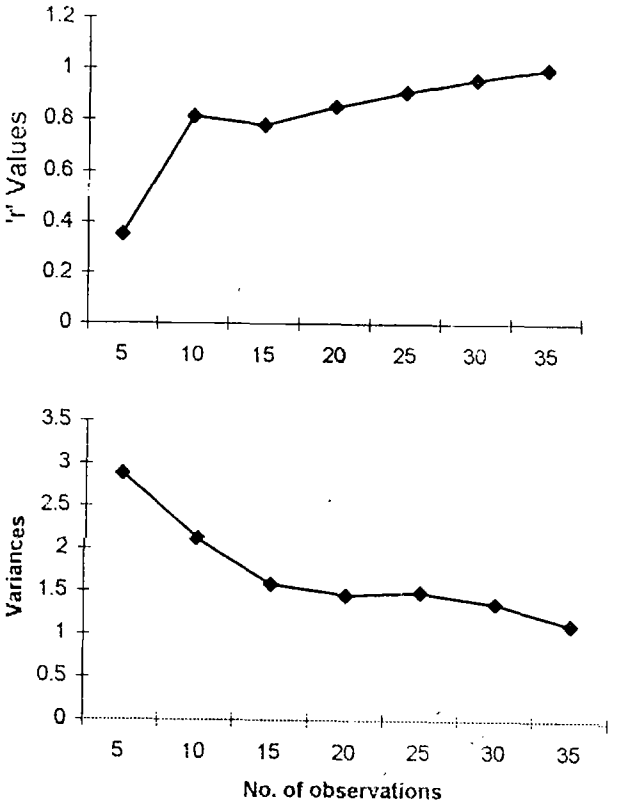

Figs 1-2. **1.** Accuracy of estimates due to increase in number of observations. **2.** Efficiency of estimates due to increase in number of observations.

from 5 to 35 for measuring length ratio of Y chromosome reduces the amount of variability of the character because of consideration of large number of metaphase plates which act as special/temporary environmental variation that ultimately appears in the total variance of the trait. The reduction of the phenotypic variance of the length ratio of Y chromosome represents the efficiency of the estimates considering the optimum number of observations required for the measurement of the trait (Fig. 2). The accuracy from 20 observation did not differ significantly from 25 observations and onward (Table 1; Fig. 1). Hence, it could be inferred that 20 observation would be the minimum and sufficient to measure the length ratio of Y chromosome.

SUMMARY

Blood samples from 20 Karan Fries (Holstein Friesian and Tharparkar crossbred) bulls, were collected for lymphocyte culture to study the length variation of Y chromosome at metaphase stage. An attempt was made to optimize and determine the minimum sample size for cytogenetic study as the availability of sufficient number of metaphase fields for estimating the length variation of chromosome is always a constraint which involves a number of steps which requires a lot of time, chemicals, manpower etc. The result showed that 20 observations i.e. metaphase plates would be the minimum and sufficient to measure the length ratio of Y chromosome.

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Effect of cottonseed feeding on economic traits of Murrah buffalo

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Key words: Buffalo, Cottonseed, Economic traits, Feeding

During early lactation increasing dietary intake fails to keep with rising milk production. The resultant is negative energy balance and higher rate of mobilization of body reserves. Whole cottonseed being a rich source of fibre and energy can overcome this energy deficiency. Therefore, the present investigation was undertaken for 150 days to study the effect of feeding varying levels of cottonseed on milk production performance of lactating buffaloes.

From first to fifth lactation, 16 lactating Murrah buffaloes were taken. Prior to the start of experiment the animals were given adjustment period of 7 days. The buffaloes were divided into 4 groups each having 4 buffaloes of similar stage of lactation, body weight, milk yield and parity. In dietary treatment T_4 (control) concentrate mixture contained 27 parts mustard-cake, 15 parts rice polish and 55 parts barley (15.9% CP and 70% TDN). Mustard-cake in concentrate mixture of control group was replaced with cottonseed to the tune of 75% in T_1, 50% in T_2 and 25% in T_3 group on crude protein basis.

The experimental animals were kept in loose house type shed and were allowed to move freely. Concentrate feeding was done at the milking individually according to their requirement (NDRI Recommendations 1985). Buffaloes were fed with seasonal dry and green fodder. Level of concentrate feeding was adjusted at weekly intervals on the basis of body weight, milk production and quality of green fodder available to the animals to maintain overall nutrient requirements.

The statistical analycis revealed that treatments had no effect on milk yield (Table 1). However, an apparent increase 2.0 to 4.8% in milk yield was noticed in T_1, T_2 and T_3 groups over control. Lactating animals fed with cottonseed produced 3.2% more milk than control group (Patel and Ray 1949, De-Peers *et al.* 1985, Horner *et al.* 1986) while feeding of cottonseed to lactating cows and buffaloes decreased the milk yield (Palmquist 1987).

