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Indian Journal of Animal Sciences 67 (4) : 357-358, May 1997

Evaluation of BHK_{21} cell-line adapted Newcastle disease vaccine

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Received : 12 June 1996

ABSTRACT

Lasota strain of Newcastle disease virus was successfully adapted to BHK_{21} cell-culture. BHK_{21} cell-culture adapted virus showed reduced virus infectivity, MDT and HA with chicken and equine RBC and elevated ICPI when compared with Lasota virus of chick embryo origin. Thermostability of the virus was unaffected. Serological response and challenge test results of the chicks vaccinated with BHK_{21}-adapted vaccine showed encouraging results.

Key words : BHK_{21}, Cell-line, Newcastle disease virus

Newcastle disease vaccines of chick embryo origin containing lentogenic strain of Newcastle disease virus (NDV) are commonly used to immunize day-old chicks. The immune response of chicks to BHK_{21} cell-adapted mesogenic strains of Newcastle disease was reported by Reddy and Srinivasan (1991, 1992). The use of BHK_{21} cell-adapted Lasota strain of NDV as a vaccine was assessed and a comparison of the serological response in chicks elicited by Lasota strain vaccine of chick embryo origin and the BHK_{21} cell-origin was made in this study.

MATERIALS AND METHODS

Cells

Baby hamster kidney (BHK_{21}) monolayer cells maintained in our laboratory were used in the study. The cells were grown in Eagle's MEM (Glasgow modification) containing 10% adult bovine serum and antibiotics (penicillin 100 IU/ml, polymyxin 100 µg/ml, neomycin 100 µg/ml).

Viruses

Lasota strain of NDV obtained from the Indian Veterinary Research Institute (IVRI), Izatnagar, was given 2 passages in SPF eggs and allantoic fluid was stored in liquid nitrogen as master bank. The challenge virus was passaged once in SPB eggs and stored in LN_2.

Embryos and experimental birds

Embryos obtained from nonimmunized flock were used for determination of virus infectivity and mean death time (MDT).

Day-old chicks hatched out from eggs of non-immunized flock were used for vaccination studies and intracerebral pathogenicity index (ICPI) determination. Chicks were maintained for 8 weeks and used for titration of virulent virus.

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Adaptation of Lasota strain to BHK_{21} cells

Confluent BHK_{21} monolayers were infected with Lasota strain of NDV and incubated at 37°C. The virus was passaged serially for 13 times and the virus harvest from 13th passage was freeze-dried and stored at -20°C till use. The freeze-dried preparation was referred as tissue culture Newcastle disease vaccine (TCNDV).

Table 1. Results of biological assays of TCNDV and AFNDV

Test	TCNDV	AFNDV
MDT (hr)	92	120
ICPI	0.4	0.2
Virus titre (EID_{50}/ml)	7.5	9.0
HA titre		
with chicken RBC	256	512
with equine RBC	16	64
Thermostability of		
HA protein at 56°C (min)	5	5

Vaccines

Lasota strain of chick-embryo origin from master bank was passaged once in specific pathogen free (SPF) eggs and the allantoic fluid was distributed in 1 ml quantities and freeze-dried. This was used as vaccine and designated as allantoic fluid Newcastle disease vaccine (AFNDV).

Virus infectivity assay

The test was performed on AFNDV and TCNDV as per Hanson (1980) and the titres expressed as EID_{50}/ml.

Vaccination and blood sample collection

The vaccination particulars are furnished in Table 2. The blood samples were collected 0, 41 and 62 days post-vaccination (DPV) for serum antibody assay.

Table 2. Serological response and challenge test results of birds (10) vaccinated with TCNDV and AFNDV

Vaccine	Dose	Route	O day titre		41 dpv titre		62 dpv titre		Challenge results
			HI	ELISA	HI	ELISA	HI	ELISA	Sur / Inc
TCNDV	10^5EID_{50}	Nasal	<2	-	102.2	5.33:1	165.1	6.68:1	10/10
AFNDV	10^6EID_{50}	Nasal	<2	-	46.2	2.73:1	213.3	7.66:1	10/10
Control	-	-	<2	-	<2	-	<2	-	0/10

* Birds in each group were revaccinated with similar dose of respective vaccine on 41st day by i/m route.

Haemagglutination (HA) and haemagglutination inhibition (HI) test

HA test was carried out using chicken and equine RBCS and for HI studies chicken RBCs were used (Hanson 1980).

Intracerebral pathogenicity index (ICPI), mean death time (MDI) and thermostability tests

All the tests were performed as per Hanson (1980).

ELISA

ELISA test was performed as per Reddy and Srinivasan (1992). The titres were expressed as mean ELISA OD ratio of vaccinated with control chicks.

Challenge test

The birds were challenged on 62 dpv intramuscularly with 10^4 bird infective doses (ID_{50}). The birds were observed for 14 days for mortality and morbidity.

RESULTS AND DISCUSSION

Initial passages of Lasota strain of NDV did not show any noticeable cytopathic effect (CPE) and cellular changes such as rounding and syncytia formation were observed from sixth passage onwards. The TCNDV showed low virus infectivity titres, MDT, HA titre with both chicken and equine RBC when compared to AFNDV (Table 1). The ICPI of TCNDV was higher than that of AFNDV. The thermostability of the HA protein of AFNDV and TCNDV at 56°C was same. Slosaris *et al.* (1989) made similar observations. The elevation of ICPI of BHK_{21} adapted Komarov strain of NDV was reported by Reddy and Srinivasan (1992).

The results of serum antibody assay indicated that the birds vaccinated with TCNDV elicited higher antibody titres when compared to AFNDV (Table 2). This variation in serological response may be attributed to mild elevation of virulence of virus on passage in BHK_{21} cells. However the birds in TCNDV group did not show any untoward reaction. Lower titre of TCNDV was used for vaccination based on earlier experiments conducted in our laboratory. Even lower doses of TCNDV induced serological response comparable to higher doses of AFNDV. On subsequent vaccination of the birds with both AFNDV and TCNDV by intramuscular route, AFNDV induced higher antibody response. The results of ELISA correlated well with HI titres and protection upon challenge. Srinivasan *et al.* (1992) observed similar results. Birds vaccinated with 2 doses of TCNDV and AFNDV withstood the challenge with virulent virus (Table 2). All the controls showed typical symptoms and died. The Lasota strain of NDV adapted to BHK cells can be used to vaccinate birds for one week safely as the vaccine induced no untoward reaction and serological response of birds to the vaccine showed encouraging results.

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Artificial immunization of rabbits against *Hyalomma dromedarii*- Effect on developmental stages of tick

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Received : 20 October 1995

ABSTRACT

The feeding and moulting performances of *Hyalomma dromedarii* larval and nymphal stages were adversely affected when fed on the rabbits immunized with the partly fed female adult tick derived midgut concealed antigens (supernate and pellet fractions). The engorgement and moult periods of these developmental stages were significantly prolonged. Immunized animals permitted significantly reduced number of larvae and nymphs to feed upon them. Engorged weight was significantly decreased in both developmental stages (larvae and nymphe) on repletion. Animals, inoculated with both the fractions emulsified with Freund's complete adjuvant (FCA) and without FCA rejected 11-38 and 10-39% of larval and nymphal ticks respectively.

Key words: *Hyalomma dromedarii,* Immunization, Rabbit, Tick

Hyalomma dromedarii is considered to be the second most common tick among *Hyalomma* species in India and is reported experimentally to have more vector potential than *H. anatolicum anatolicum* for transmitting the agent of bovine tropical theileriosis (Khurana *et al.* 1988). Of late, the immunological approaches to the tick control are soliciting the attention of investigators (Opedbeeck *et al.* 1988, Willadsen *et al.* 1989, Banerjee *et al.* 1990, Mbogo 1994). Immunological control of *H. dromedarii* tick has not been studied so far. The present study was designed to know the effect on the performance of larval and nymphal stages fed on artificially immunized rabbits.

Table 1. Effect on feeding performance of *Hyalomma dromedarii* larval ticks on immunized rabbits

Immunization regimen (group No.)	No. of animals	No. of larvae released	Engorgement period (days)	Replete larval yield	Replete nymphal yield	Engorged weight of larvae (mg)	Larval tick weight reduction (%)	Larval tick rejection (%)
Midgut supernate with FCA (group 1)	4	200	7.48±0.10** (5-12)	44.25±1.80	5.25±0.63**	0.63±0.015**	11.27	37.53
Midgut supernate without FCA (group 2)	4	200	7.57±0.07** (5-11)	49.25±0.95	10.00±1.08**	0.64±0.012**	9.80	25.24
Midgut pellet with FCA (group 3)	4	200	7.41±0.08** (4-11)	47.75±1.41	13.25±0.85**	0.68±0.010	4..23	23.03
Midgut pellet without FCA (group 4)	4	200	7.12±0.08** (4-11)	50.75±1.70	19.75±2.00**	0.69±0.010	2.82	11.04
Control (group 5)	4	200	6.09±0.07 (4-10)	48.25±1.75	31.00±1.22	0.71±0.011	-	-

Each value denotes mean ± SE and the values in parentheses represent the range.

** P<0.01; FCA, Freund's complete adjuvant.

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Table 2. Effect on feeding performance of *Hyalomma dromedarii* nymphal ticks on immunized rabbits

Immunization regimen	No. of animals	No. of nymphs released	Engorgement period (days)	Replete nymphal yield	Engorged nymphal weight (mg)	Larval tick weight reduction (%)	Tick rejection (%)
Midgut supernate with FCA (group 1)	4	50	10.31±0.15** (8-14)	23.75±1.03**	17.20±0.03**	11.98	38.71
Midgut supernate without FCA (group 2)	4	50	8.83±0.15 (6-13)	26.75±0.85**	18.07±0.20**	7.52	30.97
Midgut supernate with FCA (group 3)	4	50	8.55±0.10 (6-12)	29.75±1.38**	18.94±0.89**	3.07	23.33
Midgut supernate with FCA (group 4)	4	50	8.31±0.10 (6-11)	34375±1.49	19.01±0.26	2.71	10.32
Control (group 5)	4	50	8.55±0.08 (6-10)	38.75±1.32	19.54±0.12	-	-

Each value denotes mean ±SE and the values in parentheses represent the range.
** P<0.01; FCA, Freund's complete adjuvant.

MATERIALS AND METHODS

New Zealand White rabbits (20/3-4 months old) of both the sexes were randomly divided into 5 groups of 4 animals each. Animals in groups 1-4 were immunized with antigens, whereas group 5 was kept as control.

Tick colonies

Colonies with all the stages, viz. larvae, nymphs and adults of *Hyalomma dromedarii* were maintained in the laboratory in cotton-plugged glass-tubes at 28°C and 85% RH by conventional method. These stages fed on the ears of rabbits for their blood-meal. Rabbits were restrained from grooming with wooden neck collars.

Preparation of antigen

The tick antigen was prepared from surface sterilized partly fed female adults by following the method of Kumar and Kumar (1996). The fractions of antigens were obtained as supernate and pellet. Protein concentration in both the fractions was adjusted to contain 1 mg of antigen per millilitre for inoculation of rabbits.

Immunization schedule

Each antigen fraction was injected in 2 forms, one after emulsification with FCA and the other without FCA separately. Animals in groups 1 and 2 were administered with gut supernate antigen (GSA), with and without FCA respectively. Similarly, the animals in groups 3 and 4 were inoculated with gut pellet antigen (GSA) with and without FCA respectively. Each rabbit received 3 subcutaneous inoculations of 1 ml each on days 0, 14 and 21. Third inoculation in animals of groups 1 and 3 was given without FCA. In group 5, animals were inoculated only with phosphate-buffered saline, parallel to immunization schedule of other groups.

Table 3. Moulting peformance of *Hyalomma dromedarii* larval ticks on immunized rabbits

Immunization regimen, (group No.)	No. of animals	No. of larvae kept for moulting	Moult period (days)	Larval moult (%)
Midgut supernate with FCA (group 1)	4	176	6.13±0.08** (4-8)	84.47±2.53
Midgut supernate without FCA (group 2)	4	197	6.48±0.08** (4-10)	84.27±1.24
Midgut supernate with FCA (group 3)	4	191	5.51±0.08**	89.01±0.89
Midgut supernate without FCA (group 4)	4	203	5.35±0.08** (3-8)	87.61±1.17
Control (group 5)	4	193	4.87±0.97 (3-7)	86.23±2.77

Eachvalue denotes mean ±SE and the values in parentheses represent the range. **P<0.01; FCA* Freund's complete adjuvant.

Challenge infestation with tick developmental stages

The rabbits of each group were challenged with unfed larvae and nymphs derived from laboratory-maintained tick colonies 14 days after last inoculation. Rest period of 1 week was given in between the infestation of 2 different developmental stages. The numbers of larvae and nymphae in each infestation were 200 and 50 per rabbit respectively.

Table 4. Moulting performace of *Hyalomma dromedarii* nymphal ticks on immunized rabbits

Immunization regimen (group No.)	No. of animals	No. of nymphs kept for moulting		Moult period (days)		Nymphal moult (%)	
		2-host tick	3-host tick	2-host tick	3-host tick	2-host tick	3-host tick
Midgut supernate with FCA (group 1)	4	21	95	15.0±0.344** (13-18)	14.50±0.16** (12-18)	85.18±5.40	84.30±2.81
Midgut supernate without FCA (group 2)	4	40	107	14.19±0.27** (11-18)	14.51±0.15** (11-18)	90.83±5.34	87.10±3.33
Midgut pellet with FCA (group 3)	4	53	119	13.31±0.19** (10-17)	13.22±3.85 (10-17)	82.81±3.93	
Midgut pellet without FCA (group 4)	4	77	139	12.60±018** (9-17)	12.98±0.16** (9-17)	92.77±1.63	91.35±1.09
Control (group 5)	4	124	155	12.25±0.10 (9-15)	12.42±0.11 (9-16)	92.06±1.47	92.96±1.09

Each value denotes mean ±SE; the values in parentheses represent the range.

** P< 0.01; FCA, Freund's complete adjuvant

Observations

Daily observations were recorded on tick-challenged rabbits for drop down of larvae and nymphs. Further, feeding and moulting performances of these developmental stages studied. The data were analysed statistically using Student's 't' test (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

The present study revealed significant prolongation in engorgement and moult periods, decrease in per cent yield and engorged weight of *H. dromedarii* larvae and nymphs in response to GSA (Tables 1,2). This indicated that there may be some common antigens in different developmental stages and the immune response against these antigens may be responsible for alteration in feeding and moulting behaviour of these larvae and nymphs. This finding appears to simulate that of Brown (1988) who analysed sharing of potential antigens between different developmental stages by Western blot analysis in *Amblyomma americanum* ticks. Wong and Opdebeeck (1990) reported protection against adult *Boophilus microplus* ticks fed on cattle immunized with larval membrane antigens which further confirmed the sharing of some antigenic components in different developmental stages. Significant reduction in engorged weight of nymphs was also observed by Jongeian *et al.* (1989) in case of *Rhipicephalus appendiculatus* tick after being fed on rabbits immunized with tick gut extract. Decrease in larval engorged weight in the present study is in agreement with the findings of Essuman *et al.* (1991) in *R. appendiculatus* tick fed on cattle immunized with midgut extract. Kumar (1990) also observed similar type of findings in *H.a. anatolicum* nymphal and larval ticks as significant extesion in engorgement and moult periods, decresae in per cent yield and mean engorged weight when fed on cattle immunized with tick gut antigens.

The change in feeding and moulting performances of developmental stages (nymphs and larvae; Tables 3,4) when fed on immunized rabbits with midgut antigens may be due to acquired resistance. Both, humoral as well as cellular immune responses were demonstrated in rabbits immunized with *H. dromedarii* midgut antigen (Kumar and Kumar 1996). Perhaps both types of immune responses play important role in acquired resistance. The efficacy of immunological approach to control ticks may be enhanced further if we characterize and isolate potential immunogenic components common in different development stages and use them as immunoprophylactic agents.

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Highly virulent form of infectious bursal disease in Egypt : Some epidemiological observations

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ABSTRACT

Investigation of 82 severe outbreaks of infectious bursal disease (IBD) in broiler farms, showed differences in mortality rate among the various strains and in the age of susceptibility among the infected flocks. Also the different programmes of vaccination, type of the vaccine, and the time of onset of the disease after vaccination all affected severity of the outbreaks and the mortality rates.

Statistically nonsignificant antigenic differences were detected amongst 5 commercial vaccinal strains of standard serotypes 1 and 8 field isolates of IBD virus (IBDV). Hyperimmune rabbit antisera against vaccinal strains and field isolates were used in cross-neutralization tests. Relatedness values were calculated from geometric mean neutralizing antibody titres based on a minimum of 3 tests.

Key words : Broiler, Infectious broiler disease, Virulent IBD, Epidemiology of IBD

Infectious bursal disease (IBD) is probably one of the most important infections of chickens that causes serious economics losses. The causative virus damages the bursa of Fabricius in young chickens resulting into immunosuppression (Hopkins *et al.* 1979). At least 2 serotypes of IBD virus have been identified, serotypes I and II (Lukert *et al.* 1979, McFerran *et al.* 1980). Serotype I included most of the virus isolates from chickens and turkeys while serotype II contained virus strains isolated mainly from turkeys (Jackwood *et al.* 1982). However, viruses of serotype II could be isolated from chickens (Ismail *et al.* 1988). Subtypes or variants of serotype I were also reported from the USA (Saif *et al.* 1987).

In Egypt IBDV infection was first reported on pathological basis by El-Sergany *et al.*(1974), but the disease had been recognized since the early 1970's (Ayoub and Malek 1976).

MATERIALS AND METHODS

Flock histories

Outbreaks (82) of IBD were studied during 1989-1993. The strain of the birds and their age at onset of the disease, history of vaccination, morbidity and mortality rates varied among the flocks (Table 1). The clinical signs were typical of IBD with sudden onset, short course and sharp increase in mortality that peaked on the third day after onset, followed by a steep decrease ; mortalities reached 56% in some cases. The litter became wet due to increased water intake and there was lower feed consumption. On postmortem examination, typical lesions of IBD were seen including feverish and dehydrated carcasses, haemorrhagic and turgid bursa of Fabricius, small splash haemorrhages in the thigh and pactoral muscles, petechial haemorrhages on the mucosa of the proventirculus, enteritis and brownish kidneys with swollen tubules.

Field isolates

Isolates (82) were recovered from IBD outbreaks by inoculation of bursal homogenates from dead birds into the yolk sac and on the chorioallantoic membrane (CAM) of chicken embryo as well as in chicken embryo fibroblast (CEF) monolayers (Jackwood and Saif 1987). The bursal homogenates were also tested by the agar gel precipitation test (AGPT) as described by Faragher (1972), where they produced very clear precipitation lines when examined against known IBD antiserum.

Preparation of hyperimmune sera

Rabbit hyperimmune antisera were prepared against 8 field isolates of IBDV and 5 vaccinal strains of the standard serotype I (Jackwood *et al.* 1982). The vaccinal strains included :

(a) Winterfield strain 2512 (Gumbokal, Pliva, Zagerb, Yougosalvia; control No. 2680100).

(b) 2512 strain (IBD-Blen, CEVA laboratories, INC., Overland Park, Kansas; list no. 6236).

(c) Delvax Gumboro strain LZD 228 (Mycofarm International b.v. Boxmeer Holland; Batch No. 03166 B).

(d) Intervet Gumboro vaccine Nobilis, strain D78 (Batch/lot 43096, Intervet International B.V., Boxmeer Holland).

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Table 1. Relationship between broiler strains, age of broilers at onset of the disease and IBD mortality rate

Broiler strain	No. of outbreaks	Age at onset of disease (days)			Mortality rate (%)		
		Minimum	Maximum	Average	Minimum	Maximum	Average
Hubbard	33	13	46	27	0.7	40.	12.1
Arbor Acres	14	21	47	33	0.6	24.0	6.26
ASA	6	5	45	24	4.0	30.0	17.70
Lohmann	14	21	38	28	1.8	9.0	4.36
Hypeco-red	5	25	44	34	2.1	14.6	6.00
Native varieties	10	16	26	20	12.0	56.0	27.94
Total	82						

Table 2. Relationship between IBD vaccination, average age at onset of the disease and average mortality rate.

Strain of vaccine	Total No. of outbreaks	Age at vaccination in days									Average age at the onset of the disease	Average mortality rate %		
		First			Second			Third						
		Min.	Max.	Average	Min.	Max.	Average	Min.	Max.	Average		Min.	max.	Average
Intermediate (Int.)	32	12	15	13	-	-	-	-	-	-	26	0.6	36	9.18
Int-Int	7	9	14	12	18	24	23	-	-	-	32	0.96	24	7.49
Mild	25	7	14	13	-	-	-	-	-	-	24	2.4	56	11.66
Mild-Int	11	9	14	12	18	29	24	-	-	-	35	2.4	8.42	6.23
Mild-Int-Int	3	14	19	16	24	29	26	33	35	34	37	0.8	8.42	6.32
No vaccination	4	-	-	-	-	-	-	-	-	-	18	0.8	24	10.48
Total	82	-	-	-	-	-	-	-	-	-	-	-	-	-

(e) TAD Gumboro vac., a mild attenuated strain of cloned-purified CUIM TAD (pharmazeutisches Werk GMBH; Batch 135/1).

Cross neutralization tests

Cross-neutralization tests using CEF cultures were conducted (Jackwood and Saif 1987). Antibody titres to CEF-adapted homologous and heterologous IBDV strains were determined using the constant virus (100 $TCID_{50}$) varying antibody technique.

Determination of antigenic relatedness

The criterion of Kapikian *et al.* (1967) previously described by Jackwood and Saif (1987) was used to express the antigenic relatedness; using the formula of Archetti and Horsfall (1950). The formula is given by the function

$$R = \sqrt{r_1 \times r_2}$$

R values were calculated from a geometric mean titer obtained from 3 tests, a homologous R value is by definition 1, and an R value of 1 or close to 1 indicates antigenic similarity between two virus strains.

Statistical analysis

The data analysed by analysis of variance followed by Duncan's new multiple range test after Steel and Torrie (1960).

RESULTS AND DISCUSSION

Since the summer of 1989, severe outbreaks of IBD with unusually high mortalities appeared in almost all types of chicken farms in poultry producing areas all over the country. Investigation of 82 broiler farms (Table 1) showed that 10 native varieties, 6 ASA, 33 Hubbard, 14 Arbor Acres, 5 Hypeco-red and 14 Lohmann were affected, with average mortality rates of 27.94%, 17.70%, 12.0%, 6.26% and 4.36% respectively. This may suggest difference in susceptibility among the examined broiler strains to IBDV infection, although several other factors have certainly contributed to the differences encountered in average mortality rates. Greenfield *et al.* (1986) concluded that response to IBDV is genetically controlled. Variation in age-susceptibility from 5 up to 47 days (average 26 days) was observed. The upper age-limit (47 days) agrees with that reported by Cosgrove (1962) and El-Batrawi (1990). The lower age-limit (5 days) may suggest the important role of maternal immunity in the early protection of chicks against clinical IBD as reported by Hitchnner (1990). Nevertheless, some authors observed an early age resistance to clinical IBD, regardless of maternal antibody status (Fadly and Nazerian 1983). Ismail *et al.* (1987) suggested that the presence of a large number of highly susceptible bursal cells is very important in the development of clinical IBD.

Furthermore the different vaccination programmes (Table 2) adopted in the broiler flocks showed that the use of intermediate strains of commercial IBD vaccines was generally more protective as judged by lower mortalities than the mild vaccines. This may support the findings of Moussa *et al.* (1990) and Van Den Berg *et al.* (1991). In addition, the use of

Table 3. Relationship between type of vaccine, time of onset of outbreak and mortality rate during outbreaks of infectious bursal disease

Type of vaccine	Outbreaks occurring with 10 days of the date of primary or secondary vacination			Outbreaks occurring 10 days after the date of primary or secondary vaccination		
	No. of outbreaks/ total outbreaks	% of outbreaks	% of average mortality	No. of outbreaks/ total outbreaks	% of outbreaks	% of average mortality
Intermediate	14/32	43.75	12.81	18/32	56.25	5.63
Int.-Int.	3/7	42.58	7.92	4/7	57.14	7.20
Mild	17/25	68.0	13.13	8/25	32.0	8.20
Mild-Int.	5/11	45.45	6.78	6/11	54.54	7.05
Mild-Int.-Int.	2/3	66.66	7.61	1/3	33.33	2.43
Total	41/78*	25.56	9.65	37/78*	47.43	6.10

* Flocks in other 4 outbreaks were nonvaccinated.

Table 4. Antigenic relatedness (R. values) of several vaccinal and field strains of infectious bursal disease.

b	Antisera												
Virus	V4	V7	V10	V65	V66	V17	V16	V9	Vs.2512	Vs. winteer field	VsLZD 288	VsD78	Vs.CUIM
a	c												
V4	1.0 ±0.00	0.81 ±0.167	0.81 ±0.167	0.81 ±0.167	0.83 ±0.288	0.83 ±0.288	0.83 ±0.288	0.83 ±0.288	0.81 ±0.167	0.81 ±0.167	0.81 ±0.167	0.81 ±0.167	0.81 ±0.167
a V7	0.81 ±0.167	1.0 ±0.00	0.83 ±0.288	0.81 ±0.167	0.83 ±0.288	0.83 ±0.288	0.81 ±0.167	0.83 ±0.288	0.90 ±0.167	0.90 ±0.167	0.90 ±0.167	0.90 ±0.167	0.90 ±0.167
a V10	0.81 ±0.167	0.83 ±0.288	1.0 ±0.00	0.83 ±0.288	0.83 ±0.288	0.81 ±0.167	0.81 ±0.167	0.83 ±0.288	0.83 ±0.288	0.83 ±0.288	0.81 ±0.167	0.83 ±0.288	0.81 ±0.167
a V65	0.81 ±0.167	0.83 ±0.288	0.83 ±0.288	1.0 ±0.00	0.90 ±0.167	0.83 ±0.288	0.81 ±0.167	0.83 ±0.288	0.81 ±0.167	0.83 ±0.288	0.90 ±0.167	0.83 ±0.288	0.90 ±0.167
a V66	0.83 ±0.288	0.83 ±0.288	0.83 ±0.288	0.90 ±1.167	1.0 ±0.00	0.81 ±0.167	0.81 ±0.167	0.83 ±.288	0.90 ±0.167	0.83 ±0.167	0.83 ±0.288	0.90 ±0.167	0.83 ±0.288
a V17	0.83 ±0.288	0.83± 0.288	0.81 ±0.167	0.83 ±0.288	0.81 ±0.167	1.0 ±0.00	0.83 ±0.288	0.81 ±0.167	0.81 ±01.67	0.90 ±0.167	0.83 ±0.288	0.90 ±0.167	0.83 ±0.288
a V16	0.83 ±0.288	0.81 ±0.167	0.81 ±0.167	0.81 ±0.167	0.81 ±0.167	1.0 ±0.00	1.0 ±0.00	0.83 ±0.288	0.90 ±0.167	0.90 ±0.167	0.90 ±0.167	0.90 ±0.167	0.90 ±0.167
a V9	0.83 ±0.288	0.83 ±0.288	0.83 ±0.288	0.83 ±0.288	0.83 ±0.288	0.81 ±0.167	0.83 ±0.288	1.0 ±0.00	0.90 ±0.167	0.83 ±0.288	0.83 ±0.288	0.81 ±0.167	0.81 ±0.167
a Vs.2512	0.81 ±0.167	0.90 ±0.167	0.83 ±0.288	0.81 ±0.167	0.90 ±0.167	0.90 ±0.167	0.90 ±0.167	1.0 ±0.00	1.0 ±0.00	0.90 ±0.167	0.90 ±0.167	0.90 ±0.167	0.90 ±0.167
a VsWinterfield	0.81 ±0.167	0.90 ±0.167	0.83 ±0.288	0.83 ±0.288	0.83 ±0.288	0.90 ±0.167	0.90 ±0.167	0.90 ±0.167	0.83 ±0.288	1.0 ±0.00	1.0 ±0.00	0.90 ±0.167	1.0 ±0.00
a VsLZD288	0.81 ±0.167	0.90 ±0.167	0.81 ±0.167	0.90 ±0.167	0.83 ±0.288	0.83 ±0.288	0.90 ±0.167	0.83 ±0.288	0.83 ±0.288	0.90 ±0.167	1.0 ±0.00	0.90 ±0.167	1.0 ±0.00
a VsD78	0.81 ±0.167	0.90 ±0.167	0.83 ±0.288	0.83 ±0.288	0.90 ±0.167	0.90 ±0.167	0.90 ±0.167	0.81 ±0.167	0.90 ±0.167	1.0 ±0.00	0.90 ±0.167	1.0 ±0.00	1.0 ±0.00
a VsCUIM	0.81 ±0.167	0.90 ±0.167	0.81 ±0.167	0.90 ±0.167	0.83 ±0.288	0.83 ±0.288	0.90 ±0.167	0.81 ±0.167	0.90 ±0.167	1.0 ±0.00	1.0 ±0.00	1.0 ±0.00	1.0 ±0.00

a. The same lowercase letter indicate nonsignificant differences was at $P < 0.01$.

b. 100 TCID 50 of 4th issue culture passage were used.

c. A homologous R value is considered 1.00.

IBD vaccines for more than 1 vaccination during the broiler period was more protective (lower mortalities) than a single vaccination (Table 2). An important observation was that the outbreaks which occurred about 10 days or more after primary or secondary vaccination had generally lower mortalities (Table 3). This agrees with the findings of El-Batrawi (1990)

The results of cross-neutralization tests (Table 4) generally showed that the R value of 8 field isolates and 5 classical serotype I vaccinal strains with homologous antisera ranged between 0.81 and 1.0 indicating great antigenic similarity. This

means that there are no subtype differences between the classical serotype I vaccinal strains of IBDV and the recovered field isolates under investigation, which are responsible for the severe outbreaks of IBD. Brooksby (1967) defined the subtype as a group of strains that can be differentiated from other strains within the same serotype by serological methods or by cross-protection studies. Our results differ from that obtained by El-Sanousi *et al.* (1994), and Sultan (1995) but still in agreement with that of Van Der Marel *et al.* (1990).

Our findings suggest that the recent outbreaks of IBD can be attributed to very virulent IBDV belonging to standard serotype I. We also suggest that the failure of the different vaccination programmes to give adequate protection against IBDV field challenge may be attributed to many factors other than antigenic variance, such as proper timing of vaccination, handling and administration of the vaccines, type of the vaccine, as well as the vaccine dose (El-batrawai 1990, Moussa *et al.* 1990, El-Batrawi and Bastami 1990, Van Den Berg and Meulemans 1991). Van Den Berg *et al.*(1991) found that the severe IBD outbreaks were attributable to very virulent IBDV of standard serotype I. Serological typing with monoclonal antibodies showed an antigenic drift in some of these pathogenic strains (Van Den Berg *et al.* 1991, El-Sanousi *et al.* 1994).

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Haemolytic complement activity in normal and duck-plague-virus-vaccinated ducks

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ABSTRACT

The haemolytic complement (C') level of the ducks of different age groups was estimated by using micro-complement fixation test (micro-CFT) and radial haemolytic assay (RHA) technique. In 15-day-old duckling the mean C' titre was very low; however, in adult ducks the titre was significantly high. The C' titre decreased from 60 hr post-challenged infection with the duck plague virus (DPV).

Key Words: Duck plague virus, Ducks, Haemolytic complements activity

The complement (C') is an essential element in the host defence process of animals and man. The levels of C' activity in the chicken were established by the standard tube assay (Barta and Barta 1975) and the radial haemolytic assay (RHA) technique (Skeeles *et al.* 1979). In chicken C' titres increase from birth to adult life (Skeels *et al.* 1989). However, the levels of the haemolytic C' in ducks have not been studied extensively, so far. The study was carried both to establish the baseline levels of the haemolytic C' activity in the ducks at different ages. Additionally, the role of the duck C' in the viral immunity was investigated.

MATERIALS AND METHODS

One week-old crossbred Khaki Campbell ducklings (15; group 1) and 5 adult ducks (group 2) were reared in the cage providing sufficient water and feed. All birds were clinically healthy.

Sera

Blood samples were collected for serum either by heart puncture or venipuncture. Blood was allowed to clot at 4°C for about 3 hr and the serum was collected by centrifugation at 4°C. Blood samples were collected at 15, 30, 45 days of age from ducklings and adult ducks. Ducks of group 2 were vaccinated with 4.5 DLD_{50} duck plague virus (DPV) by i/m route at 3 months of age and revaccinated after 1 month of the first vaccination. Vaccinated ducks were challenged with 2.5 DLD_{50} virulent DPV by i/m route at 21 day of the booster vaccination. Blood samples were collected just before challenge and at 20,60,90 and 150 hr of post-challenge infection.

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Complement assay

In C assay, the rabbit erythrocytes were sensitized with 1:400 dilution of duck haemolysin. The radial haemolytic assay (RHA) was carried out according to Skeeles *et al.* (1979). Each 11 ml reagents contained 6 ml of melted agarose, 4 different volumes (0.5, 1.0, 1.5, 2.0 ml) of 1% sensitized erythrocytes and required volume of gelatin- veronal buffer (GVB+, pH 7.2). The sensitized erythrocytes of 1.5 ml were found suitable and added to the melted agrose maintained at 60°C in a water-bath. The immunodiffusion plate (75 mm × 75mm) were prepared and after solidification the wells of 4 mm size were cut in 4 rows of 6 wells each. Serum samples in 2-fold dilutions were made in NaCl-veronal buffer containing calcium and magnesium and 20 µl of each dilution was added in wells in duplicate. An additional 20 µl was added into the wells in duplicate. Plates were then placed in the water-bath for 1 hr at 37°C and results were read immediately. The highest dilution of the serum with visible haemolytic ring around the well was considered as the end point.

Table 1. Mean complement (C) titres (±SE) of ducks at different ages

Age (days)	No. of ducks	Mean C' titre		
		Micro-CFT	RHA(20 µl)	RHA (40 µ l)*
15	9	3.55±0.27	2.00±0.00	3.55±0.27
30	9	8.00±0.00	4.00±0.00	7.55±0.41
45	9	23.11±2.65	12.44±1.32	24.00±3.07
Adult	9	53.33±10.05	20.44±5.20	40.88±10.40

*Additional 20 µl each diluted serum sample was added.

Duplicate serum samples were subjected to the micro-CFT Fadly and Nazerian (1983) following with modification to permit the use of GVB+ and 1% sensitized rabbit erythrocytes. To

each 20 µl of diluted serum, an equal volume of sensitized erythrocyte was added. The plates were incubated at 37°C in the water-bath for 1 hr. The highest dilution showing visible haemolysis was considered as the titre of the serum.

RESULTS AND DISCUSSION

The standard tube assay has been used earlier to establish the levels of C' in the chicken (Barta and Barta 1975). However, this assay technique has limitation in handling large number of serum samples. Comparison of the results (Table 1) of RHA and micro-CFT revealed that the micro-CFT was approximately 1 dilution more sensitive than that of the RHA. However, RHA values corresponded with the titre of the micro-CFT when an additional 20 µl of each diluted serum sample was added into the respective wells. The results confirmed that both RHA and micro-CFT were rapid in estimation of C' titre and required small volume of reagents (Gewurz and Suyehira 1976, Skeeles *et al.* 1979, Fadly and Nazerian 1983). Although RA is slightly less sensitive, yet is simpler and practical in the study of C'. In RHA repeated standardization of the reagents is not necessary and prepared slides could be preserved at 4°C for some time.

Table 2. Mean complement (C') titres (±SE) in duck-plague-vaccinated ducks at different postchallenge hours

Postchallenge period (hr)	No. of ducks	Mean C' titre	
		Micro-CFT	RHA (20 µl)
0	5	51.20±7.01	25.60±3.50
20	5	51.20±7.01	25.60±3.50
60	5	35.20±7.01	17.60±3.50
90	5	16.00±0.00	8.00±0.00
130	5	19.20±2.86	9.60±1.43

The C' titre in healthy normal ducks increased with the age (Table 1). The levels of C' were significantly high ($P<0.01$) in the adult ducks as compared to the ducklings. The mean C' titre in 15-day-old ducklings was 3.55 and 2.00 in the micro-CFT and RHA, respectively, while the corresponding values in adult ducks were 53.33 and 20.44. The present study establishes baseline levels of the haemolytic C' in the duck at various ages. Increase of the levels of C' from the ducklings to the adult strongly suggest to the age dependent development of C' activities. The mean radial haemolytic C' titres were low in ducks (Table 1) as compared to the chicken of 14 - to 43- days old where mean titre ranged from 14 to 33 (Skeeles *et al.* 1980). This variation in C' titre could be attributed to phylogenic differences of the 2 species.

The host immune status in most cases is regulated by the C' (Sarma and Sharma 1989). Ohta *et al.* (1986) demonstrated the elimination of the early pox virus infection by alternative pathway of the C'. Chicken plasma showed the complement resistance to avian *E. coli* infection (Wooley *et al.* 1991). In this study, the adult ducks infected with DPV showed the gradual decrease of the C' titre from 60 to 130 hr post-infection (Table 2). The mean C' titre dropped from 25.6 to 9.6 in RHA and 51.2 to 19.2 in micro-CFT. The decline of the C' titre in immune ducks challenged with DPV suggests the involvement of the C' in the immune complexes. In contrast the sera of the repeatedly immunized ducks sometime lacked complement fixing activities (Higgins *et al.* 1993). Although the role of C' was not clearly indicated but the solid matrix duck hepatitis B virus antibody antigen complex could eliminate the serum duck hepatitis B virus antigen (Wen *et al.* 1994). Therefore the role of C' in infection needs to be elucidated further.

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Antigenic specificities of several Indian stocks of *Trypanosoma evansi* by Western blot analysis

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ABSTRACT

Western blotting was used in the analysis of major antigens in the whole cell lysate (WCL) preparations of 7 stocks of *Trypanosoma evansi* isolated from buffaloes, equids and camels from different localities of northern India. Most of the major polypeptides (in sodium dodecyl sulphate polyacrylamide gel electrophoresis) were detected as major antigens employing homologous as well as heterologous hyperimmune sera (HIS). The total number of major antigens detected ranged between 17 to 30, most of which were in the M.W. range of 14.6 to 99.5. Homologous HIS detected more antigens in 5 stocks than the number by heterologous HIS. The observation in stock-3 and stock-7 was somewhat different, heterologous HIS detecting more antigens than those by homologous HIS. Antigenic profiles of the WCL did not show resemblance between any 2 of the 7 stocks. Antigenic cross-reactivity of the most prominent polypeptide, that presumably represents the major variable surface glycoproteins (VSG) was very high indicating the possibility of single or a few iso-VAT (variable antigen type) population in these 7 stocks.