Fat corrected milk yield was significantly ($P<0.01$) higher in T_1 (8.85 kg) than other groups (Table 1). However, FCM yield in T_2 and T_3 did not differ significantly. The higher FCM yield in cottonseed fed groups was due to higher fat content in their milk. Similar results were obtained for solid corrected milk also. The present findings are in agreement with De-Peters (1975); Horner *et al.* (1986). No significant change was found in fat corrected milk yield of animals fed at different levels of cottonseed in the ration (Anderson *et al.* 1979).

Milk fat content increased with inclusion of cottonseed into the ration and was higher ($P<0.01$) for all groups except control. The fat percentage was increased ($P<0.01$) with increasing proportions of cottonseed in the ration. The high fat content in cottonseed feeding groups may be due to higher amount of fat content in cottonseed. Consequently, cottonseed feeding increases the fat content in milk.

SNF percentage was significantly ($P<0.01$) higher in T_1 and T_2 groups when compared with T_3 and T_4 groups. This showed that only feeding high level of cottonseed influences SNF content of milk, which tally with Tendon (1974) and Anderson *et al.* (1979). However, Tomlinson *et al.* (1981)

Table 1. Average daily milk yield (kg) and milk composition in different dietary treatments

Treatments	Milk yield	6 % FCM	SCM	Fat %	TS %	SNF %	Protein %
T_1	7.348±0.22	8.85±0.28^a	11.57±0.35^a	7.58±0.09^a	18.09±0.10^a	10.51±0.03^a	4.47±0.01^b
T_2	7.27±0.17	8.29±0.19^b	10.89±0.24^b	7.23±0.08^b	17.71±0.04^b	10.48±0.02^a	4.48±0.01^b
T_3	7.45±0.19	8.20±0.20^b	10.80±0.27^b	6.90±0.05^c	17.29±0.06^c	10.39±0.02^b	4.47±0.01^b
T_4	7.14±0.21	7.70±0.21^c	10.19±0.29^c	6.72±0.05^d	17.17±0.07^c	10.36±0.03^b	4.58±0.01^a

Means ($P<0.05$) having different superscripts differ significantly.

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reported significant reduction in SNF content in milk with cottonseed feeding. The mean values of total solids content of milk under different treatments were 18.09±0.10, 17.71±0.04, 17.29±0.06 and 17.17±0.07% for T_1, T_2, T_3 and T_4 respectively. Total solid contents of the milk increases ($P<0.01$) with the increasing level of cottonseed in the feed of buffaloes (Table 1). No significant difference was observed between T_3 and T_4 groups. This indicates that the level of cottonseed feeding influenced the total solid content in milk and it tallys with Tomlinson *et al.* (1981) and De-Peters *et al.* (1985). However, Mulla and Iqbal (1981) and Horner *et al.* (1986) found no improvement in total solid percentage of milk in animals which were fed different levels of cottonseed in the ration.

The protein content was significantly ($P<0.01$) higher in control group than T_1, T_2 and T_3 groups (Table 1). The exact mechanism for the decrease in milk protein in cottonseed feeding is not fully understood. However, a decrease in casein synthesis and role of insulin and glucose availability was observed. Reduced milk protein concentration was found because of Insulin (De-Peters *et al.* 1985). Palmquist and Moser (1981) further suggested that fat feeding may induce insulin resistance and cause reduced transfer of amino acids into mammary gland and hence reduces milk protein synthesis. The results of the present study are in line with the findings of these workers.

SUMMARY

Cottonseed feeding was found beneficial for improving fat corrected milk yield. As the stage of lactation advanced the fat%, solid not fat%, total solid%, and protein % of milk increased.

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Nutritive evaluation of inoculated fermented wheat straw as complete feed for buffaloes

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Key words: Buffaloes, Fermentation, Nutrients utilization, Urea, Wheat straw fermentation

The naturally fermented wheat straw (FWS - straw treated with 3.5% urea at 40% moisture and stacked for 9 days) supplemented with minerals and vitamin A could meet the basal energy and protein requirements for maintenance of adult ruminants (Bakshi *et al.* 1986, 1987). Efforts were made to enhance the fermentation by using freshly prepared FWS as crude inoculum in the subsequent batches of urea wheat straw. The straw preparations were evaluated as complete feed for buffaloes.