Key words : Antigens, Polypeptides, SDS-PAGE, *Trypanosoma evansi*, Western blotting

Results from cross-immunity studies indicated the existence of a number of serologically distinct stocks of *Trypanosoma evansi* (Hoare 1972), but information on the antigenic specificities and distribution of the different stocks is lacking. New techniques of immunology and molecular biology provided the means for the characterization of strains/isolates/stocks from diverse sources. A search for potential protective non-variable *T.evansi* antigens could be of interest from both an immunoprophylaxis and immunodiagnosis point of view. Jones and Mckinnell (1985b) suggested limited antigenic diversity among *T.evansi* stocks compared to the tse tse fly transmitted salivarian trypanosomes. It is desirable to identify the number of serodemes in a discreate endemic area (Gardinder P R , personal communication). As nothing is known so far regarding the antigen specificities of different stocks/strains of *T.evansi* in India, the present study attempted to provide fundamental information through polyacrylamide gel electrophoresis (PAGE) on whole cell lysate antigen of several stocks isolated from diverse sources of northern India.

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MATERIALS AND METHODS

T. evansi *stocks*

Stocks (7) from different hosts and localities (Table 1) were maintained in batches of albino rats through serial passages of infected blood at 72 hr intervals.

Trypansomal protein samples

Whole cell lysate (WCL) of trypanosomes of each stock was prepared after collection from the experimentally infected albino rats and separation through DEAE cellulose column (Lanham and Godfrey 1970). Trypanosomes were sonicated at 150 W for 3 min 3-4 (keeping on ice) by ultrasonic disintegrator. The supernatant fluid was collected and the protein content adjusted to 1.0 mg/ml with PBS, pH 8.0 (Lowry *et al.* 1951). The WCL antigens were stored in aliquotes at -20°C till further use.

Hyperimmune sera (HIS)

Anti-trypanosome HIS were raised separately stock wise by multiple site injections of rabbits with 1.0 mg/ml of the WCL antigen. The antigen was mixed with equal volume of Freund's incomplete adjuvant for first injection and Freund's incomplete adjuvant for the subsequent 2 injections. After a minimum of 3 weekly booster immunizations, the rabbits were bled by cardiac puncture and the sera were separated and stored in aliquots at -20°C till further use.

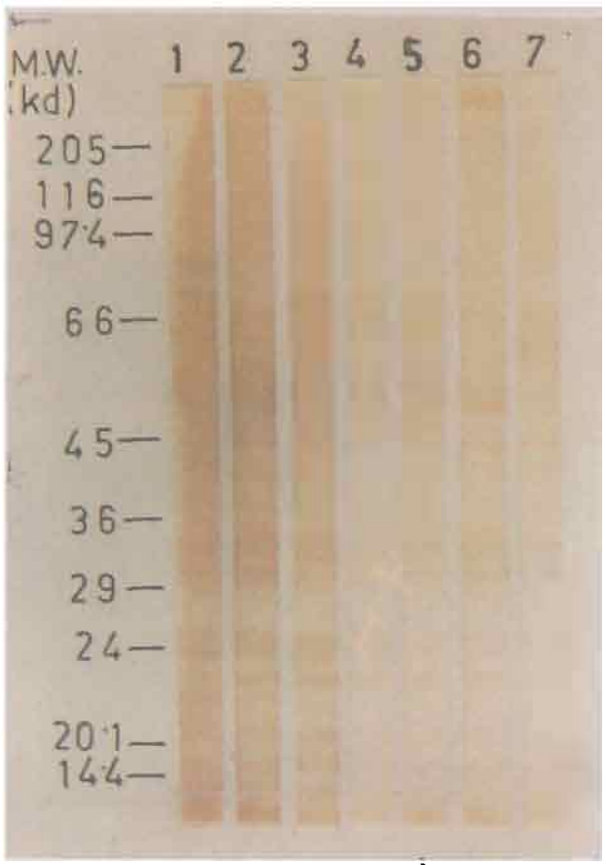


Fig.1. Western blot of the whole cell lysate antigens of stock- 6 of *T. evansi*, using homologous (Lanes-6 and heterologous), Lanes 1 - 5 and -7 HIS. Positions of the molecular weight markers are shown on the left side of the photograph. Lane 1 : Antigen profile of WCL of stock-1, Lane 2 : Antigen profile of WCL of stock-2, Lane 3 : Antigen profile of WCL of stock-3, Lane 4 : Antigen profile of WCL of stock-4, Lane 5 : Antigen profile of WCL of stock-5, Lane 6 : Antigen profile of WCL of stock-6, and Lane 7 : Antigen profile of WCL of stock-7.

Trypansomal WCL proteins were solubilised by boiling them for 3 min in sample buffer containing a final concentration of 5% (v/v) 2- mercaptoethanol and 2.3% sodium dodecyl sulphate. A 0.5 ml volume of the reduced proteins in sample buffer were loaded into 1 large well spanning the width of the 3% acrylamide stacking gel. The proteins were separated in 10% acrylamide resolving gel under reducing conditions by using Laemmli's discontinuous buffer system (Laemmli 1970). The molecular weight marker proteins (Mw-SDS-200 and MW-SDS-70L) ranging between 14.4 KD and 205 KD, 10 µl of each were loaded in the adjacent (Border) wells in all the runs. All gels were 1.5 mm thick. The electro-phoresis was performed at room temperature (20°-25°C) for about 6 hr in vertical slab gel electrophoresis unit at a constant current of 20 mA for stacking and subsequently at 30 mA for approximately 4 hr for resolution in the separate gel at a time, when the tracking dye front reached approximately 5 mm above the bottom edge of the gel.

Western blotting

Trypanosomal proteins resolved by SDS-PAGE were electrophoretically transferred at 24V for 4 hr from the gel to a 0.45 µm pore-size nitrocellulose membrane (Towbin *et al.* 1979). Following transfer, the nitrocellulose membrane-contained-blotted trypanosoma protein was cut into a number of 5 mm wide strips. All incubation steps and washing of these strips were performed on a shaker at room temperature. One of these strips was stained with 0.1% Coomassie brilliant blue R-250 in 45% methanol, 10% acetic acid and 45% distilled (glass) water to detect all transferred trypanosomal proteins and molecular weight markers run along the sample. The remaining strips were immediately placed in phosphate-buffered saline solution (10 mM phosphate buffer, pH 7.2, 150 mM NaCl) containing 0.05% Tween 20 (PBST) and 3% bovine serum albumin fraction V for 2 hr to block excess protein - binding sites. Seven anti-trypanosome rabbit sera (1 from each of the *T. evansi*) stocks were diluted 1:100 in PBST and incubated without serum to act as the negative control. Following for 5 min washes with PBST, the strips were incubated for 1 hr with HRP anti-rabbit IgG conjugate at a 1:2000 dilution in PBST. After 5 washes as described above, the strips were developed with freshly prepared substrate solution, 3,3' - diaminobenzidine tetrahydrochloride 50 mg and 20 µl H_2O_2 (30% v/v) in 100 ml of PBS. The reaction was allowed to proceed for about 5 min and stopped by washing the strips with distilled water.

Molecular weights for the trypanosomal proteins that were reactive either serum antibodies (HIS) estimated by comparing their migration, relation to those of marker proteins of known molecular weight, that run on the same gel.

The relative mobilities (R_f) were calculated for each of the marker proteins and the resolved antigens of *T. evansi* stocks by the formula:

$$R_f = \frac{\text{Migration distance of protein/antigen (mm)}}{\text{Migration distance of tracking dye (mm)}}$$

Molecular weight determination of the individual antigen in the profile of WCL was carried out using regression analysis in a programme generated in Fortran -77 language on Micro-32 computers.

The regression equation was :

$Y' = a + bx$

Where,

$Y' = \log^Y = \log$ molecular weight

$a =$ constant factor

$b =$ regression coefficient

$x = R_f$

RESULTS AND DISCUSSION

Western blot analysis of WCL stocks 1 to 7 of *T. evansi* using homologous and heterologous HIS (Figs 1 and 2; Table 2) indicating that most of the major polypeptides in the WCL were detected as major antigens by homologous as well as heterologous HIS. However, some of the major polypeptides

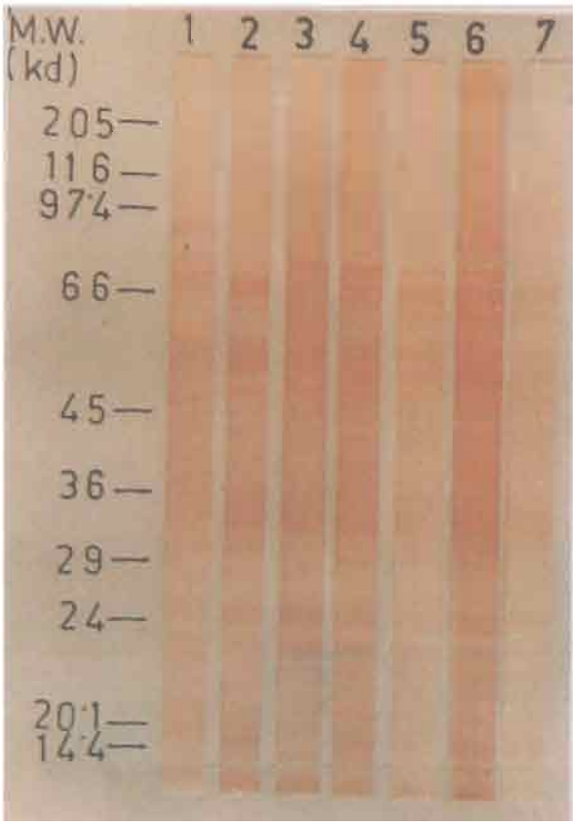


Fig.2. Western blot of the whole cell lysate antigens of stock- 7 (Mule, Ludhiana) of *T. evansi*, using homologous (Lane-7 and heterologous Lanes 1-6) HIS. Positions of the molecular weight markers are shown on the left side of the photograph. Lane 1 : Antigen profile of WCL of stock-1, Lane 2 : Antigen profile of WCL of stock-2, Lane 3 : Antigen profile of WCL of stock-3, Lane 4 : Antigen profile of WCL of stock-4, Lane 5 : Antigen profile of WCL of stock-5, Lane 6 : Antigen profile of WCL of stock-6 and Lane 7 : Antigen profile of WCL of stock-7

were detected only in homologous or heterologous HIS. A minimum of 24, 17, 25, 21, 30 and 24 antigens could be detected in stocks 1 to 7, respectively, using HIS against these 7 stocks separately. Homologous HIS detected a larger number of antigens compared to heterologous HIS in all but stocks 3 and 7.

Table 1. Geographic location and host species of the seven stocks of *Trypanosoma evansi* under study

Stock No.	locality where isolated	Host species (or origin)
1	Bareilly	Buffalo
2	Bikaner	Camel
3	Hisar	Horse
4	Karnal	Buffalo
5	Karnal	Horse
6	Lucknow	Buffalo
7	Ludhiana	Mule

The most prominent polypeptides, viz. 58.7 KD in stock-1, 60.1 KD in stock-2, 63.0 KD in stock-3, 61.5 KD in stock-4 and 63.0 KD in stock-7 was selected by all the 7 HIS. In stock-5 the prominent polypeptide of 63.0 KD was detected by HIS against stock-5 (homologous) and 6 and 7. In stock-6 the most prominent band of 63.0 was detected by HIS against stock-6 (homologous) and stocks 1,2,5 and 7.

In stock-1, 6 polypeptides of 58.7, 58.3, 50.0, 42.9, 26.5 and 14.6 KD were detected by all 7 HIS. In stock-2 6 polypeptides of 60.1, 58.7, 55.9, 49.6, 29.2 and 14.6 KD were detected by all the 7 HIS. In stock-3, 10 polypeptides of 72.8, 71.1, 69.4, 63.0, 53.0, 47.2, 45.0, 34.6, 32.9 and 14.6 KD M.W. were detected by all HIS. In stock-4, 5 polypeptides of 80.2, 74.6, 61.5, 57.3 and 40.9 KD M.W. were detected by all the 7 HIS. In stock-5, 7 polypeptides of 66.1, 50.8, 43.9, 41.9, 32.2, 29.9 and 21.9 KD M.W. were detected by all the 7 HIS. In stock-6, 5 polypeptides of 74.6. 58.7, 57.3, 25.9 and 23.5 KD were detected by all the 7 HIS. In stock-7, 9 polypeptides of 72.8, 66.1, 63.0, 47.2, 45.0, 40.9, 30.7, 26.5 and 19.4 Kd M.W. were detected by all the 7 HIS.

Table 2. Western blot analysis of whole cell lysate antigens detected by homologous and heterologous hyperimmune sera against seven stocks of *T. evansi*

HIS employing against stock No.	Whole cell lysate antigens of stock No.						
	1	2	3	4	5	6	7
1	21	15	21	21	18	24	20
2	15	15	17	22	12	16	22
3	15	15	14	8	9	15	21
4	18	12	22	25	16	26	14
5	16	11	18	23	21	16	15
6	20	8	19	21	15	28	18
7	18	8	11	23	21	17	16
Overall antigens detected by all the seven HIS	24	17	25	27	23	30	24

Most of the major polypeptides in the WCL of different stocks were detected as major antigens by homologous as well as heterologous HIS. However, some of the major polypeptides could not be detected by homologous and heterologous HIS. A few major polypeptides were not detected by their homologous HIS in case of stock-2 (52.0 and 43.4 KD), stock-4 (60.1 KD) and stock -5 (101.9, 80.2, 63.0 and 61.5 KD). There are several possible explanations for these anomalies. Firstly antibody titre in these HIS was much lower as compared to other HIS, since fixed dilution (1:100) of all the HIS used in this study, less number of antigens could have been detected by these HIS. Secondly, the immune response against some of the polypeptides not detected by homologous HIS might have been predominantly against conformational epitopes against these antigens. Since immunoblotting technique is incapable of detecting conformational epitopes (it detects only linear epitopes of

denatured proteins), the number of antigens detected by HIS were less. Either one or both the postulated reasons could be accepted for such an anomaly.

Many minor polypeptides were also detected as major antigens in western blot analysis of the 7 stocks, probably because of their high antigenicity. Some of the major polypeptides not being detected by homologous HIS and most of the heterologous HIS was possible either due to their poor antigenicity or their having predominantely conformational epitopes or broth.

Epitopes which are involved in antigenic variations in most cases are conformational in nature. For antigenic analysis methods that fail to destroy such epiotopes are used. These methods used more frequently for VAT determination of trypanosomes, include immunolysis, immuno-fluorescence and in certain cases radio-immuno precipitation. Since we used SDS-PAGE for resolving proteins and subsequently transferred to nitrocellulose membrane for immunoblot analysis such conformational epitopes were not expected to be detected by HIS. *T. evansi* may be more limited antigenically than tse tse transmitted trypanosomes because of the absence of developmental stages in the vector which precludes an opportunity for genetic exchange in *T.evansi* (Jones and Mckinnell 1958a).

Serological characterization of 15 isolates of *T. evansi* by their predominate VATs showed the existence of only 3 serodemes among trypanosomes isolated from camels in the Sudan (Jones and Mckinnell 1985b).

Furthermore, in a limited study in Indonesia only 1 serodemes circulating in cattle and buffalo of 1 district was found (Luckins 1988). Therefore, on the basis of these findings, it can be speculated that among the seven *T. evansi* stocks under study the number of serodemes appeared very limited. The apparently limited antigenic diversity in *T. evansi* infection can have a bearing on control programmes based on immunoprophylaxis. The idea of a local vaccine based on a cocktail' of metacyclic antigens was proposed after the identification of a limited and serodeme-specific-metacyclic-VAT heterogeneity in African trypanosomes (Vickerman and Barry 1982, Masake *et al.* 1987). This 'cocktail vaccine' theory has always depended on the identification and enumeration of trypanosome serodemes circulating in nature. Strict monitoring of circulating serodemes would have to occur over a period of time before any evaluation of control measures could be taken.

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Experimental mastitogenicity of *Mollicutes* originated from bovines with reproductive disorders in rat mammary gland model

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ABSTRACT

Rat mammary gland model was used to test mastitogenicity of *Mollicutes (Mycoplasma, Acholeoplasma)* from various bovine reproductive disorders. The progress of infection was monitored in terms of gross and histopathological lesions of mamary tissues and reisolation of inoculated mollicutes at necropsy. The tested *Mollicutes* could be categorized into 2 groups : (i) *M. mycoides* subsp. *mycoides* (LC), *M. bovis, M.bovigenitalium, M.bovoculi, M.arginini* (type strain G-230), *A.granularum* as mastitogenic, and (ii) *A. axanthum, A.laidlawii, A.oculi* and *M.*sp. (ungrouped) as non-mastitogenic to rat mammary gland. Thus. rat-mammary gland model is recommended for use as a suitable and economical model for determining the mastitogenicity of mollicutes.

Key words : Mammary gland, Mastitogenicity, *Molllicutes, Mycoplasma, Acholeoplasma*

Role of mycoplasmas in reproductive disorders of animals is of emerging interest. Earlier, pathogenicity of *Mollicutes* has been tested *in-vivo* by mammary gland inoculation of cows, goats and mice (Pal *et al.* 1983, Mackie and Ball 1984, Kumar *et al.* 1983, Mackie and Ball 1984). Garg *et al.* (1991) and Kumar *et al.* (1994) have evaluated experimental pathogenicity of *Mollicutes* from equine metritis and bovine mastitis, respectively, inoculating the rat mammary gland. This paper describes the mastitogenicity of *Mollicutes* isolated from bovines with various reproductive disorders in rat mammary gland model.

MATERIALS AND METHODS

Mollicutes

Eight *Mollicutes,* viz. *M. mycoides* susp. *mycoides* LC type (BA-17/83, SC); *M.bovis* (BA-75/85, I0); *Mycoplasma* sp. (US-10/83, FT); *A. laidlawii* (BA-45/83, HB) from cases of bovine abortion : *M. bovoculi* (SBC-7/84, I0) from bovine still birth; *M.bovigenitalium* (FS-82/85) from frozen bull semen ; *A. oculi* (RB-21/83), *A. granularum* (RB-23/83) from repeat breed cow and 2 type strains, viz *M. arginini* (G-230), *A. axanthum* (S-743) in lieu of field isolates of *M. arginini* (FS-19/83) from frozen bull semen and *A.axanthum* (VSBA-33/83) from an aborted cow, were studied to evaluate the mastitogenicity in rat mammary gland model.

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Media

All the *Mollicutes* were maintained by serial passages in PPLO-broth medium (Kapoor and Garg 1987). Inoculum was prepared by growing the culture for 48 hr in PPLO-broth medium without thallium acetate.

Experimental animals

Norway albino female rats (*Rattus norvigus* strain *wistar)* at 7-10 days lactation were from the Disease Free Small Animal House, CCS Haryana Agricultural University, Hisar. Their offsprings were weaned 1-2 hr prior to the experiment of rat mammary-gland.

Infection of rat mammary gland

The rat was inoculated via intra-mammay route (Garg *et al.* 1991); the third, fourth and fifth pectoral mammary glands of each side inoculated with 0.1 ml (10^6 cfu/gland) of *Mollicutes.* Each infected as well as control mammary gland was cut into 2 halves; 1 half being fixed in neutral buffered formalin for histopathological examination. The other half was ground with sterile sand in 1.8 ml examination. PPLO-broth (Kapoor and Garg 1987) containing 50 µg/ml ammonium reineckate to recover the inoculated organisms.

Mammary glands on the left side were inoculated with test culture, whereas, 2 right glands served as control and one right gland served as control inoculated with sterile broth. Five rats per culture were infected and observed daily and one rat each was sacrificed at 1-, 2-, 3-, 4- and 6-day post infection (PI) and autopsied.

Table 1. Mastitogenicity potential of *Mollicutes* originated from bovine genital tract in rat

Mollicutes infected in rat mammary gland	Org. recovery	Gross lesion	Degree of histopathological changes										Mastitogenic status
			Inflammatory cell response in						Avelor lining		Fibro	-Hyper-	
			Lumen of alveoli			Interalveolar tissue			Desq.	Disto.	balstic infiltration	plasia	
			N	L	M	N	L	M					
M.mycoides ss *mycoides* (LC)	4*	4*	+++ (3)*	-	-	+ (3)	++ (6)	++ (6)	++ (3)	+++ (6)	++ (6)	++ (6)	Mastitogenic
M.bovis	4	6	+++ (2)	+ (4)	++ (4)	++ (1)	+ (6)	++ (6)	+ (3)	++ (6)	++ (6)	++ (6)	Mastitogenic
M.bovigeni-talium	4	4	+++ (3)	-	+ (4)	+ (3)	++ (6)	+ (6)	+ (4)	++ (6)	+++ (6)	+ (4)	Mastitogenic
M. bovoculi	6	6	+++ 2	-	-	+ 4	++ 6	++ 6	+ 4	++ 6	+++ 6	++ 4	Mastitogenic
M.arginini	6	4	+++ (2)	-	-	+ (2)	++ (6)	+++ (4)	+++ (2)	+++ (6)	+++ (6)	+	Mastitogenic
A. granularum	6	5	+++ (2)	-	-	+ (2)	++ (6)	++ (6)	++ (5)	+++ (6)	+++ (6)	++ (6)	Mastitogenic
A. axanthum	*4*	*3*	+ (3)	-	-	-	+ (6)	+ (6)	+ (1)	+ (6)	+ (6)	+ (6)	Non-masti-togenic
A. laidlawii	4	3	+ (4)	+ (3)	-	-	+ (6)	+ (6)	+ (5)	++ (6)	++ (6)	++ (6)	Non-masti-togenic
A.oculi	4	4	+ (2)	-	-	-	++ (6)	++ (6)	++ (6)	++ (6)	++ (6)	+ (6)	Non-masti-togenic
Mycoplasma sp.	4	4	-	-	-	-	+ (6)	+ (6)	-	++ (6)	++ (6)	++ (6)	Non-masti-togenic

1+ to 3+ Indicates intensity of cell infiltration in described histopathological change; N, neutrophils; L, lymphocyte cells; M, macrophages; Desq', Desquamation; distor, distortion; *Figures indicate observation at day-post infection.

RESULTS AND DISCUSSION

All the infected rats appeared clinically healthy just as control one. All the isolates of *Mollicutes* inoculated into the rat mammary gland could be reisolated in pure form 4 to 6 day-PI (Table 1) and no other bacteria were ever found associated with *Mollicutes* during entire period of observations. No gross change except mild to dark red area surrounding the glandular tissues on reflected skin could be observed. The main histopathological features were: mild to marked neutrophilic infiltration in alveolar lumen, desquammation of alveolar cell-lining with mild to moderate infiltration of lymphocytes and macrophages in interalveolar tissue followed by distortion of alveoli leading to narrowing of lumen due to fibroblastic and macrophage proliferation and lymphocytic infiltration in the interstitium. Occasionally, mild to moderate degree of hyperplasia of intra-lobular duct was also observed (Table 1).

Lesions observed in the rat-mammary gland and the recovery of inoculated organisms from mice mammary gland could be conveniently used to group the *Mollicutes* of bovine reproductive tract origin to indicate their mastitogenic status into 2 categories : (i) Mastitogenic, viz. *M. mycoides* subsp. *mycoides* (LC), *M.bovis*, *M.bovigenitalium*, *M.bovoculi*, *M.arginini*, *A.granularum* which induced moderate to marked (++ to +++) histopathological lesions (and mollicutes could be reisolated from rat mammary glands till 4 to 6 days post infection), and (ii) non-mastitogenic, viz. *A. axanthum*, *A. laidlawii*, *A.oculi*, *M.*sp. (ungrouped) which produced no or insignificant mild histopathological lesions (- to ++) and establishment of these mollicutes, however, was indicated by their recovery till 3 to 4 days postinfection.

This study suggested that histopathologically the basic inflammatory response in rat mammary tissues infected with *Mollicutes* was neutrophilic similar to that seen in mouse mammary gland infected with *Mycoplasma* (Hale *et al.* 1962, Jasper, 1982, Kumar *et al.* 1994) and *Ureaplasma* (Howard *et al.* 1975). *M. bovis*, *M. baigenitalium* and *M. arginini* are known to produce mastitis in cow (Roberts 1968, Bennet and Jasper 1977, Jasper 1981). *M. mycoides* subsp. *mycoides* (LC), *M. bovoculi*, *A. granularum* and *A.oculi* have not been tested earlier in udders of cows, mice and other animals. *A. laidlawii* and *A. axanthum* are known to produce only transient inflammatory response in mammary glands of lactating cows and mice (Gourlay *et al.* 1979, Kumar *et al.* 1994). The experimental observations also suggested that though *M. mycoides* subsp. *mycoides* (LC), *M. bovis*, *M. bovigenitalium*, *M. bovoculi*, *M. arginini* and *A. granularum* used in our study were associated with reproductive disorders in bovines,

these may also cause mastitis in milching animals. Such mycoplasmal cross infections have already been shown to occur experimentally in bovine calves with *M. mycoides* subsp. *mycoides* (LC) (Kapoor 1993)

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Gross and histomorphological effects of antiarthritic drug in experimental acute traumatic arthritis in donkeys

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ABSTRACT

Acute aseptic arthritis was induced in 8 healthy donkeys aged 3-4 years and weighing 80-100 kg. The animals were randomly divided into 2 groups A and B of 4 animals each. Group A served as a control whereas in group B antiarthritic drug (AD) was administered orally @ 20 g from day 2 to 26. In AD treated animals, the gross changes in the joint capsule, synovial membrane and articular cartilage were far less than those in control. Microscopically joint capsule showed complete longing of the intima layer and subintimal layer showed severe inflammatory reaction or even complete necrosis of the intima layer and fibrous capsule and synovial membrane in untreated animals. In AD-treated animals various layers of joint capsule showed organization and proliferation and inflammatory cells were not traceable. Synovial membrane of untreated animals showed less of alcinophilic material and was more PAS-positive as compared to treated animals. Calcium cartilage deposition was not evident in joint capsule of the treatment group. Degeneration of articular was observed microscopically in control group animals as marked by fibrillation and desquamation of pericohndrial tissue with different layers or even complete sloughing of the articular cartilage. Severity of articular cartilage changes in AD-treated animals was very much less than that in control group and the changes were restricted to tangential layer only.

Key words : Antiarthritic drug, Arthritis, Donkeys, Histomorphological effect,

Most of the times, injuries due to accidents or from racing and jumping may lead to traumatic arthritis in equines if the joint area is involved. Any delay in treatment can ensure drastic physiological /biochemical changes in the joint resulting in secondary degenerative joint disease. Antiarthritic drug (AD) is an herbal preparation (containing *Allium sativum, Cyperus rotundus* and *Zingiber officinalis*) and possesses remedial properties for arthritis. On the basis of clinical, radiological, haematological and synovial fluid observations, it was concluded that the drug has healing effect in acute arthritis in equines as confirmed by decreased periarticular swelling and arthralgia as evident by early return to normal stance, decreased lameness and pain on flexion as well as normalization of various synovial and blood biochemical parameters (Singh 1994). The present study was conducted to assess the effect of this drug at cellular level in acute arthritis in donkeys.

MATERIALS AND METHODS

The study was conducted on 8 healthy donkeys aged 2-4 years and weighing 80-100 kg. A fortnight before the start of the experiment, all animals were given 2.5 ml of absorbed tetanus toxoid intramuscularly. Deworming was also done with albendazole @ 5 mg/kg body weight. During experimental period, the managemental and feeding practices were similar and constant for all the animals . A day before the start of the experiment, the donkeys were clinically examined along with radiograph of the carpal joints and declared clinically healthy for the experiment.

Pilot trials were conducted on 4 additional donkeys to standardize the dose of turpentine oil for induction of acute arthritis in the intercarpal joint. A dose of 0.5 ml of turpentine oil mixed with 0.5 ml of gentamicine sulphate (containing 20 mg of gentamicine) was sufficient to create aseptic traumatic arthritis in the left intercarpal joint of the animals.

In all animal, the left carpal joint was prepared aseptically using soap and a chlorhexidine and cetrimide solution. After 2-3 washings, about 1 ml of lignocain hydrochloride was injected subcutaneously in the depression felt over the intercarpal joint at dorsomedial aspect of the carpal. This was followed by final scrubbings of the carpal joint and painting with povidone iodine solution. In standing position, left knee was flexed and intercarpal joint was entered aseptically with a sterilized disposable 1" 20 gauge needle in the depression medial of carpi radialis tendon. About 2 ml of synovia was aspirated with a disposable syringe. After collection of

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synovia, 0.5 ml of turpentine oil mixed with 0.5 ml of gentamicine was injected into the intercarpal joint through the same needle. The animals were left loose to move around freely on the subsequent day till the end of the experiment.

All the 8 donkeys, after induction of acute arthritis were randomly divided into 2 groups A and B of 4 animals each. Group A served as control in which no treatment was given. The animals of group B were given 20 g of AD daily for 25 days, starting 48 hr after the induction of the arthritis. The drug was mixed in *gur* (treacle) and given orally in the form of electuary. As per recommendation of the manufacturers 15 ml of PF S concentrate was given orally 7 days before the start of AD administration.

All the animals of both the groups were sacrificed on 30th. day after induction of arthritis. The affected carpus and the contralateral carpus were harvested by cutting proximal and distal bones of the carpus. The joints were de-skinned with a scalpel blade no. 20 and the intercarpal joints of both affected and normal joints were opened carefully avoiding any damage to the articular cartilage. The joint capsule and articular cartilage were observed for gross pathological change. To study the psychohistopathological and histochemical changes, the tissue samples of articular cartilage and joint capsule of diseased and contralateral normal joints were harvested. Articular cartilage was collected from the base with a fine saw. All samples were fixed in 10% buffered neutral formaline and in Bouin's fixative.

After processing, 5 μ thick sections of collected tissue were obtained and stained with the following procedures :

1. Hematoxylin and Eosin stain for histomorphological studies (Luna 1968)
2. Alcian blue (1pH) periodic Acid Schiff stain to study mucopolysaccharides (Luna 1968).
3. Masson's trichrome stain to study fibrous tissue/collagen (Luna 1968)
4. Best's Caramine stain to demonstrate glycogen (Luna 1968)
5. Vonkossa counter stain with Nuclear Fast Red to demonstrate calcium deposits (Luna 1968).

RESULTS

Macroscopic findings

In gourp A, the affected carpal joint showed thickened periarticular tissue and the joint capsule. The synovial membrane was having haemorrhagic spot with the presence of blood clots all around its surface. In group B, the periarticular tissue and the joint capsule was less swollen as compared to group A. The synovial membranes had fewer haemorrhagic spot and was without any blood clot on its surface. The articular cartilage of the affected joint of group A was reddish black in appearance. The cartilage became thin and at locations, it was eroded with haemorrhagic spots (Fig.1). Reddish fibrinous material was also seen adhered to the surface. Articular cartilage of group B was smooth (without any erosion) whitish yellow with some shine (Fig. 2). Few focal haemorrhagic spots were seen on the articular surface.

Microscopic findings of joint tissue

In group A, intimal layer of synovial membrane was completely sloughed off. Subintimal layer showed edema, necrosis and leukocytic infiltration (Fig.3). There were increased number of blood vessels with haemorrhagic spots. The deeper layer of joint capsule was also oedematous and connective tissue bundles appeared to be loosened. In 1 animal, synovial membrane and part of fibrinous capsule showed necrosis. At certain locations even the calcified mass was encountered over tunica intima and subintimal layer (Fig.4). The tangential layer of articular cartilage in this group showed marked fibrillation and desquamation of perichondrial tissue (Fig.5). The chondrocytes were necrosed. The intermediate layer showed vacuolation and necrosis of chondrocytes and lacunae could be seen without chondrocytes. The deeper layer also showed necrosis of chondrocytes and separation from underlying calcified layer was evident. The osseocartilagenous tissue was a little disorganized. In one animal, there was complete sloughing off articular cartilage leaving the calcified cartilage exposed.

In group B, the intima, subintima and fibrous layers of joint capsule showed organization and proliferation of granulation tissue (Fig. 6). No inflammatory cells could be seen. The articular cartilage from AD treated animals showed necrosis of superficial layer and part of intermediate layer but radiate layer had normal chondrocytes (Fig. 7). The perichondrium was strongly eosinophilic as compared to intermediate and radiate layer of articular cartilage. The tangential layer of perichondrium showed fibrillation and desquammation. The lower area of tangential and upper area of intermediate layer also showed varying degree of necrosis. The total thickness of cartilage was comparable to control.

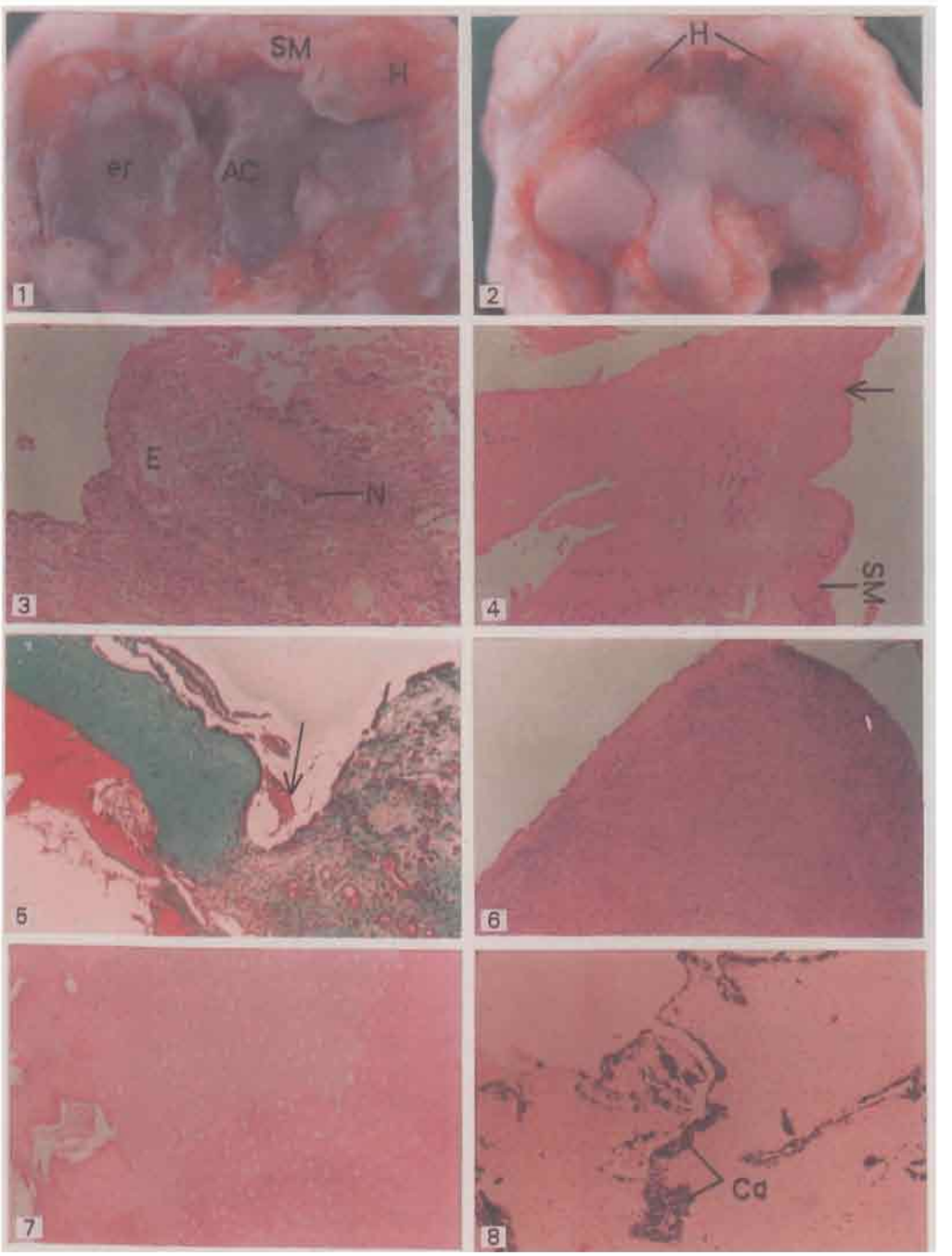
Histochemical changes

Synovial membrane from group A arthritic animals showed loss of AB material but it was more PAS-positive. Overall reaction was poor than normal. In articular cartilage of this group, the AB reaction was more in the intermediate layer but it was also seen in both tangential and radiate layers. The PAS-reaction could not be seen in the matrix of lacunae and chondrocytes.

In AD-treated animals, the joint capsule showed very weak alcinophilic reaction and were more PAS-positive. The articular cartilage of this group showed better alcinophilic reaction in intermediate and radiate layers. The tangential layer was weakly PAS-positive.

As compared to normal joint capsule, the intima and subintima and fibrous layer of joint capsule in group A showed weak reaction of glycogen but a loss of glycogen in chondrocytes of articular cartilage was seen.

A weak reaction in intimal and subintimal layer was also seen in group B. There was a slight improvement in glycogen



Figs 1-8 : **1.** Intercarpal joint from donkey with induced acute arthritis showing haemorrhagic (H) synovial membrane (SM) and thin eroded (er) haemorrhagic articular cartilage (AC). **2.** Induced acute arthritis of intercarpal joint from donkey treated with AD showing focal haemorrhagic spot (H) on synovial membrane with smooth articular cartilage. **3.** Section of joint capsule from the donkey with induced acute arthritis showing necrosis of intima, edema (E) and necrosis (N) in the subintimal layer. H & E × 70. **4.** Section of joint capsule from the donkey with induced acute arthritis showing necrosis (arrow) of synovial membrane (SM) and joint capsule. H & E. × 70. **5.** Section of articular cartilage of intercarpal joint from donkey with induced acute arthritis showing marked degenerative changes and complete sloughing off the articular (arrow). Masson's cartilage Trichrome × 70. **6.** Section of joint capsule from the donkey with induced acute arthritis and treated with AD showing well organized granulation tissue in intimal and subintimal layer. H & E × 70. **7.** Section of articular cartilage of intercarpal joint form donkey with induced acute arthritis and treated with AD showing normal chondrocytes in lower part of intermediate and in radiate layer. H & E × 70. **8.** : Section of joint capsule from donkey with induced acute arthritis showing calcium deposition (Ca) in intimal and subintimal layers of synovial membrane and focal deposition in fibrous capsule. Vonkossa counter stain with Nuclear Fat Red × 70.

content of chondrocytes of articular cartilage.