In a batch, 14 kg urea dissolved in 200 litres water was sprayed/sprinkled on 386 kg wheat straw, mixed and stacked for 9 days (FWS_9). The freshly prepared FWS_9 was used as crude inoculum (@ 5% on DM basis) in the subsequent batches of urea wheat straw (3.5: 96.5 at 40% moisture). Each batch was stacked for 6 days ($IFWS_6$) or 9 days ($IFWS_9$) for natural fermentation and 2 experiments were conducted. In the first experiment 10 male Murrah buffaloes (average body weight 475 ± 25 kg) divided into 2 equal groups were fed complete diet containing either FWS_9 or $IFWS_9$ with 2 kg available green fodder and mineral supplement. A 7-day metabolism trial was conducted after 30 days adaptation period. In the second experiment, another set of 10 male Murrah buffaloes (average body weight 325 ± 29 kg) divided into 2 groups were fed *ad lib.* either $IFWS_6$ or $IFWS_9$ with 2 kg available green fodder and mineral supplement, for 30 days followed by a 7 days metabolism trial. Biochemical changes in the rumen were assessed by feeding each straw preparation (FWS_9, $IFWS_6$ or $IFWS_9$) separately i.e. one at a time to 3 rumen fistulated male buffaloes for 21 days. Rumen liquor samples were collected before feeding and then at 2 hr interval up to 12 hr post-feeding. The strained rumen liquor (SRL) was preserved after adding few drops of saturated mercuric chloride and stored at 4°C. The finely ground samples of feed, feed residue and faeces were analysed for total ash and CP (AOAC 1984), cellulose (Crampton and Maynard 1938) and other cell wall constituents (Robertson and VanSoest 1981). The SRL was analysed for total-N (AOAC 1984) and TCA precipitable-N (Cline *et al.* 1958). The data of the 2 experiments were analysed separately by using completely randomized design (Snedecor and Cochran 1968).

The hemicellulose content was lower in $IFWS_9$ compared to FWS_9 indicating that microbes utilized it for their proliferation thereby enhancing the fermentation rate and resulting in increased crude protein content of $IFWS_9$ (Table 1). The significantly ($P<0.05$) higher dry-matter intake, CP digestibility and N-retention in animals fed $IFWS_9$ than those fed FWS_9 indicated that inoculation had improved the nutritive value of fermented straw. It was associated with considerably higher concentration of TCA precipitable N in the SRL of animals fed $IFWS_9$ (66.55%) than that fed FWS_9 (60.95%). Reverse trend was observed in NPN concentration in the SRL of animals fed $IFWS_9$ or FWS_9 (33.45 or 39.05% respectively). The higher ($P<0.05$) apparent biological value in $IFWS_9$ than FWS_9 group indicated better efficiency of utilization of absorbed-N.

The higher CP content in $IFWS_6$ than $IFWS_9$ without any impact on rest of the constituents indicated that period of fermentation in inoculated straw could be reduced from 9 to 6 days. The CP digestibility was significantly ($P<0.05$) higher in $IFWS_6$ than if the fermentation was allowed to continue up to 9 days ($IFWS_9$). The reduction in fermentation period did not have any adverse effect on dry matter intake, digestibility of CWC, N-retention, TCA precipitable N in SRL except low apparent biological value which could be due to relatively higher urinary-N excretion.

The results of the two experiments conclusively revealed that nutritive value of FWS_9 can be improved by inoculation. Moreover, the fermentation period could be reduced to 6 days without any adverse effect on the nutritive value of straw preparation.

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SUMMARY

The freshly prepared naturally fermented wheat straw

Table 1. Effect of feeding fermented straw preparations on the nutrient utilization in adult buffaloes

Parameter	Experiment 1			Experiment 2		
	FWS_9	$IFWS_9$	Pooled SE	$IFWS_6$	$IFWS_9$	Pooled SE
Composition (%)						
CP	7.12	7.69	-	9.00	8.30	-
Cellulose	41.25	43.20	-	41.85	41.90	-
NDF	82.10	80.60	-	81.05	80.80	-
Hemicellulose	26.42	22.65	-	24.45	23.07	-
DMI (kg/day)	9.42[a]	10.18[b]	0.71	6.47	6.67	0.18
Digestibility coefficients (%)						
OM	59.26	54.88	1.31	52.71	54.13	1.43
CP	40.41[a]	53.63[b]	0.89	61.10	57.26	1.16
Cellulose	74.38	75.51	1.02	72.17	72.59	1.08
NDF	64.45	63.31	1.43	61.56	62.63	1.22
Nitrogen balance (g/day)						
Intake	108.30[a]	125.67[b]	0.68	99.00	94.93	2.91
Outgo						
Faecal	64.52[a]	70.84[b]	1.08	38.13	40.59	0.79
Urinary	35.82[b]	15.49[a]	2.11	35.87[b]	26.19[a]	1.48
Total	100.34[b]	86.33[a]	3.02	74.00	66.78	2.12
Retained	7.96[a]	39.34[b]	3.05	25.00	28.15	3.38
Apparent BV (%)	17.54[a]	71.64[b]	5.94	37.47[a]	51.77[c]	5.38

(wheat straw treated with 3.5% urea at 40% moisture, stacked for 9 days) was used as crude inoculum in the subsequent batches of urea wheat straw. The straw preparations were evaluated as complete feed for buffaloes. Better utilization of crude protein was observed in buffaloes fed inoculated than uninoculated fermented straw. Reduction of fermentation period from 9 to 6 days in inoculated group did not have any adverse effect on the utilization of nutrients.