In group A, large amount of calcium deposit could be seen on intimal and subintimal tissue and focal in some location of fibrous capsule (Fig. 8). However, no calcium could be seen in any part of articular cartilage except ossified cartilage which is a normal site of deposition.

In AD-treated animals, the joint capsule and articular cartilage were devoid of any calcium deposits.

DISCUSSION

Due to irritant nature of turpentine oil, its intra-articular administration in the intercarpal joint inflicted chemical injury on the soft tissues specially joint capsule thereby resulting in acute arthritis as observed clinically.

At necropsy, the periarticular tissue including joint capsule was thickened and the synovial membrane severely haemorrhagic in control animals. In AD-treated animals, the gross changes in fibrous capsule and synovial membrane were very much less than those in control animals. This indicated healing effect of AD on soft tissue of the affected joint. As compared to reddish black extensively eroded thin and haemorrhagic articular cartilage of control animals, the gross appearance of articular cartilage of the treatment group was more near normal though it was whitish and less shinning. The articular cartilage with loss of sheen, yellowing, thinning, foci of fibrillation, small pits and grooves, erosion with partial or total loss of cartilage with marginal osteophytes were the gross findings of osteoarthrits (DJD) in cattle (Van Pelt and Langham 1966, 1970) and in dogs (Pedersen and Pool 1987). In aseptic acute arthritis, the joint capsule thickened with haemorrhage in calves and dogs whereas the articular cartilage was grossly thinned and eroded in calves (Bhatia 1990, Bhatia *et al.* 1992a, 1992b). In equines, acute aseptic arthritis resulted in yellow to yellow-brown discolouration (Bowman *et al.* 1983), haemorrhage and thickening of joint capsule (Bowman *et al.* 1983, Welch *et al.* 1991, Gustafson *et al.* 1992).

In this study, the microscopic changes of joint capsule of control (untreated) animals showed complete sloughing off the intima and subintimal layer evinced severe inflammatory reaction or even complete necrosis of fibrous capsule and synovial membrane. At places calcification of different layers of joint capsule or calcification of necrotic mass was also observed reflecting the severity of inflammatory reaction produced by turpentine oil. In AD treated animals, various layers of joint capsule showed organization and proliferation without inflammatory cells. Zaitsev (1982) reported that inflammed synovial membrane and inadequate production of synovial fluid initiate the degenerative process in the cartilage. Thus administration of AD helped in resolution of inflammation of synovial membrane.

Synovial membrane of untreated animals showed loss of AB material and was more PAS-positive indicating loss of acid mucopolysaccharides. Alcinophilic reaction was also weak in AD-treated animals and there was more of neutral mucopolysaccharides in all the layers of synovial membrane.

Degenerative changes of articular cartilage was observed microscopically in untreated animals. Similar changes in articular cartilage were reported in various arthritic conditions in horses (McIlwraith and Van Sick 1981, Yovich *et al.* 1987, Gustafson *et al.* 1989, Ono *et al.* 1989, Trotter *et al.* 1989, Gustafson *et al.* 1992). Olsson (1971) explained that chondromalacia, the earliest sign of degeneration of articular cartilage was due to decreased content of sulphated mucopolysaccharides in the ground substance as demonstrated by loss of alcinophilia and basophilia in the sections. Severity of articular cartilage changes in AD-treated animals was very much less than untreated group and were restricted to tangential layer only. The total thickness of articular cartilage was comparable to normal cartilage in this group. AD is also claimed to be a potent immunomodulator which regulates lysosome secretion from macrophages and progressive degeneration of articular cartilage is prevented. The present study confirms that AD do have a role in restricting degenerating changes in the articular cartilage.

Alcinophilic reaction was more in intermediate layer of articular cartilage of control group indicating loss of sulphated/ acidic mucopolysaccharides (chondroitin sulphate) in tangential layer as well as radiate layer of articular cartilage. Acid mucopolysaccharides are responsible for holding collagen fibres together in the cartilage and hence maintain the integrity of the cartilage (Yovich *et al.* 1987). Due to loss of the acid mucopolysaccharides, the cartilage becomes more prone to degenerative changes (Bhatia *et al.* 1992a). A very high degree of degenerative changes were noted in the articular cartilage of control animals in the present study. AD-treated animals showed more alcinophilic reaction in the intermediate and radiate layer of the articular cartilage but the superficial layer was weakly PAS-positive. Although sulphated glycosaminoglycans of the cartilage matrix are PAS-negative, but Banks (1981) reported it to be PAS-positive. This indicates that considerable healing of the articular cartilage had taken place in AD-treated animals.

As compared to normal joint capsule, the synovial membrane of control and AD-treated animals showed weak reaction of glycogen but there was no change in outer fibrous layers. The decreased glycogen content of synovial membrane indicated still poor nutrition to the synovial membrane. A loss of glycogen in chondrocytes of articular cartilage was seen in control group whereas a slight improvement in glycogen content of chondrocytes of articular cartilage was seen in animals treated with AD.

In control animals, calcium deposition could be seen in various parts of joint capsule. Subintimal calcium deposits had also been reported in traumatic arthritis in cattle (Van Pelt 1968) and DJD in dogs (Pedersen and Pool 1978). This indicated advanced inflammatory changes in control group animals in which necrosed tissue had tendency for calcification. The joint capsule of animals given AD showed no calcium

deposits reflecting normalization of joint capsule. Calcium deposits were not traceable in any part of the articular cartilage except calcified cartilages in both the groups.

It is concluded that AD-treated animals showed very encouraging results as reflected by healing of synovial membrane and articular cartilage. If given at early stage, AD may help in restricting the degenerative changes in the articular cartilage.

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Disposition kinetics of gentamicin in normal and febrile goats following single dose intramuscular administration

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ABSTRACT

A study of the disposition kinetics of gentamicin was conducted in goats following single dose im administration (5 mg/kg). The effect of fever (induced by *E. coli* endotoxin) was observed on absorption, distribution and elimination of gentamicin. Serum concentrations of gentamicin were detectable up to 6 hr in both groups of goats. Significant differences in serum concentrations of gentamicin were not observed at any time after injection between 2 groups of animals. The data were adequately described by a one- compartment model both in normal and febrile goats. Elimination half-life of 104.8 min in fertile goats was calculated. The volume of distribution (Vd/F) was 192.5 ml/kg in febrile goats whereas in normal goats it was 158.0 ml/kg. Based on these pharmacokinetic parameters a dosage regimen for gentamicin was calculated.

Key words : Febrile goats, Goats, Gentamicin, Kinetic disposition

Pharmacokinetic studies of drugs are generally conducted in normal animals, the implied hypothesis being that disease status do not change the pharmacokinetic behaviour of a drug. However, pharmacokinetic properties vary during fever (Ladefoged 1977), pneumonia (Burrows 1985), nephropathy (Frazier *et al.* 1988) etc. Gentamicin is usually administered in severe bacterial infections associated with fever, and fever alters the elimination of drugs (Song *et al.* 1972).

The present investigation was designed to determine the effect of *Escherichia coli* endotoxin-induced fever on the pharmacokinetics of gentamicin in goats following intramuscular administration.

MATERIALS AND METHODS

Female goats (8) weighing on an average 10.3±1.1 kg were used in the experiment. Injections containing 40 mg/ml of gentamicin sulphate were used in the study.

The drug was administered @ 5 mg/kg in the gluteal muscles. The pharmacokinetic study was first conducted in normal goats and then 4 to 6 weeks later in the same goats after producing fever with *E. coli* endotoxin (0.2 µg/kg i v single dose).

The drug was injected in febrile goats after they developed a minimum of 1° - 1.5° C rise in temperature, which usually occurred 2-3 hr after injection of endotoxin. The mean rise in rectal temperature is depicted in Fig. 1. Diurnal variation in body temperature was not significant.

Blood samples were collected from the jugular vein at 5,10,15 and 30 min and at hourly intervals until 8 hr after drug injection. Blood samples were allowed to clot at room temperature (30° - 32°C) for 4-6 hr. The serum was centrifuged and the supernatant was stored at -20° C until assayed.

Table 1. Pharmacokinetic values (range and median) of gentamicin in normal (n = 8) and febrile (n=8) goats following single i m dose (5 mg/kg)

Parameter	Unit	Normal goats			Febrile goats		
		Min	Max	Median	Min	Max	Median
A	µg/ml	20.3	64.2	31.65	19.8	42.6	26.05
Ke	min^{-1}	0.005	0.008	0.0075	0.005	0.01	0.008
Ka	min^{-1}	0.055	0.12	0.092	0.06	0.27	0.11
t ½ ke	min	79.1	133.9	88.8	62.7	132.5	104.8
t ½ ka	min	2.7	12.6	7.6	2.6	12.6	6.6
Vd/F	ml/kg	77.9	246.3	158.0	117.4	252.5	192.5
cl/F	ml/min.kg.	0.7	2.1	1.3	1.1	2.7	1.2
AUC	µg.min/ml	2372.2	7416.0	3795.25	1839.7	4382.2	416.3

A = Zero time plasma drug concentration coefficient of absorption phase ; Ke = elimination rate constant ; Ka = absorption rate constant; t ½ (ka) = absorption half life; Vd/F = apparent volume of distribution when the fraction of drug absorbed F is not known, Cl/F = clearance of durg from the body upon extra vascular administration when the fraction of drug absorbed F is not known; AUC = area under the serum concentration time curve ; t ½ (ke) = elimination half life.

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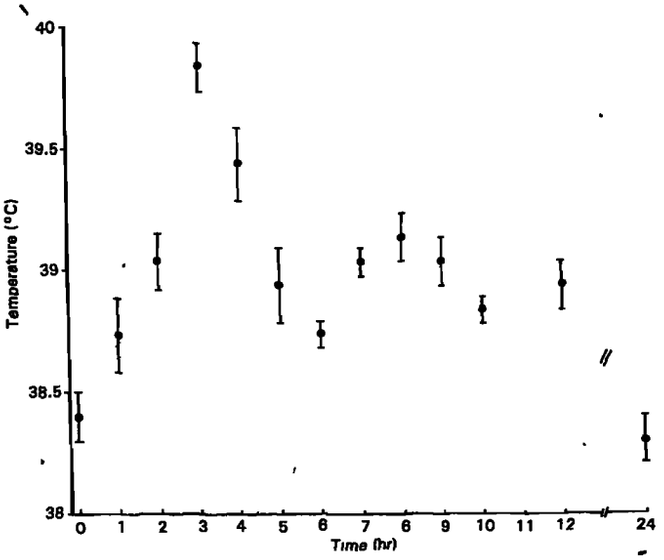


Fig. 1. Mean±SE of rectal temperature in goats (n = 8) in which fever was produced by intravenous injection (0 hr) of *E. coli* endotoxin. Gentamicin was injected (2-3 hr) for the study of pharmacokinetics.

The serum samples were assayed microbiologically for gentamicin concentration by the cylinder-plate method (Arret *et al.* 1971) using *Bacillus subtilis* as test organism and Trypticase soy Agar as medium. The lower limit of quantification was 0.62 µg/ml. The intra and inter-assay coefficients of variation were 5.6% and 7.4% respectively. The linear correlation of gentamicin concentration and zone of inhibition was 0.911.

The statistical comparison of serum concentrations of gentamicin at various time intervals between normal and febrile goats were done by analysis of variance (Snedecor and Cochran 1967). However, the pharmacokinetic data were compared by non-parametric Wilcoxon's rank sum test (Steel and Torrie 1960).

Table 2. Dosage regimens (IM) of gentamicin based on the mean kinetic data of normal goats and febrile goats

Dosage regimens (mg/kg every 8 hr)	Expected serum concentration (µg/ml) C_{ss}^{-}	C_{max}^{ss}	C_{min}^{ss}
	Normal goats		
3.5	5.6	21.5	0.75
4.0	6.4	24.6	0.85
5.0	8.0	30.74	1.1
	Febrile goats		
3.5	4.7	19.1	0.7
4.0	5.4	21.8	0.75
5.0	6.8	27.2	0.95

C_{ss}^{-}, Average serum drug concentration at steady state; C_{ss}^{max} maximum serum drug concentration at steady state; C_{ss}^{min} minimum serum drug concentration at steady state.

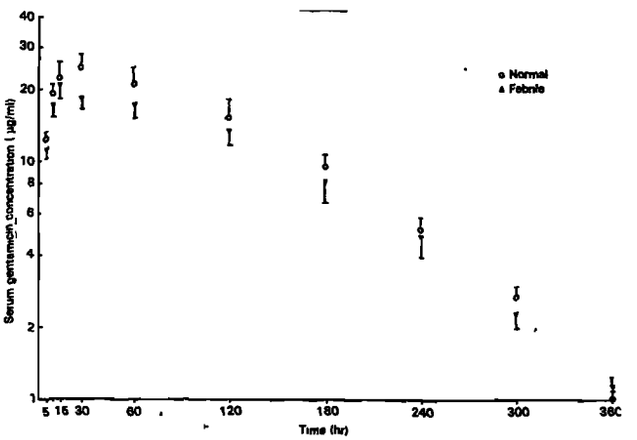


Fig. 2. Serum concentration time profile (mean ± SE) of gentamicin sulphate in goats after single dose intramuscular administration of the drug @ 5 mg/kg body weight in normal (n = 8) and febrile (n = 8) goats.

The pharmacokinetic analysis of gentamicin data for each goat was performed with the aid of a nonlinear interative curve fitting computer programme. The weighting mode for fitting the serum concentration vs time curve was kept as zero.

RESULTS AND DISCUSSION

The plots of mean serum concentration against time for normal and febrile goats are shown in Fig. 2. A one compartment model adequately fitted the serum concentration time data in both normal and febrile goats. The correlation coefficients of fit of curve were 0.96±0.008 and 0.96±0.005 in normal and febrile goats respectively.

The absorption rate constant (ka) was 0.11 min in febrile goats (Table 1) as compared to 0.092 min in the normal ones, however, slightly lower absorption half life (t½ ka) was observed in febrile goats (6.6 min) as compared to normal goats (7.6 min). The apparent volumes of distribution (Vd/F), neglecting the absorption phase (F), were 192.5 and 158.0 ml/kg in febrile and normal goats respectively. The relative availability (AUC endotoxin / AUC control) of gentamicin following im administration in febrile goats was 85.3% of that in normal goats, indicating that less drug was available for antibacterial action in febrile goats than in the normal ones. The clearance value in febrile and normal goats were 1.2 and 1.3 ml/min kg respectively.

Based on the pharmacokinetic parameters, dosage regimens for gentamicin in normal and febrile goats are depicted in Table 2. There was no significant difference between the steady state concentrations calculated from any dosage regimen in the 2 groups.

Increased body temperature enhances the absorption of $^{24}Na^{+}$ following s c and i m injections (Ballard 1974) and of orally administered sulphonamides (Van Miert *et al.* 1976). This enhancement in the absorption is due to cardiovascular

changes associated with fever during which peripheral circulation increases (Pennington *et al.* 1975) or more specifically an increase in blood flow to absorption sites under febrile conditions (Halkin *et al.* 1981). In this study a slightly more rapid rate of absorption of gentamicin was observed in febrile goats, although the increase was not statistically significant. There might not have been a substantial enough increase in blood circulation following endotoxin administration to cause a significant change in absorption.

An increase in elimination half-life in febrile goats as compared to normal ones was observed, which, however, was not statistically significant. There have been varying reports regarding elimination half lives in normal and febrile animals. In rabbits, half life of gentamicin increased during endotoxaemia (Halkin *et al.* 1981), whereas in dogs it decreased (Pennington *et al.* 1975). Apparent volume of distribution showed a statistically nonsignificant increase in febrile goats as compared to normal ones. During fever this increase is expected as fever may enhance the capacity of the drug to penetrate cellular barriers (Baggot 1980). However, Wilson *et al.* (1984) had reported no difference in apparent volumes of distribution of gentamicin in febrile ewes compared to normal ones. The changes in the pharmacokinetic parameters of gentamicin between normal and febrile animals may be attributed to alterations associated with fever and subsequent shift of gentamicin from plasma to other body fluid compartments (Pennington *et al.* 1975). Although, in this investigation, statistically significant changes in the pharmacokinetic parameters were not observed in febrile goats as compared to normal ones, this does not rule out the possibility of shifting of fluid between compartments. The shift of fluid between compartments may have been of low magnitude as indicated by unaltered packed cell volume in this study.

As there is a very narrow gap between therapeutic and toxic doses of gentamicin, the use of a dosage regimen appropriate to the clinical circumstances is of great concern (Conzelman 1980). The various pharmacokinetic parameters calculated in this study were used to calculate the dosage regimen. The minimum concentration of gentamicin required for *in vitro* inhibition of a large majority of gram negative aerobes is 3-5 µg/ml (Conzelman 1980). To achieve and maintain a therapeutically satisfactory plasma drug level of 4 µg/ml on im dosage regimen of 3.5 mg/kg gentamicin every 8 hr was calculated as suitable for goats. Plasma levels of gentamicin in the range of 7-10 µg/ml were nontoxic (Gyselynck *et al.* 1971), however, peak serum concentrations consistently higher than 15 µg/ml are associated with nephrotoxicosis and ototoxicosis (Falco *et al.* 1969). The dosage regimen (3.5 mg/kg every 8 hr) recommended in this study would be expected to give peak serum concentration higher than 15 µg/ml, and might therefore cause toxicity. This possibility, however, needs to be investigated by measuring actual peak serum concentrations during prolonged therapy (5-10 days).

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Acute toxicity of triazophos and its effects on biochemical parameters in buffalo calves

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ABSTRACT

Single oral administrations of 1.0, 2.5 and 5.0 mg/kg body weight of triazophos produced mild to moderate toxic symptoms of anticholinesterase poisoning in buffalo calves. The lowest dose was not lethal, whereas 2.5 and 5.0 mg/kg produced 50 and 100 % leathality within 32-120 and 6.5-31 hr after dosing respectively. Triazophos in different doses inhibited erythrocyte cholinesterase (50.3 - 82.4%) and plasma carboxylesterase (37.4 - 54.5%). The inhibition of erythrocyte cholinesterase was dose-dependent and correlated well with severity of toxicity. There was marked elevation in the plasma levels of total proteins and blood urea nitrogen, following triazophos intoxication.

Key words : Biochemical parameters, Buffalo calves, Toxicity, Triazophos,

The activity of triazophos {0,0-diethyl 0-(1-phenyl H-1, 2, 4-triazol-3-yl) phosphorothioate}, an indirectly-acting organophosphorus insecticide, is not just limited to insects only but its indiscriminate use poses inadvertant serious health hazards in man, livestock, game and wild life (Kidd and Hartley 1987). The acute oral toxicity rating of triazophos under group I (very high) and group II (high) by Environmental Protection Agency (EPA) for birds and mammals, respectively, (Briggs 1992) has not prevented its widespread use in agriculture. Some studies on triazophos have been reported in laboratory animals and birds (Kidd and Hartley 1987, Thompson *et al.* 1991), but these do not provide information useful for extrapolation to buffaloes. It was therefore considered important to investigate the acute toxicity of triazophos and its effect on some biochemical parameters in buffalo calves.