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Performance of mithun calves on Napier grass based feeding

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Key words: Digestibility, Feed intake, Growth rate, Mithun, Napier grass

Present experiment was conducted to study the nutrient intake and their utilization by mithun on cultivated fodders (Napier grass) along with concentrate feeding.

Young female mithun calves (4) of 140 kg average body weight were fed a ration consisting of concentrate mixture and Napier grass (*Pennisetum perpureum*). The concentrate mixture was fed @ 3-4 kg per animal a day and Napier grass in vegetative stage was offered *ad lib.* as roughage source. The experiment was conducted for 35 days as the availability of grass in the area was limited. Body weight at weekly interval was recorded throughout the experimental period. A 6-day digestion trial was conducted to evaluate nutritive value of the ration. Proximate principles of feeds, residues and faeces were estimated as per AOAC (1990) to determine the digestibility and intake of nutrients in mithun calves.

The chemical composition of Napier grass and concentrate mixture fed to the animals are presented in Table 1. Napier grass contained high level of crude protein, ether extract and total carbohydrates which may be due to cutting of grass in vegetative stage as the stage of growth is the most important factor influencing the composition and nutritive value of grass herbage. Banerjee (1988) had reported similar higher level of protein in Napier. Young plants are very rich in protein, on the dry basis, than the same plants at the later stages of growth (Morrison 1984). So young Napier grass that was growing actively usually contained 16.51% crude protein. The crude protein and total carbohydrate content in concentrate mixture were 19.00 and 67.06 % respectively. Concentrate mixture contributed higher proportion of DM in the ration, thus supplying higher level of nitrogen and readily available energy to the animals for rapid growth. The digestibility coefficients of various nutrients indicate that the mithun calves are efficient feed utilizers. The digestibility value of fibre fractions was low and of crude protein and ether extract was high which may be due to intake of higher proportion of concentrate than roughage by the animals. The roughage to concentrate ratio in the ration was 29: 71 and a daily body weight gain of 500g was found by feeding of this ration (Table 2). Growth rate reported in past for cattle and buffaloes in different Indian studies varied from 200 to 550g/ day (Pathak *et al.*1987, Saha *et al.* 1993). DM, DCP and

Table 1. Chemical composition of concentrate mixture and Napier grass (% DM basis) and digestibility (%) of nutrients

Items	Concentrate mixture	Napier grass	Digestibility (%)
Organic matter	92.74	90.23	66.91 ± 1.27
Ash	7.26	9.77	63.30 ± 1.23
Crude protein	19.00	16.51	70.91 ± 3.43
Ether extract	6.68	3.74	73.90 ± 2.56
Crude fibre	4.92	22.67	57.66 ± 2.54
Nitrogen free extract	62.14	47.31	66.40 ± 1.06
Total carbo-hydrate	69.90	69.98	65.14 ± 0.68
Acid detergent fibre	9.61	32.80	37.86 ± 4.47

Composition of concentrate mixture: crushed maize, 30%: wheat bran, 15%; rice polish, 20%; mustard oil-cake, 33%; mineral mixture, 1%; salt, 1%.

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Table 2. Plane of nutrition, growth rate and feed conversion efficiency of mithun calves

Particulars	Mean ± SE
Body weight (kg) during digestion trial	157.58 ± 7.50
Roughage: concentrate	29: 71
DM intake (kg/day)	3.73 ± 0.12
DM intake (% B.wt.)	2.38 ± 0.05
DM intake (g/Wkg$^{0.75}$/day)	83.94 ± 1.17
DCP intake (g/day)	492.00 ± 40.00
TDN intake (kg/day)	2.50 ± 0.11
Body weight gain (g/day)	500.01 ± 98.97
Feed conversion efficiency (kg feed intake/kg gain)	5.32 ± 0.78
Nutritive value of the ration	
DCP%	13.20±0.71
TDN%	67.11±1.80

TDN intake of mithun calves were higher in compare to NRC (1988) recommendation of buffalo calves for 150kg body weight and 500g daily body weight gain which may be because of higher initial body weight of the animals. DCP and TDN values of the ration were 13.20±0.71 and 67.11±1.80 respectively. The ration had higher nutritive value due to higher proportion of concentrate in the ration and feeding of Napier in vegetative stage which was soft, tender and had much less fibre and, therefore, more digestible.

On the basis of this study it would be inferred that feeding of Napier in vegetative stage along with concentrate feed @ 3-4 kg/day could meet the nutrient requirements and give a growth rate of 500 g/day.

SUMMARY

Napier grass (*Pennisetum perpureum*) was fed to the growing mithun calves to find out the growth rate, voluntary feed intake and nutrients utilization. A daily body weight gain of 500g was observed on feeding of this ration. Voluntary feed intake was recorded to be 2.38kg per 100kg body weight. Mithun calves consumed 492g DCP and 2.50kg TDN daily. The study indicated that the ration containing 29% roughage DM and 71% concentrate DM is sufficient to meet the body requirement of mithun calves and to obtain 500g daily body weight gain while feeding the calves with Napier (vegetative stage) and concentrate feed.

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Effect of supplementary feeding during late gestation on production performance of ewes grazing on community rangeland

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In semi-arid region of the country, sheep are reared mainly on community rangeland and/or stubble grazing on cropped land after harvesting of crop with top feed supplementation during lean season to meet their nutritional requirements. These rangeland are covered with a wide variety of vegetation mainly grasses, bushes, shrubs and trees. The biomass yield of community rangeland is low and stocking density is high (Mann and Mehta 1998, Sankhyan *et al.* 1999) and sheep grazing on such land are underfed for most part of the year. Majority of the sheep farmers in semi-arid region do not supplement concentrate to their sheep even in critical physiological stages, while limited concentrate supplementation in addition to free grazing on cenchrus dominated pasture improves production performance of ewes (Shinde *et al.* 1996). Therefore, the present study was undertaken to demonstrate the beneficial effects of concentrate supplementation to ewes during late gestation on their production performance at farmers' doorstep.

Table 1. Meteorological condition of the location during the experimental period

Attributes	October	November	December	Average
Dry bulb temperature (°C)				
0830 hr	23.7	17.0	8.7	16.5
	(20.0-28.50	(9.0-25.5)	(5.0-16.0)	(5.0-28.5)
1430 hr	32.3	29.4	26.8	29.5
	(25.5-36.5)	(27.0-33.5)	(24.5-30.0)	(24.5-36.5)
Minimum temperature (°C)				
0830 hr	19.1	12.0	6.5	12.5
	(12.0-23.0)	(7.0-20.0)	(3.5-10.0)	(3.5-23.0)
1430 hr	19.0	11.9	7.1	12.7
	(12.0-23.0)	(7.0-19.0)	(4.0-24.5)	(4.0-24.5)
Maximum temperature (°C)				
0830 hr	32.7	29.5	26.7	29.6
	(25.5-35.0)	(27.0-33.5)	(21.5-30.0)	(21.5-35.0)
1430 hr	32.6	29.5	26.9	29.7
	(25.0-36.5)	(27.5-33.5)	(23.5-29.0)	(23.5-36.5)
Relative humidity (%)				
0830 hr	74.5	68.2	78.3	73.7
	(46.0-96.0)	(45.0-95.0)	(55.0-94.0)	(45.0-96.0)
1430 hr	42.5	32.2	30.3	35.0
	(23.0-96.0)	(19.0-50.0)	(21.0-46.0)	(19.0-96.0)
Wind velocity (km/hr)	3.5	1.9	0.9	2.1
	(0.6-53.0)	(0.7-5.6)	(0.4-2.0)	(0.4-53.0)
Total rain fall (mm) in 24 hr	1.2	0.0	0.0	0.4
	(0.0-24.0)	(0.0-0.0)	(0.0-0.0)	(0.0-24.0)
Sun shine (hr)	8.3	8.4	8.9	8.53
	(0.0-10.3)	(2.2-9.8)	(5.2-9.8)	(0.0-10.3)

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Table 2. Ingredients and chemical composition of concentrate mixture and pasture consumed by ewes

Ingredients	Parts
Physical composition	
Barley	30
Wheat bran	30
Deoiled rice bran	18
Mustard-cake	20
Mineral mixture	1
Salt	1
Vitablend (A and D_3)	0.02

Chemical composition (on% DM basis)

	DM	OM	CP	NDF	ADF	Cellulose	Lignin	GE (Mcal/kg)
Concentrate mixture	90.10	87.50	17.40	41.67	25.92	14.08	6.2	4.60
Pasture	62.50	84.70	10.10	60.50	38.80	24.20	11.30	3.90