MATERIALS AND METHODS

Healthy male buffalo calves (20), weighing 80-160 kg, purchased from local market were kept under standard housing conditions and maintained on green fodder and tap water *ad lib.*

A preliminary trial was conducted to assess the approximate lethal, sublethal and non-lethal doses of triazophos. Based on these results single doses of 1.0, 2.5 and 5.0 mg/kg of triazophos were selected and administrated to 3 groups of 4 buffalo calves each. The requisite quantity of triazophos was suspended in 100 ml of tap water and given by drench. All the calves were fasted 16-18 hr prior to insecticide administration. The animals were observed for toxic effects for 28 days.

For determining various biochemical parameters, blood samples were collected at 0, 2, 8, 12, 24, 48, 168 and 672 hr after insecticide administration. Erythrocyte cholinesterase (E.C. 3.1.1.7) and plasma carboxylesterase (E.C. 3.1.1.1) were measured (Voss and Sachsse 1970, Mendoza *et al.* 1971). Total plasma proteins and blood urea nitrogen (BUN) were determined (Wootton 1964). The data were analysed by student's t-test. A probability level of $P< 0.05$ and $P< 0.01$ was considered statistically significant.

RESULTS AND DISCUSSION

Triazophos in single oral doses of 1.0, 2.5 and 5.0 mg/kg produced dose-dependent toxicity and mortality. Mild to severe toxic effects characteristic of anticholinesterase poisoning, viz. restlessness, salivation, lacrimation, twitching of muscles, tremors, frequent urination, diarrhoea, incoordination, difficult respiration, prostration and death were recorded. The lower dose (1.0 mg /kg) caused no mortality, whereas 2.5 and 5.0 mg/kg were 50 and 100% lethal respectively. The onset of toxic symptoms (20-70 min) and mortality (390-7200 min) following different doses of triazophos administration were considerably delayed which may be because of slower absorption of insecticide from the gastro-intestinal tract or bioactivation of this insecticide in the body like other A+S containing OP compounds (Main 1984). The triazophos in buffalo calves appears to be more toxic than monocrotophos, phosphamidon and chlorypyriphos (oral LD_{50}, 20, 40 and 50 mg/kg, respectively (Awal *et al.* 1988, Sandhu and Malik 1988, Kaur 1993) and less toxic than phorate (oral LD_{50} 0.8 mg/kg) (Sultana 1986).

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Table 1. Effect of single oral administration of triazophos on erythrocyte cholinesterase and plasma carboxylesterase in buffalo calves

Dose	Time after administration (hr)							
(mg/kg)	0	2	8	12	24	48	168	672
	Erythrocyte cholinesterase (n mol acetylthiocholine hydrolysed / min/ ml)							
1.0	2533±183.7	1782*±142.4	1260**±218.8	1482*±231.4	1612*±222.7	1534*±198.7	2128±312.0	2657±116.5
2.5	2637±154.1	887.7**±237.2	620.1**±209.0	580.9**±212.8	535.3**±62.6	548.3***±108.8	992.2[b]	2384.0[b]
5.0	2370±81.4	894.3**±240.3	535.3**[b]	417.8**	470.0[c]			
	Plasma carboxylesterase (n mol indophenol formed / min / ml)							
1.0	218.8±21.6	202.8±23.2	180.3±28.8	166.0±25.0	172.2±34.8	175.0±25.2	191.0±20.9	219.4±9.28
2.5	231.9±14.2	169.5*±5.32	164.2±20.7	169.0±21.1	158.3±18.4	145.1**±17.0	186.1[b]	184.7[b]
5.0	183.3±19.1	144.5±25.3	91.67*[b]	83.34*[b]	111.11[c]			

Values represent the mean ±SE of 4 animals unless otherwise stated. a : value is mean ± SE of 3 animals, b : Value of mean of 2 animals, c: Value of 1 animal, * $P < 0.05$, ** $P < 0.01$.

Table 2. Effect of single oral administration of triazophos on total plasma proteins and blood urea nitrogen in buffalo calves.

Dose	Time after administration (hr)							
(mg/kg)	0	2	8	12	24	48	168	672
	Total plasma proteins (g/ 100 ml)							
1.0	8.32±0 19	8.32±0.49	8.54±0.09	8.32±0.19	8.32±0.07	8.16±0.16	8.78±0.20	8.62±0.21
2.5	9.01±0.15	8.80±0.27	9.21±0.65	9.10±0.30	9.44±0.40	9.78a±0.36	9.77*[b]	9.12[b]
5.0	7.91±0.13	8.66±0.42	9.45**[b]	9.63**[b]	9.81[c]			
	Blood urea nitrogen (m mol / litre)							
1.0	4.63±0.23	4.77±0.48	6.12±0.65	5.75±0.68	5.96±0.53	5.85±0.86	4.78±0.29	4.72±0.42
2.5	5.60±0.31	6.59±0.75	9.34±2.31	9.01±1.75	12.19*±2.34	9.50**±0.30	6.71[b]	6.20[b]
5 0	5.19±0.51	6.71±0.64	9.92*[b]	11.84**[b]	19.46[c]			

Values represent the mean±SE of 4 animals unless otherwise stated. a : Value is mean±SE of 3 animals, b : Value is mean of 2 animals, c : Value of 1 animal, * : $P < 0.05$, ** : $P < 0.01$.

Triazophos in single oral dose of 1.0, 2.5 and 5.0 mg/kg produced significant inactivation of erythrocyte cholinesterase to the extent of 50.3% (8 hr), 79.7% (24 hr) and 82.4% (12 hr) respectively. OP insecticides cause their toxic effects by inhibition of cholinesterase enzymes, which is considered a better index to ascertain their toxic effects (Halbrook *et al.* 1992). The inhibition of erythrocyte cholinesterase enzyme following higher doses (2.5 and 5.0 mg/kg) of triazophos correlated well with the severity of toxic symptoms. Similar results were also reported for other organophosphorus insecticides in domestic animals (Malik *et al.* 1978).

The higher doses (2.5 and 5.0 mg/kg) of triazophos produced significant inactivation of plasma carboxylesterase up to 37.4 and 54.5% at 48 and 12 hr respectively. But no inactivation of this enzyme was seen with the lower dose (1.0 mg/kg) of insecticide. Plasma and hepatic carboxylesterase enzymes have been associated with the metabolism of OP compounds (Taylor 1996). This study indicates that triazophos as such has lesser inhibitory effect on plasma carboxylesterase enzyme, so its prior exposure is less likely to potentiate the toxicity of OP insecticides that are selectively biodegraded by this enzyme system.

Triazophos caused elevation in total plasma proteins in animals intoxicated with 2.5 and 5.0 mg/kg doses to the extent of 8.4 and 24.0% recorded on day 7 and 24 hr respectively. Elevation in the proteins levels in the blood following organophosphorus compounds may have occurred due to dehydration, hemoconcentration and/or stimulation of acute phase protein (APP) synthesis and corresponding m RNA's in the body tissue (Sevaljevic *et al.* 1990). Increased production of stress proteins in response to toxins, poisons and pollutants also protect cells from harm (Welch 1993).

Higher doses of 2.5 and 5.0 mg/kg also caused significant increase (69.6 - 121.2%) in blood urea nitrogen (BUN) levels (Table 2). Increase was very high (275.0%) in 1 animal which survived for 31 hr in the maximal dose group. Increased levels of blood urea nitrogen are related to either kidney disease or urinary obstruction leading to decreased excretion of urea from the body. Hypovolemia associated with dehydration causing pre-renal retention of nitrogenous wastes (Finco 1989) and hepatic dysfunctions are also accompanied by increased BUN levels. Though the exact casue(s) of the increased BUN

levels is not certain in this study, elevated levels of blood urea nitrogen indicates that pre-renal, renal or post-renal azotemia did exist in the animals poisoned with triazophos.

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Inflammatory response of skin autografting in the chicken

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ABSTRACT

Pathology of skin autografting in the chicken showed grossly cyanosis and oedema in the beginning. Histopathologically, endothelial swelling and vascular wall degeneration, endothelia swelling and spongiosis, and focal degeneration, were observed. These changes were gradually reduced and the graft appeared healthier at seventh day. The vascular and cellular changes conformed with the pattern of reaction in the chicken in response to incised wounding. Noteworthy finding was the formation of prominent perivascular lymphoid aggregates.

Key words : Chick, Inflammatory response, Skin autografting

Various events of the avian inflammatory responses have been studied using living, non-living and immunological stimuli (Vegad and Katiyar 1995). However, information on the nature of inflammation associated with transplantation reaction in the chicken is scanty. The paper reports the inflammatory response of skin autografting in the chicken.

MATERIALS AND METHODS

White Leghorn chicken, of either sex, and aged 12-16 weeks. were used.

One day before grafting, feathers from the luteal hip regions were plucked, and the skin cleaned with sterile distilled water. On the following day, the skin was cleaned by 1% ampicillin solution. An area of 2 cm × 1 cm was demarcated lightly with grease pencil on both the hips (Fig. 1).

Just before grafting, anaesthesia was induced by inhalation of chloroform, soaked in swap, and it was maintained by periodic inhalations.

The demarcated skins were incised with a Bard-Parker knife, freed from the site and removed. The 2 graft pieces, thus obatined, were kept in 2% ampicillin solution at 4°C in individual containers. The wound on the right side was kept covered with sterilized cotton swabs soaked in ampicillin solution. The wound on left side was cleaned for grafting. The right graft was then implanted on the left hip. First, 4 corners of the graft were sutured with the corresponding corners of the graft-bed, using simple interrupted 1/0 cotton sutures. Then, the borders were sutured. While suturing, sponging solution. The grafting was performed observing strict aspetic precautions. The left graft was then implanted similarly on the right wound of each time interval, viz. 1,3,5,7 and 9 days. six grafts were obtained.

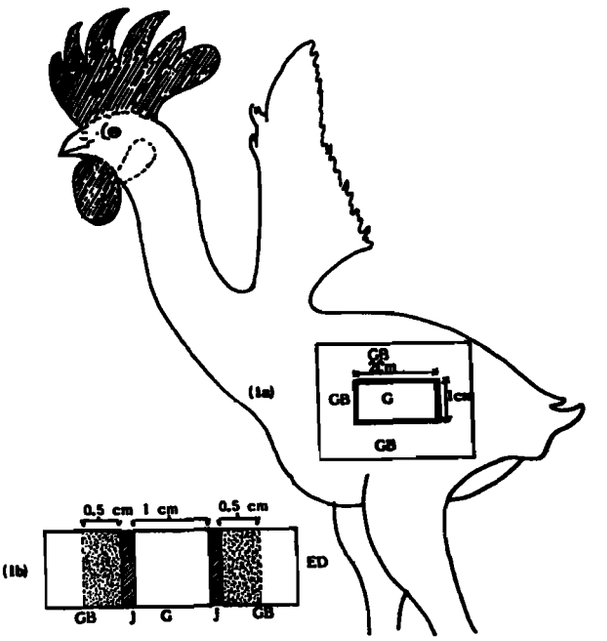
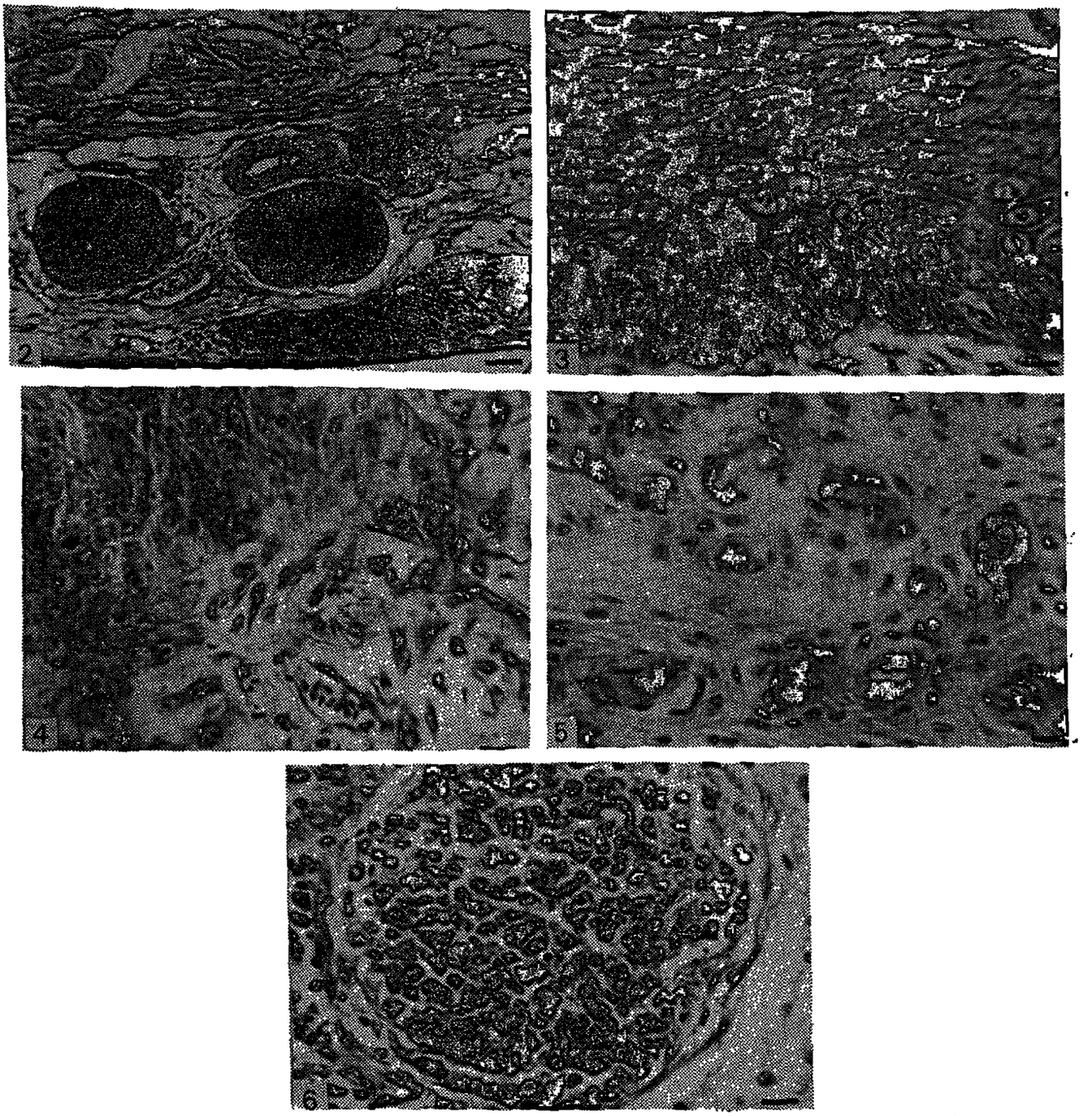


Fig. 1. Diagrammatic representation of skin grafting in the chicken. Ia - lateral hip region where the graftng was done. Ib- the pattern of histopathological study including mast cell, basophil and lymphoid aggregate counts.

Each bird received 10 mg of ampicillin kg^{-1} body weight daily, for the entire period. In addition, the grafted sites were moistened with ampicillin solution twice a day.

The sequential histopathological changes were studied in grafts showing signs of acceptance. Sections of 5µm thickness were stained with haematoxylin and eosin, 0.05% solution of toluidene blue in acetate buffer (pH 3.8 for 72 hr) for

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Figs 2-6. **2.** Section of grafting showing vascular stasis 3 days after grafting. **3.** Section of graft-bed showing marked acanthosis 3 days after grafting. **4.** Section of graft-bed showing a number of activated macrophages in the dermis 1 day after grafting.H. & E. bar = 10 μm. **5.** Section of graft showing angiogensis 3 days after grafting. H. & E. bar = 10μm, **6.** Section of graft-bed showing lymphoid aggregates of varying size 1 day after grafting. H. & E.; bar = 40 μm.

basophils and must cells (Dhodakpar *et al.* 1984), and Mallory-Heidenhan for collagen and fibrin, using standard techniques.

Mast cells and basophils were counted in the toluidine blue stained sections. Also, lymphoid aggreagates were counted in the grafts and graft-bed in the H& E studied sections.

Using micrometer, thickness of the epidermis and skin in the graft-bed was measured at 2 places : (1) at the graft-bed margin, and (ii) 0.5 cm lateral to the margin (Fig.1). Likewise, lymphoid aggregates were also measured.

RESULTS AND DISCUSSION

The graft was soft and slighlty cyanotic at 1 day.From day 3 onward it looked pinkish and microscopically some venules showed blood stasis (Fig.2). The junction between the graft and graft-bed was hyperaemic and swollen on 1 day. The swelling disappeared gradually. The junctional gap was filled up by 7 days. The undersurface of the graft revealed intense hyperaemia and haemorrhage on 1,3 and 5 days. These changes were markedly reduced on day 7, and suggested the development of adequate snostomosis (van Dam *et al.* 1978). Jennings (1984) concluded from skin grafting in the horse that during the adherence phase the graft is nourished by to-and-fro exchange of fluid between graft and graft-bed.

Table 1. Micrometric measurements of the graft-bed in skin autograft

Age of graft (days)	Thickenss of graft-bed epidermis 0.5cm away from the junction(μm)	Thickness of graft-bed epidermis at the junction (μm)	Thickness of graft-bed 0.5 cm away from the junction (μm)	Thickness of graft-bed at the junction (μm)
1	21.39±2.16	87.76±24.75	634.10±38.46	873.31±86.01
3	20.54±2.41	52.77±14.96	664.06±92.72	845.40±155.55
5	18.23±2.08	38.96±9.76	476.71±59.75	728.87±101.22
7	19.35±2.18	43.87±7.52	605.62±145.45	862.39±210.07
9	18.65±1.98	35.06±9.27	554.27±76.35	728.52±56.75

Histologically, the epidermis revealed spongiosis, degenerative changes and focal necrosis during early phase. The spongiosis is frequently associated with the inflammatory process in the skin (Lever 1975, Cotran *et al.* 1994). The degenerative changes observed in this study may be due to anoxia, untill the development of anastomosis. van Dam *et al.* (19789) also reported that cell injury is caused by ischaemia at the time of graft.

Acanthosis was observed right from 1 day of grafting up to 9 days, near the edges of the graft and graft-bed (Table 1, Fig. 3). with evidence of activated macrophages (Fig.4). Acanthosis during healing by primary union occurs in 24 hr at the cut edges of incised wounds as a result of increased mitotic activity in the basal layer (Cotran *et al.* 1994). The present results of acanthosis are in conformity with these observations. Angiogenesis was observed from day 3 onward and was more pronounced in the upper dermis (Fig. 5). It is well established that activated macrophages play an important role in the process of healing by releasing several growth factors, including epidermal growth factors, i(EGF) (Cotram *et al.* 1994, Vegad 1995). It is suggested that the EGF derived from the activated macrophages may have caused epidermal proliferation, acanthosis and angiogenesis.

There were margination and emigration of lymphocytes, monocytes and basophils in all stages, in the graft and graft-bed. Presence of large number of mast cells was seen in the dermis. The number of basophils and mast cells was 5.33±2.17 and 6.0±1.65 noticed at day 1, respectively. Their number was maximal on day 5; 7.0±0.36 and 13.6±0.36,respectively. Thereafter the number was reduced gradually. At no stage eosinophils and giant cells were present.