A demonstration was laid on a farmer's sheep flock maintained on natural rangeland at Chanda Ki Dhandi, district Tonk, Rajasthan. The location of field research was about 2 km from the Central Sheep and Wool Research Institute, Avikanagar, located in hot semi-arid region of the country at 75° 28' E latitude and 26° 17' N longitude and 320 m above mean sea level. The experiment was conducted for 108 days extending from end of the monsoon season to winter (22 September 1998 to 8 January 1999). During the experimental period, the average minimum and maximum temperature and relative humidity of the location at 0830 and 1430 hr was 12.5 and 12.7, 29.6 and 29.7°C and 73.7 and 35.0% respectively (Table 1). Malpura ewes (20), 2-3 year old, in their late gestation (68 days before parturition) and weighing 32.93±0.73 kg were selected and divided into 2 equal groups of 10 each (G1 and G2). Ewes in both the groups were grazed on natural rangeland from 0700 to 1800 hr followed by night shelter in side opened improvised animal shed. G1 ewes in addition to grazing received concentrate mixture @ 1% of their body weight, while the G2 ewes were maintained on sole grazing. All the ewes in G1 were supplemented up to lambing. The body weight of ewes at the start and end of the experiment and at parturition was recorded. Birth weight of lambs and weekly changes in body weight up to the age of 75 days were also recorded. The biomass yield of the community rangeland was assessed by clipping 4 random quadrates (1 m^2) according to Tadmore *et al.* (1975). The samples of concentrate mixture and rangeland pasture were analyzed for dry matter (DM), organic matter (OM) and crude protein (CP) as per AOAC (1984). Cell wall constituents by the method of Van Soest *et al.* (1991). The gross energy of the samples was estimated using ballistic bomb calorimeter. The data were statistically analyzed following the procedures of Snedecor and Cochran (1967).

The vegetation cover of natural rangeland was dominated by *Cynodon dactylon* (*doob*) and *Cenchrus biflorus* (*bharbhut*) grasses, *Zizyphus nummularia* (*pala*) and *Calotropis procera* (*aak*) shrubs and fodder trees *Azardirachta indica* (*neem*), *Acacia nilotica* (*babool*) and *Prosopis cineraria* (*khejri*). The biomass yield of the community rangeland utilized in mixed grazing round the year was 6.40q DM/ha during winter. The biomass yield of public rangeland as observed in this study is almost similar to that reported by Chaturvedi *et al.* (2000), whereas it was lower than that reported by Sankhayn *et al.* (1999). Such difference in biomass yield could be attributed to fertility of land, type of pasture, type of grazing and stocking density on the rangeland. The chemical composition of community rangeland pasture and concentrate mixture is presented in Table 2. The chemical composition of pasture was similar to that reported earlier (Sankhayn *et al.* 1999, Chaturvedi *et al.* 2000) during winter. The digestible crude protein (DCP) and total digestible nutrients (TDN) content of concentrate mixture was calculated as 13.9 and 70.2% respectively.

The initial body weight of pregnant ewes in G1 and G2 was 33.3 and 32.6 kg respectively. With supplementation of concentrate mixture @ 1% of the body weight, the ewes in G1 group gained 2.0 kg, whereas the G2 group (without supplementation) lost 0.4 kg during the same period (Table 3). The present findings indicate that the ewes in G2 group were not able to meet their nutrient requirements exclusively from grazing. As a result, to meet out the added nutrient requirement of growing foetus, they mobilized their body reserves leading to loss in their body weight. The body weight gain (kg) of ewes during lactation period was higher ($P<0.05$) in G2 (2.9) than that of G1 (1.5). The ewes in G2 and G1 gained 8.8 and 4.2% in 34 and 40 days of lactation period respectively (Table 3). The lower plane of nutrition of ewes in G2 during late gestation in comparison to G1 led to compensatory gain in former group during lactation period (Ryan 1990, Santra and Pathak 1999). The birth weight of lambs born to the ewes of G1 group was significantly ($P<0.01$) higher (3.6 kg) than that in G2 (2.8 kg). One lamb of low birth weight (1.5 kg) born from G2 ewe died 4 days after his birth. In G1 lambs, the higher birth weight was due to concentrate supplementation to their mother during pregnancy

Table 3. Production performance of ewes and their lambs

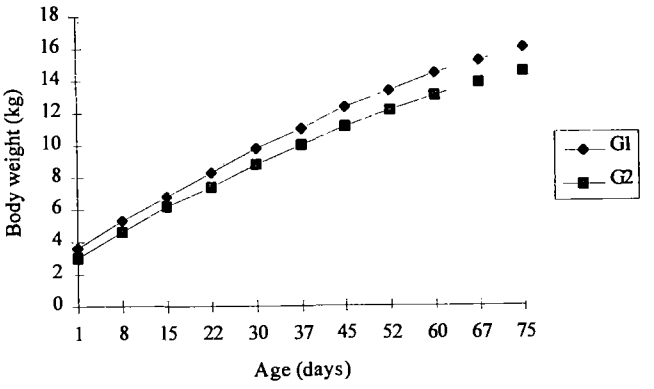
Attributes	G1	G2
Production performance of ewes		
Start of experiment before parturition (days)	66.44±4.78	69.67±8.09
Initial body weight of ewes (kg)	33.27±1.08	32.60±0.91
Body weight of ewes at parturition (kg)	35.28±1.28	32.22±1.02
Gain/loss in body weight at parturition (kg)	2.01±0.80	-0.38±0.89
Final body weight of ewes after lambing (days)	40.44±4.82	34.44±8.42
Final body weight of ewes at end of expt. (kg)	36.78±1.29	35.05±1.12
Body weight gain of ewes during lactation (kg)*	1.50±0.17	2.83±0.45
Per cent increase in weight of ewes during lactation**	4.30±0.52	8.85±1.54
Birth wt of lambs (kg)		
Male**	3.70±0.27	2.75±0.25
Female**	3.54±0.17	2.92±0.19
Pooled**	3.62±0.14	2.83±0.20
Body weight of lambs at 75 days (kg)		
Male	13.85±1.66	12.50±0.50
Female	14.88±0.52	13.05±1.48
Pooled	14.36±0.75	12.77±1.09
Total body weight gain of lambs in 75 days (kg)		
Male	10.15±1.26	9.75±0.53
Female	11.34±0.51	10.13±1.23
Pooled	10.74±0.70	9.94±1.00
Average daily gain (g/d)		
Male	135.33±19.42	130.00±10.00
Female	151.20±7.61	135.11±17.95
Pooled	143.26±9.31	132.55±13.30

*P<0.05, **P<0.01.

(Shinde *et al.* 1996). Although, the body weight of lambs at 75 days, total gain in body weight of lambs during 75 days and average daily gain was higher in G1 than that of G2, the difference was statistically nonsignificant (Table 3). The growth rate of the lambs during preweaning stage was higher in G1 than G2 (Fig. 1). Further, the birth weight, body weight at 75 days, weight gain in 75 days and average daily gain was similar between the male and female lambs (Table 4). It is concluded from the study that the biomass yield of the community rangeland is low which was insufficient to meet

Table 4. Sex-wise growth performance of lambs born from ewes

Attributes	Male	Female
Birth weight (kg)	3.11±0.35	3.20±0.16
Weight at 75 days (kg)	13.4±1.10	13.88±0.85
Weight gain in 75 days (kg)	10.29±0.94	10.68±0.77
Average daily gain (g/d)	137.20±12.60	142.4±10.24



Supplemented (G1), $Y = 3.40 + 0.243X - 0.001X^2$

Nonsupplemented G2), $Y = 2.78 + 0.231X - 0.001X^2$

Fig. 1. Growth pattern of lambs in farmer's field during preweaning stage.

the nutrient requirement of ewes during late gestation. However, concentrate supplementation @ 1% of body weight to ewes during the period improved their production performance and growth rate of their lambs.

SUMMARY

A demonstration was laid on a farmer's sheep flock maintained on natural rangeland located in hot semi-arid region of the country. Malpura ewes (20) in their late gestation were selected and divided into 2 equal groups of 10 each (G1 and G2). Ewes in both the groups were grazed on natural rangeland from 0700 to 1800 hr followed by night shelter. G1 ewes in addition to grazing received concentrate mixture @ 1% of their body weight up to lambing. It is concluded from the study that the biomass yield of the community rangeland is insufficient to meet the nutrient requirement of ewes during late gestation. However, concentrate supplementation @ 1% of body weight to ewes during the period improved their production performance and growth rate of their lambs.

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Rumen digesta manipulations on Holstein steer's activities

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Past studies did not incorporate the influence of digesta manipulations on time spent eating, ruminating and idling. This study examined the influence of digesta manipulations on time spent on these activities by Holstein steers over 24hr.

Three Holstein steers (638, 744 and 850kg) fitted with large rumen cannulae of about 110mm internal diameter were used. The steers were fed *ad lib.* on diet consisting of predominantly of timothy grass (*Phleum pratense*). The hay was hammer-milled through a 50mm screen and transferred to a Butler Oswalt Ensilmixer trailer. A solution of 1.5% urea and 0.13g a hydrous Na_2SO_4 was sprinkled onto the hay while in the mixer. The dry matter and nitrogen content of the hay were 898g and 12kg^{-1} respectively. Mineral supplement was offered during the work.