In this study the nature of cellular response was in conformity to the pattern of reaction in the chicken occurring during inflammatory-reparative response in the later phase (Katiyar *et al.* 1993, Vegad and Katiyar 1995).

An interesting observation was the formation of prominent perivascular lymphoid foci of varying size (Fig. 6, Table 2). Their largest size was observed on day 1. The formation of lymphoid aggregates is the characteristic feature of vain inflammation (Vegad and Katiyar 1995). However, in this study the aggregates were usually large.

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Table 2. Number and size of the lymphoid aggregates in the skin autograft

Age of graft (days)	No. of lymphoid aggregate/cm		Size of lymphoid aggregate (μm)							
			Graft				Graft-bed			
	Graft-bed	Graft	Largest		Smallest		Largest		Smallest	
			Max.L.	Max.W.	Max.L.	Max.W.	Max.L.	Max.W.	Max.L.	Max.W.
1	3.42	1.50	29.04	16.30	5.70	4.96	190.40	70.55	46.75	34.85
	±1.00	0.34	±14.40	±6.05	±3.57	±3.14	±86.70	±32.30	±23.02	±14.45
3	2.33	2.33	28.33	28.33	4.17	10.62	95.62	70.83	29.07	14.17
	±0.95	±0.02	±11.57	±11.57	±5.79	±4.34	±49.66	±37.53	±11.81	±3.04
5	2.75	0.83	11.76	9.21	10.62	7.79	154.42	50.50	29.75	18.42
	±0.79	±0.34	1.11	±1.31	±0.95	±0.71	±57.61	±12.94	±10.23	±5.22
7	1.33	6.33	12.75	6.37	9.21	7.79	191.96	48.87	22.67	8.50
	±0.33	±1.83	±2.69	±0.95	±0.71	±0.71	±118.67	±27.19	±12.50	±1.09
9	1.83	1.33	26.21	12.75	5.67	3.54	68.00	32.58	12.75	10.62
	±0.82	±0.61	±16.80	±6.85	±3.75	±2.31	±39.56	±17.06	±6.11	±5.23

Values given : mean ±SE; Max. Maximum; L - length; W - width.

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Superovulation response, embryo recovery, their quality and related progesterone profile in repeat breeding crossbred cattle

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ABSTRACT

Ovarian response, embryo recovery, their quality and related progesterone in repeat breeding crossbred cattle superovulated with PMSG was studied. The mean ovulation rate, average number of embryos/ova and transferable embryos recovered per donor did not differ significantly between the repeat breeding and normal control cows. However, number and percentage of unfertilized eggs in repeat breeding cows were higher than the normal cows. Plasma progesterone concentration on the day of gonadotrophin treatment, on the day of PGF_2 alpha injection, at estrus and on the day of flushing also did not differ significantly between repeat breeding and normal control cows.

Key words : Crossbred cattle, Repeat breeding, Superovulation response, Embryo recovery, Progesterone profile.

Incidence of repeat breeding, an important gynaecological problem of dairy animals, is 10 to 25% in exotic and indigenous cattle (Sharma *et al.* 1983, Bartlett *et al. 1986).* Fuente and Monge (1988) reported lower ovulation rate, embryo recovery and their quality in repeat breeding cattle; however, O'Farrell (1986, 1987) failed to demonstrate such differences. In this paper ovarian response, embryo recovery, their quality and related progesterone profile in repeat breeding crossbred cows superovulated with PMSG are reported.

MATERIALS AND METHODS

Crossbred cattle (16 : Hariana × Holstein-Friesian, Brown Swiss × Jersey) were selected from the Institute's dairy herd. Experimental animals having no gross abnormalities of genital organs maintained under standard feeding and managemental practices were selected. They were divided into 2 groups.

Group A (Repeat breeder group; n=8) : Cows which failed to settle on 3 or more inseminations following parturition and a history of repeat breeding.

Group B (Normal control group; n=8) : Cows which had no record of repeat breeding and were cycling normally following parturition.

All animals of groups A and B were superovulated with a single intra-muscular injection of 2 000 IU PMSG (pregnant mare serum gonadotrophin) between days 10 and 12 of the oestrous cycle followed by a single intra-muscular injection of 25 mg PGF_2 alpha 48 hr later. The animals were subjected to detection of estrus after the injection of PGF_2 alpha twice daily, i.e. morning and evening, with the help of a vasectomized teaserbull and visual observation of external oestrus symptoms. The donors were inseminated with frozen semen at 0, 12 and 24 hr after the onset of estrus. The ovarian response was determined by rectal palpation of corpus luteum and unovulated large follicles (≥10 mm diameter) on the ovaries. The palpation was made on the day of flushing. The donors having 3 or more than 3 corpus luteum (≥ 3CL) were flushed by non-surgical method on day 6 or 7 post-estrus using modified Dulbecco's phosphate buffered saline (DPBS) containing 0.2% BSA fraction V (Bovine serum albumin) as flushing medium. All the recovered eggs after their location were lifted

Table 1. Ovarian response in repeat breeding crossbred cows superovulated with PMSG

Group/parameters studied	Repeat breeding cows	Normal (control) cows
Superovulation response		
Animals treated	8	8
Animals responding to superovulation (≥3CL)	8(100%)	7(87.50%)
Animals exhibiting estrus	8(100%)	8(100%)
PG to estrus interval (hr)	45.00±0.92	47.31±4.54
Ovulation rate	6.25±3.01	5.87±3.68
UQF at flushing (≥10 mm dia)	4.62±1.99	4.00±2.72

Difference in the mean of above variable did not differ significantly ($P<0.05$)

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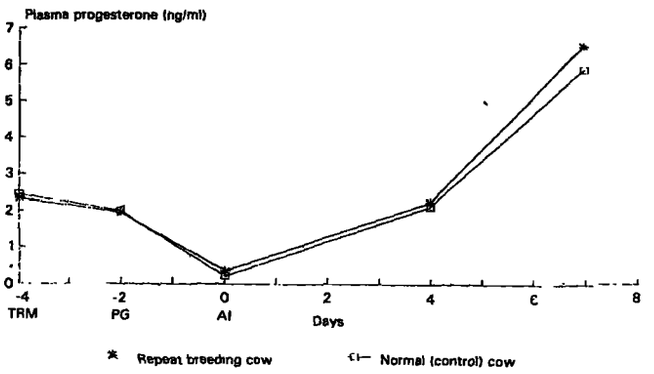


Fig. 1. Effect of type of animal on plasma concentration of progresterone

and transferred into holding media (DPBS+0.4% BSA). The quality and stage of embryonic development of ova/embryo was determined morphologically and graded as excellent, good, fair, poor, degenerated and unfertilized. Excellent, good and fair quality embryos were considered as transferable.

Plasma progesterone profile

Blood samples were collected in heparinized vials by direct puncturing of jugular vein on the day of gonadotrophin treatment, PGF_2 alpha injection, at estrus, day 4 and on the day of flushing, Plasma was separated from the blood and stored at -20°C till analysis. The plasma progesterone profile was analysed by radio imunoassay (RIA) as per WHO protocol of Sufi *et al.* (1883)

RESULTS AND DISCUSSION

Superovulatory response, embryo recovery and their quality

All animals (100%) in repeat breeding and control groups exhibited estrus following PGF_2 alpha injection. The difference in the time taken for onset of estrus following PGF_2 alpha injection between the 2 groups was statistically nonsignificant (Tables 1,2). Onset of estrus within 45 to 50 hr followed the administration of PGF_2 alpha in normal and repeat breeding superovulated cattle (Linares *et al.* 1980, Agarwal *et al.* 1993) which is in agreement with the findings of the present study. The mean ovulation rate, unovulated large follicles percentage of animals responded to superovulation did not differ significantly between repeat breeding and normal (control) cows (Table 1). Donors yielding eggs, average number of embryo/ova and number of transferable embryo per donor also did not differ significantly between the repeat breeding and control cows. However, number and percentage of unfertilized eggs in repeat breeding cows were higher than the normal cows indicating lower rate of fertilization in repeat breeding cattle. In repeat breeding donors the lower fertilization rate might be due to hostile uterine/oviductal environment (affecting sperm transport and their survival) and release of inferior quality oocytes. Certain morphological and biochemical defects in the oocytes lead to fertilization failure

Table 2. Embryo recovery and their quality in repeat breeding crossbred cattle superovulated with PMSG

Group/parameters studied	Repeat breeding cows	Normal (control) cows
Embryo production		
Donors flushed	6	7
Total number of estimated ovulations (0)	42	46
Donors yielding egg (%)	5(83.33%)	6(85.71%)
Total embryos/ova recovered (E)	19	16
Per cent embryo recovery (% E/O)	45.23	34.78
Average number of embryo/ ova recovered per donor	3.16±2.48	2.28±1.97
Embryo quality		
Fertilized eggs	1.83±1.60	1.57±1.27
(%)	(57.89)	(68.75)
Transferable embryos	0.66±0.81	0.57±1.13
(%)	(21.05)	(25.00)
Degenerated embryos	1.16±1.16	1.00±1.00
(%)	(36.84)	(43.75)
Unfertilized eggs	1.33±1.96	0.71±1.49
(%)	(42.10)	(31.25)

Difference in the mean of above variable did not differ significantly ($P< 0.05$)

in repeat breeding animals (Greve 1980, Johnson 1984). Aberrations in the LH surge interferes in the nuclear and cytoplasmic maturation of oocytes and as such inferior-quality of oocytes are liberated (Morre *et al.* 1984). Greve (1980) also reported the lower fertilization rate in repeat breeding donors. O'Farrell and Hartigen (1989) did not observe any significant difference in the fertilization rate of eggs between the repeat breeding and normal cows. As observed in this study O'Farrell (1986, 1987) also did not observe any differences in the ovulation rate, superovulatory response and number of embryos recovered donor between the repeat breeding and normal cows. However, Fuente and Monge (1988) observed lower ovulation rate, superovulatory response and number of embryos recovered/donor in repeat breeding cows than the normal cows. O' Farrell (1987) also reported no difference in quality of embryos between repeat breeding and normal cows which is in agreement with the findings of this study. However, Fuente and Monge (1988) reported poor quality of embryos in repeat breeding cows.

In this experiment a number of unovulated large follicles (UOF) were palpated on the day of flushing in both the groups. These UOF were presumably those which continued to grow due to the longer biological half-life of PMSG and failed to ovulate because of incomplete maturation at the LH surge (Monniaux *et al.* 1983).

Progesterone profile

Type of animal i.e. repeat breeding vs normal did not influ-

ence the plasma concentration of progesterone (Fig. 1). This may be due to the fact that the donors in both the groups were selected prior to superovulation treatment on the basis of presence of a good corpus luteum. Plasma progesterone concentration did not differ significantly on the day of gonadotrophin treatment and the day of PGF_2 alpha treatment between the 2 groups, indicating that administration of gonadotrophin had no luteotrophic effect. Barnes *et al.* (1982) reported similar results. However, Yadav *et al.* (1986) and Kweon *et al.* (1987) reported increase in the plasma progesterone. Plasma progesterone concentration declined after the administration of PGF_2 alpha and reaches at its basal levels at estrus. Kweon *et al.* (1987) gave similar results. Following superovulatory estrus concentration of progesterone started increasing significantly in both the groups until day of flushing indicating formation and development of corpora luteta from the ovulated follicles. Plasma progesterone concentration on the day of flushing did not differ significantly between the repeat breeding and normal cow. No difference in the ovulation rate between the repeat breeding and normal cows observed in this study would further support the above observations.

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In vitro production of blastocysts in goats, sheep and buffaloes

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ABSTRACT

This study was carried out to establish a common culture system for *in vitro* maturation, *in vitro* fertilization and *in vitro* production of goat, sheep and buffalo blastocyst embryos. Follicular oocytes of goats, sheep and buffaloes were matured in TCM-199 medium supplemented with 10% foetal bovine serum (FBS) and 5 µg/ml follicle-stimulating hormone (FSH). Some of the oocytes were stained by 2% Giemsa stain and observed for nuclear maturation and the others were fertilized with capacitated sperms. The maturation rates ranged from 65 to 78%, with no significant difference among the species. The cleavage rates were highest in buffaloes (59%), followed by sheep (56%) and goats (41%). When these cleaved oocytes were cultured in TCM-199 medium supplemented with 10% FBS and 5 µg/ml insulin for 9 days, 34% buffalo oocytes reached blastocyst stage. This was significantly higher ($P<0.01$) than the corresponding values in goat (8.1%) and sheep (4.7%).

Key words : Buffaloes, Goats, Sheep, Blastocyst, *In vitro* blastocyst production

A few reports are available on production of blastocyst embryos in buffalo (Totey *et al.* 1992, Madan *et al.* 1994) and goats (Pawsh *et al.* 1994). Recently, there has been an increase in interest for the generation of *in vitro* produced goat, sheep and buffalo embryos for cloning, micromanipulation etc. for production of multiple offsprings from a single embryo. This could significantly influence production of these important farm animals.

The present study was therefore taken with the aim of establishing a common culture system, for *in vitro* maturation, *in vitro* fertilization and *in vitro* production of goat, sheep and buffalo blastocyst embryos.

MATERIALS AND METHODS

Collection and culture of oocytes

Goat, sheep and buffalo ovaries were collected from slaughterhouse and were transported to the laboratory in isotonic saline containing 200 IU penicillin and 200 ug/ml streptomycin at 32°-37°C within 1-4 hr. The mature oocytes were recovered either by slicing the ovary or by aspirating the follicles with 20 G needle in aspiration medium (TCM-199, supplemented with 10% serum and phosphate buffer saline supplemented with 0.3% bovine serum albumin in 1:1 ratio). Cumulus oocyte complexes (COC) with evenly granulated homogenous cytoplasm with a complete corona layer and from 2 to 6 or more cumulus layer from goat (n=206), sheep (n=264) and buffalo (n=219) were chosen for maturation. In addition, oocytes with degenerated cumulus were discarded. The maturation medium used for this study was TCM-199 supplemented with 10% foetal bovine serum and 5 µg.ml FSH. After washing 3 times with the maturation medium the COC oocytes were introduced into a 50 ul droplet of maturation medium in groups of 10-12 oocytes and covered with paraffin oil in a 35 mm petri-dish, and incubated for 26 hr in a CO_2 incubator (5% CO_2 in air) at 38.5°C.

At the end of the incubation period, the oocytes were stained by Giemsa staining. Nuclear status was evaluated, under the compound microscope to see the maturation stage (Metaphase II) by karyotyping.

Sperm preparation

Frozen thawed semen was used for buffalo sperm capacitation. Goats and sheep caudal epididymes collected from the slaughterhouse were used for obtaining semen preparations. Semen was emptied into a 15 ml air-tight tube and washed with Brackett and Oliphant (BO) medium (Brackett and Oilphnat 1975). The semen was further processed for *in-vitro* capacitation (Madan *et al.* 1994). The drops of processed sperms (100 µl each, 10 to 12 million cells/ml) were placed under the mineral oil in culture dishes for *in-vitro* capacitation at 38.5° C and CO_2 for 1 hr. Capacitation was additionally judged by 'whiplash' motion of the sperm tail. The sperm suspension thus obtained was used for *in vitro* fertilization.

Establishment of oviductal cell culture

Oviductal epithelial cells were separated from the oviducts

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of (goats, sheep and buffalo) that had newly formed corpora lutea on their ovaries. Separated oviductal cells, present both as single cells and as rafts of large cell clumps, and showing movement due to ciliary activity, were suspended in TCM-199 supplemented with 10% FBS. A 0.25 ml pellet of these cells obtained by gravity sedimentation was resuspended in 5 ml of above-mentioned medium and washed twice with the same medium. The cell suspension was then dispensed in aliquots of 200 µl each, in dishes covered with mineral oil and cultured at 38.5°C in 5% CO_2 in air for 24 hr before the addition of the oocytes.

Table 1. Development of cleaved IVM-IVF oocytes in TCM-199+10% FBS and 5 µg/ml insulin cocultured with oviductal epithelial cells after 9 days of culture

Species	Oocytes cleaved	Morula (%)	Blastocyst (%)
Goat	49	28 (57.1)	4[a] (8.1)
Sheep	106	52 (49.0)	5[a] (4.7)
Buffalo	88	37 (42.0)	30[b] (34.0)

a,b, Superscripts in the same column differ significantly ($P<0.01$).

In- vitro *fertilization and embryo culture*

At the end of 30 hr of culture the oocytes were washed in BO media. These oocytes were then introduced into 100 µl droplets of capacitated sperm suspension (10 to 12 oocytes/ drop and were left for 6 hr) for fertilization to occur. The oocytes were then washed in culture medium (TCM-199 supplemented with 10% FBS and 5 µg/ml insulin) and subsequently cultured in 100 µl droplet of the culture medium; 40 µl out of this was subsequently withdrawn and replaced with oviductal cell culture suspension, which had been prepared and incubated earlier. The oocytes were then cultured at 38.5°C for 5 days. The statistical significance of the results was evaluated by using chi-square test.

RESULTS AND DISCUSSION

Oocyte maturation

The maturation rate of COC ranged from 65 to 78% among the oocytes of the 3 species which were cultured in media supplemented with 10% FBS and FSH. The maturation rate was highest in buffalo (78.4%) followed by goat (68.8%) and sheep (65.7%). The differences were not statistically significant. The method of *in -vitro* maturation of sheep and cattle oocyte in TCM-199 supplemented with 20% foetal calf serum (FCS) was developed by Gordon and Lu (1990) with a maturation rate of 79% for cattle oocytes. Addition of FSH and LH to Ham's F12 medium contain 20% FCS, improved the maturation and fertilization rates of cattle oocytes (86%) matured *in vitro* (Fukushima and Fukui 1985). However, the highest maturation rates were reported by Sirad *et al.* (1988) for the maturation of cattle oocytes (98%) in TCM-199 media with FSH, LH and estrogen.

Oocyte cleavage

The cleavage rate for buffalo (59.0) sheep (56.6) and goat oocytes (41.5%) was not significantly different. However, Gordon and Lu (1990) achieved cleavage rate of 83% for cattle oocytes matured in TCM-199 with FCS, FSH and LH. This difference may be because of differences in the quality of oocytes taken in their experiment or due to species difference. Completion of nuclear maturation does not necessarily accompany normal cytoplasmic maturation (Moor and Trounson 1977). There are several essential components required for completion of both nuclear and cytoplasmic maturation, the most important being the presence of cumulus cells, which secrete glycosylated polypeptide(s) after gonadotrophin stimulation (Moor and Crosby 1987). The beneficial effects of FBS on oocyte maturation may be mediated through cumulus cells. FBS contain gonadotrophins, oestrogen and a number of growth factors (EGF and TGF alpha), which may have an important role in the regulations of oocyte maturation, particularly via cumulus cells (Skinner 1990).

Blastocyst development

Oocytes matured and fertilized *in-vitro,* developed successfully to the morula and blastocyst stages after day 7 when they were cultured with oviductal epithelial cells. Oviductal cells began monolayer formation on day 3 of *in-vitro* culture with *in-vitro* fertilized oocytes. The monolayer occupied 50 to 60% of the bottom area of the 50 µl droplet of culture medium.