The steers were housed individually in stalls and were allowed unrestricted access to drinkable water and food with a 14-day acclimatization period. Each steer was served at 08: 30hr with fresh diet sprinkled with 50g mineral supplement. The experiment consisted of seven 5-day periods comprising 4 control periods (1, 3, 5 and 7) and 3 treatment periods (2, 4 and 6). The steers were randomly allocated to treatments in a 3 × 3 change over latin square design with 1 steer to a treatment. The 3 treatments imposed were: (i) increasing rumen digesta by 15% referred to as digesta addition, (ii) decreasing rumen digesta by 25% referred to as digesta removal, and (iii) emptying the rumen and returning all that were emptied from the rumen referred to as digesta return. The influence of digesta manipulations on activities was determined by keeping the steers under 24hr (08: 30hr-08: 30hr) surveillance on the last day of each period and time spent on each activity (eating/ ruminating/ idling) recorded every 5 min.

During the first 3hr post-digesta addition treatment (14.30-17: 30hr), time spent eating decreased though not significantly ($P>0.05$; Table 1), this trend was expected since 15% of the emptied digesta were added in addition to the initial rumen digesta. Both digesta removal and return treatments recorded an increase but only the increase from digesta removal was found to differ significantly ($P<0.05$) from the control.

The eating response from steers during digesta removal was expected since some digesta were removed from the rumen. Over the 24hr, digesta removal treatment had the highest eating time; probably this was due to the availability of space in the rumen to accommodate additional food (Blaxter *et al.* 1961, Campling and Balch 1961). The first 3hr post-digesta removal were observed to increase time spent ruminating by the steers and was significantly ($P<0.05$) different from the control but not with other treatments. This trend perhaps was due to the increased time spent eating between 14: 30 and 17: 30hr, which presumably increased rumen digesta that needs further chewing/breakdown. Other treatments recorded a decrease in time spent on rumination during this period. However, digesta removal recorded the lowest time spent ruminating and could either be attributed to the possibility of microbial degradation complementing rumination (Ulyatt *et al.* 1984) as means of particle size reduction or decrease in dry matter intake by the steers.

Though the steers were observed to spend more time eating during digesta removal, herbage intake is quantified as product of number of bites and weight of bites. The number of bites per minute during digesta removal was probably lower than what obtained in other treatments. During the first 3hr post treatments both digesta removal and return treatments recorded a decrease in time spent idling. Though the decreases were not significant either between treatments or control, the trend was expected since the steers responded by increasing the time spent on eating and ruminating. Digesta addition recorded an increase in time spent idling was highest during digesta removal treatment and was significantly ($P<0.05$) different when compared with other treatments and control. The results from this study seemed to suggest that time spent eating and ruminating cannot be altered by short period of rumen digesta manipulations. Future studies should consider the influence of rumen digesta manipulations over a long period

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Table 1.Time spent eating and ruminating over 24hr period

Activities	Addition (+15%)	Removal (-25%)	Return (- and +)	SE(d)
Eating				
Control (08: 00-11: 30hr)	56	72	62	
Treatment	38	50	29	17
Control (11: 30-14: 30hr)	32	26	31	
Treatment	23	35	39	7
Control (14: 30-17: 30hr)	30	22	26	
Treatment	24	50	46	16
Control (17: 30-20: 30hr)	44	52	46	
Treatment	68	48	24	28
Control (20: 30-08: 30hr)	80	64	79	
Treatment	70	65	52	13
Total	-5	24	-49	41
Ruminating				
Control (08: 00-11: 30hr)	40	32	35	
Treatment	44	58	51	21
Control (11: 30-14: 30hr)	74	72	69	
Treatment	80	53	73	10
Control (14: 30-17: 30hr)	74	66	66	
Treatment	70	60	60	26
Control (17: 30-20: 30hr)	65	68	59	
Treatment	60	60	82	30
Control (20: 30-08: 30hr)	308	288	299	
Treatment	335	268	338	30
Total	42	32	102	61
Idling				
Control (08: 00-11: 30hr)	84	76	80	
Treatment	98	72	100	5
Control (11: 30-14: 30hr)	74	81	80	
Treatment	77	92	68	6
Control (14: 30-17: 30hr)	76	91	80	
Treatment	86	70	71	7
Control (17: 30-20: 30hr)	71	70	74	
Treatment	72	82	68	26
Control (20: 30-08: 30hr)	332	364	334	
Treatment	315	386	332	18
Total	22	82*a	25	18

SE (d), [a] and * imply standard error of difference between means, significant ($P<0.05$) when compared with the controls and significant ($P<0.05$) when compared with other treatments and control respectively.

on time spent eating, ruminating and idling.

SUMMARY

There are no reports on the influence of digesta manipulations on time spent eating, ruminating and idling. This study considered the influence of digesta manipulations on time spent on these activities by Holstein steers over 24hr with the sole aim of determining whether the time spent eating and ruminating could be significantly altered by digesta manipulations imposed over a short period of time.

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