Embryo co-culture with oviductal epithelial cells has emerged as an important method capable of supporting development of a high proportion of *in-vitro* bovine and ovine zygotes thought the preimplantation interval to the blastocyst stage (Eyestone and First 1989, Madan *et al.* 1994). Co-culture may exert a positive influence on early development by the secretion of embryotrophic factors into the culture medium and/or by reducing the negative effects of toxic components of the culture environment on preimplantation development (Bavister 1988, Eyestone and First 1989). An oestrus-associated 90-92 KDa oviductal glycoprotein characterized in sheep is capable of binding to the zona pellucida and blastomere surface (Gandolfi *et al.* 1989) and help the cleaved sheep oocytes to progress beyond the 16-cell stage and reach up to the morula/blastocyst stage.

Insulin stimulates DNA and RNA protein synthesis and cell division in mouse embryos *in vitro* (Harvey 1988). The exogamous addition of insulin to culture medium has advantagenous effects in cattle (Zhang *et al.* 1991). Chauhan *et al.* (1995) also reported that inclusion of insulin in medium during the co-culture enhanced the production of goat blastocyst embryos. Based on these reports we have taken insulin as an additive for the development of cleaved oocytes up to blastocyst stage. In our study when the cleaved oocytes were culture in TCM-199 supplemented with 10% FBS and 5µg/ml insulin and co-cultured with oviductal cells, the rate of development up to morula stage was not significantly different among the 3 species (Table 1.) However, the rate of de-

velopment up to blastocyst stage in buffalo (34.0%) was significantly higher ($P<0.01$) than goat (8.1%) and sheep (4.7%). The low rate of development of sheep and goat IVM-IVF oocytes up to blastocyst stage as compared to that in buffalo, as observed in our study suggests that the culture system with insulin is more suitable for development of buffalo oocytes, as compared to goat and sheep oocytes. Further research is, therefore, needed to improve the development of IVM-IVF oocytes up to blastocyst stage in these 2 small ruminant species.

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Camel semen during breeding and non-breeding seasons

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ABSTRACT

Camel semen production, evaluation and seminal characteristics during breeding and non-breeding seasons (hot humid and hot dry) have been reported. The colour, consistency, pH, sperm concentration, per cent live sperm and motility did not show much variation during different seasons. Sperm abnormalities increased during non-breeding season and the most predominant abnormalities were found in the mid-piece. Gel formation occurred immediately after ejaculation which liquified within 2 hr when kept at 37°C. The results are discussed in relation to sexual behaviour and hormonal levels in the serum of the camel.

Key words : Camel, Breeding season, Non-breeding season, Semen

The normal breeding period of Indian camels is from November to March (Khanna *et al.* 1990). The testes of the camel undergo seasonal changes-strongly active spermatogenesis during winter and relative quiescence during summer (Singh and Bharadwaj 1978). In Egyptian and Sudanese camels also, there are fluctuations in the sexual activity and number of spermatogonia, spermatids and spermatozoa due to seasonal variations and the rate of spermatozoa and the rate of sperm production is higher in spring and lower during autumn (Osman and El-Azab 1974). Khan and Kohli (1973) reported seminal attributes of Indian camels. Ranga and Rai (1992) investigated ultrastructure of the camel spermatozoa through scanning electron microscopy during breeding season. The present study reports the semen production in camel during breeding and non-breeding seasons.

MATERIALS AND METHODS

Adult male fertile camels (10) of above 8 years old, maintained under intensive management system at the National Research Centre on Camel, Bikaner, were used in the present investigation. Semen samples were collected in bull artificial vagina (AV) during breeding (November-March), hot dry (May-July) and hot humid seasons (September, October). A rubber sphincter was used on the liner of AV to provide suitable constriction. The temperature of the AV was maintained at 45-50°C. The semen collection was done twice a week in the morning (8-9 AM). The semen was collected directly into tris-egg-yolk citrate glycerol buffer (Tris-EYCG) at pH 6.8. Few semen samples were also collected without buffer to study liquefaction of gel. The physical attributes, viz. ejaculation time, volume, colour consistency and pH, were recorded. Sperm count was determined (Belsey *et al.* 1980) in diluted semen with extender (1:2). The per cent live dead spermatozoa and the abnormalities were recorded by observing the smear stained with nigrosin-eosin (Mann and Luwa-Mann 1981). The libido of the camel was also recorded . Student's 't' test was employed for statistical analysis.

RESULTS AND DISCUSSION

The ejaculation time varied between 3 and 9 min during breeding season and it decreased to 1.5-4.5 min in hot dry season and 1.5 to 5.0 min in hot humid season. The total volume of the ejaculated semen also showed a decreasing trend from breeding to non-breeding season ranging from 3-10.5 ml during breeding season 1.5 to 4.5 ml during hot dry season, and 1.5 to 4.0 ml during hot humid season. The colour, consistency, pH, sperm concentration, per cent live sperm and motility did not show much variation during different seasons. However, the per cent sperm abnormalities increased during non-breeding season, viz. hot dry/hot humid (Table 1).

The camel semen samples collected without buffer had a tendency to undergo gel formation immediately after ejaculation. The time taken for liquefaction of the gel varied from 0.5 hr to 12 hr when incubated at 37°C. About 70% of the semen samples liquefied within 2 hr. Seasonality did not show any effect in the pattern of liquefaction. The sperm motility was ++++ to +++++ grade in all the seasons.

The present investigation on the physical attributes of camel semen was similar to that of Khan and Kohli (1973) in Indian dromedary camels and Chen and Yuen (1979) in bactrian camels. However, in these studies seasonal effects were not

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attempted. Chen and Yuen (1979) reported a higher semen volume (7.72 ml/ejaculation) and sperm count (615×10^6/ml) but lower pH (range 6.8 to 7.4) in the bactrian camel which might be due to species specificity and environmental factors. Khan and Kohli (1973) observed no motility up to third ejaculation; further ejaculation showed motility. Agarwal *et al.* (1995) observed no motility perhaps due to some procedural difficulty, particularly pressure and temperature in the AV. The motility of spermatozoa was ++++ to +++++ grade in all the semen samples and it was comparatively better in the samples colleted in buffer than without buffer. The gel formation immediately after ejaculation arrested the motility of camel spermatozoa in neat samples.

Table 1. Physical evaluation of camel semen during breeding season and non-breeding seasons

Parameters	Breeding season	Non-breeding season	
		Hot dry (May-July)	Hot humid (September)
Ejaculation time (min)	6.2±0.401	5.5±0.434	4.3±0.329*
Volume (ml)	5.3±0.473	3.5±0.300*	3.2±0.304*
Colour	Milky white-Creamish	Milky white-Creamish	Milky white-Creamish
Consistency	Medium-thick jelly	Medium-thick jelly	Medium-thick jelly
pH	8.2±0.094	7.7±0.083	7.9±0.083
Sperm conc. (x 10^6/ml)	5.7±0.244	5.1±1.732	4.7±0.234
% Live sperm	89.9±1.073	89.0±1.732	89.3±1.233
Motility	+++++	++++	+++++
Abnormal spermatozoa	0.01%	0.1%	2-3%
Sexual behaviour	Aggressive	Mild	Mild

D * ($P<0.05$)

The morphology of the spermatozoa showed a sharp demarcation of head, neck, mid-piece and tail regions. The head was ovoid to elliptical in shape containing smooth surface. The abnormalities of the spermatozoa observed were double head, double tail, detached head, mid-piece swelling, mid-piece bending, curling of tail, cytoplasmic droplets and sperm agglutination. However, the most prominent abnormalities were found in the mid-piece. The presence of protoplasmic droplets at the anterior end of mid-piece was quite often noted in camel which may be due to lesser stay of spermatozoa in the epididymis (Ranga and Rai 1992).

The libido of the camels varied during breeding and non-breeding seasons. During breeding season the libido was exhibited by restlessness, loss of appetite, grinding of teeth blowing soft palate, copious poll gland secretion and gurgling sound. In non-breeding seasons, these behavioural signs appeared in subdued form in several cases. The studs, however, exhibited willingness for mating and the normal coital behaviour such as biting and fondling the females, salivary frothing, jerking movements etc. and donated semen in all the seasons except for one or two occasions during non-breeding seasons.

The anatomical and morphological changes in the testes of camels during breeding and non-breeding seasons were reported (Singh and Bharadwaj 1978, Tingari *et al.* 1984) Abdel - Raouf *et al.* (1975) reported that non-breeding season in camel was characterized only by reduced spermatogenesis, never by complete aspermatogenesis. Although, the weight of the testis was elevated during breeding season, spermatogenesis occurred throughout the year (Charnot 1964). Yagil and Etzion (1980) observed definite changes in the serum androgen levels during non-breeding and breeding seasons (2 ng/ml vs 30 ng/ml). The fact that the enzymes and chemical components of the testes were not affected by the seasons (Osman *et al.* 1976) contradicts the theory of seasonaity in breeding behaviour of camel studs. In this study, the seminal attributes exhibited only minor fluctuation in the volume, pH and ejaculation time during 3 different seasons, which indicated that sperm production continues in the non-breeding season also with a subdued sexual behaviour. Rutting in male camels is mainly influenced by higher testosterone level in the serum (Yagil and Etzion 1980, Agrawal *et al.* 1987, Rai *et al.* 1996). Singh and Bharadwaj (1978) suggested that the female camels could be seasonal breeders depending on the environment but not the male camels, who could serve whenever the she-camels were in heat. This observation was corroborated in the present study also as the camels could be mated successfully even during non-breeding season (Rai *et al.* 1995).

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Comparative evaluation of macrophage migration inhibitory factor activity from buffalo lymphocyte cultures by three assay methods

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Key words : Macrophage migration inhibitory factor activity, Lymphocytes, Macrophages

Inhibition of random migration of macrophages is a commonly employed assay for determination of migration inhibitory factor (MIF) activity in culture supernatants of activated lymphocytes. In the present study 3 types of assay modifications to the basic migration inhibition assay were employed to determine their efficiency, sensitivity and reproducibility, using culture supernatants produced from activated buffalo lymphocyte cultures on 3 types of test indicator cells.

Adult male buffaloes (3) were inoculated subcutaneously in the back region with live *Mycobacterium bovis* (BCG strain) suspension in incomplete Freund's adjuvant (50 mg/buffalo). Animals were tested for sensitization by intra dermal test using PPD (*M. bovis* BCG strain) following the method of Lesslie *et al.* (1975). Three other male buffaloes were also included in the study for getting lymphocytes to be stimulated by mitogens.

Production of MIF from buffalo lymphocyte cultures

Mononuclear cells from buffalo peripheral blood were separated (Boyum 1968) with some modification . Blood was collected in 2.7% EDTA flasks (20 : 1 ratio) and kept at 4 °C for 30 min. Leuckocyte rich plasma was aspirated and centrifuged at 1 000 g for 15 min. The cell pellet was suspended in phosphate buffer saline (PBS, Ca^{++}, Mg^{++} free, pH 7.2), and mononuclear cells were separated using histopaque as density gradient material as described in standard procedure. The cell viability was determined and cells were suspended in Iscove's medium at required concentration. *In vitro* cultures were set up using these cells and were stimulated by mitogens either ConA or PHA (10 µg/ml) and from sensitized animals by PPD (10 µg/ml, Weiser *et al.* 1981).

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The culture supernatants were harvested at 24 hr post-incubation and freezed at -20°C in aliquots until assayed. Control cultures were also set up as above but without providing any stimulations.

Isolation of alveolar macrophages

Alveolar macrophages from buffalo and goat lungs were isolated from freshly slaughtered animals (Holt 1979). Warm PBS (pH 7.2 , Ca^{++}, Mg^{++} free) was poured inside the lungs and kept as such for 10 min. The lavages were collected through muslin strainers and centrifuged at 1000g for 10 min at 4°C. The cell pellets so obtained were given 3 washings in PBS and suspended in Iscove's medium (IMDM) and were incubated in flat-bottom flasks at 37°C for 2 hr. The supernatant media was discarded and adherent cells were collected by using a rubber policeman and were used in the assays.

Collection of guineapig peritoneal exudate cells (PEC)

Peritoneal exudate cells from guineapig ,were obtained by the method of Lavergene and Harrington (1978), and were used in the assays.

Capillary migration assay was performed as described by Little and Naylor (1977). The results were recorded by projecting the images of migrated cells onto a graph paper using a camera lucida. Area of migration was recorded and migration ratio (MR) was calculated as :

$$MR = \frac{\text{area of migration in test sample}}{\text{area of migration in control sample}}$$

Finally per cent migration inhibition (% MI) was calculated as

$$\% MI = (1 - MR) \times 100$$

samples showing % MI > 20 were considered positive for MIF activity.

Agarose droplet assay was performed as per Harrington and Stastny (1973). Different dilutions of culture supernatants were tested and values were calculated as above. Plasma droplet assay was performed as described by Sandru and Sandru (1978).

Table 1. A comparative evaluation of per cent migration inhibition produced by 3 assay systems on test indicator cells using different stimulants

Type of assay	Indicator cells								
	Buffalo alveolar cells			Goat alveolar cells			Guinea pig pertional exudate		
	Stimulation provided with (10 µg/ml)								
	ConA	PHA	PPD	ConA	PHD	PPD	ConA	PHA	PPD
Capillary	52.99	49.26	52.99	52.01	48.00	51.12	51.14	48.60	48.6
Agarose	50.00	46.51	45.00	52.40	40.47	52.40	48.80	40.42	48.8
Plasma	54.54	50.00	52.27	54.54	50.00	52.16	53.19	50.00	51.0

The results of 3 assay systems were analysed for their reproducibility and sensitivity using 3 types of test indicator cells and MIF rich culture supernatants. It was observed that in capillary migration inhibition assay on an average 48.00-52.99 % MI was recorded. Similarly in agarose droplet assay 40.42-52.40 % MI and in plasma droplet assay 50.00-54.54% MI was recorded. Since the 3 assay systems were based on the same principle of migration inhibition, the results indicated that there was no significant difference in the %MI recorded by each assay. The results were reproducible and the methods were sensitive enough for comparison.

Agarose droplet assay and plasma droplet assay were easy to perform and permitted assay of small volumes of test supernatant, and large number of samples could be tested in a short time and simultaneously. Sandru and Veraguth (1979) also observed similar type of reproducible results in plasma droplet and agarose droplet assays separately. It was also observed in this study that buffalo and goat alveolar macrophages responded to the MIF effect equally and showed no species specificity. Thus by employing these alveolar cells one could avoid the use of laboratory animals for getting peritoneal exudate cells and save precious labour, time and money for earing these animals. Bice *et al.* (1977) have also reported the use of alveolar macrophages in migration inhibition assays successfully. Thus it could be inferred that when large number of samples with small volumes of test liquid were to be tested in a short span of time one could employ either agarose droplet or plasma droplet assays using alveolar macrophages as test indicator cells and reduce the cost expenditure in such experimental studies.

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Infectivity of *Chlamydia psittaci* in McCoy cells

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Key words : *Chalmydia psittaci*, McCoy cells

Chlamydiae are obligate prokaryotic parasites that replicate intracellularly in eukaryotic cells. Different cell-lines have been reported to be efficient, economical and quick tools for the isolation and identification of chlamydiae. Johnson *et al.* (1983) found McCoy cells more sensitive for isolation of *C. psittaci* from clinical materials of abortions compared to staining of Ziehl- Neelsen method. The present study was undertaken to observe the suitability of McCoy cell-lines to support the growth of chick-embryo-adapted *C. psittaci* isolates.

Sample processing

The chicken-embryo adapted isolates of *C. psittaci* from kid pneumonia, kid enteritis, sheep abortion and goat abortion in McCoy cell-lines were studied. The inoculum for infection of cell-culture monolayer was prepared from fresh heavily infected yolk-sac membranes triturated in 2 ml of Bovarnick's buffer (Bovarnick 1950). The homogenates were centrifuged at 100 × g for 10 min at room temperature (20°-25° C). The interface material(s) between the cell debris and lipid layer were collected and suspensions were diluted 1 : 100 in minimum essential media (MEM Eagles) with non-essential amino acids and Earle's balanced salt solutions supplemented with 10% (V/V) heat inactivated foetal calf serum to infect McCoy monolayers.

Infection of McCoy monolayer

One millilitre of McCoy cell suspension containing 2.5× 10^5 cells/ml was seeded on to 12 mm diameter glass coversilp in 4 to 6 flat-bottom test-tubes for each sample and incubated at 37°C till the monolayer was completed. The monolayer were inoculated with 0.5 ml of inoculum of each isolate individually. To enhance the attachment of chlamydiae to the cells, inoculumn on to the monolayers was centrifuged at 2 000 × g for 60 min at 37°C (Andersen and Tappe 1989). Thereafter, the excess inoculum was removed and replaced with freshly constituted 1 ml of growth media from containing cyclohexamide (1 µg/ml). The infected monolayers were incubated at 37°C for 3 days.

Staining of monolayers

The coverslips were stained by modified Gimenez technique for the demonstration of elementary bodies/chlamdial inclusion. The infected monolayers on glass coverslips were subjected to indirect immunofluorescense examination as per the method of Anserson and Tappe (1989) employing anti-chlamydia group-specific monoclonal antibodies (NJ/D/3). The coverslips were examined under Nikon Epifluorescence microscope (model Optiphot-2) at magnification of 100, 200 and 400×.

The chlamydiae and cultured cells have mutually repulsive surfaces due to net negative charges at neutral pH. Therefore, the centrifugation of chlamydial inoculum onto the cell monolayer reduced repulsive forces and enhanced infectivity at 37°C. Dennis and Storz (1982) also observed that infectivity of *C.psittaci* of bovine origin was increased by centrifugation of the chalmydial inoculum onto mouse L-cell monolayers. The treatment of monolayer with cyclohexamide suppressed the replication of the cells thus making more nutrients available for replication of chlamydiae. The cells treated with cyclohexamide differed from the untreated cell and were fibroblastic, slightly larger, the cell sheet was also less dense. After 72 hr elementary bodies/ inclusions were visible in both treated and untreated cultures of McCoy cells. The inclusions were intracytoplasmic, pink and finely granular ball-like structures with a bluish-green background when stained by modified Gimenez method. These observations corroborate with those reported by Anderson (1986).

The immunofluorescent reaction of McCoy cells revealed intracytoplasmic chlamidial elementary bodies/inclusions (kid pneumonia, kid enteritis and sheep abortion isolates). Interestingly, the isolate of goat abortion origin had extra cellular elementry bodies thereby suggesting that McCoy cells could not support the replication of goat abortion isolate. Perez-Martinez and Storz (1983) already documented non-replication of ovine abortion isolates in L cell-lines. The findings of this study recommended that McCoy cell-lines are suitable

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for the maintenance of *C. psittaci* isolates in the laboratory.

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Viability of normal saline deep frozen homologous plasma and glycerin saline preserved equine tendon grafts

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Key words : Equine tendon grafts, Viability of preserved grafts

Studies are lacking with regard to the viability of banked tendon grafts preserved under different conditions for a specific period of time. Viability of the equine banked grafts preserved in plasma or glycerin at refrigeration temperature and by deep freezing has been investigated.

The flexor tendons (both superficial and deep) from all the limbs of about 10-12 cm in length, were collected aseptically from 4 donkeys. The collected tendons were washed in sterile saline solution thrice to make them blood free and were divided into smaller pieces of 1-2 cm length. These were stored by any of the following 3 methods of preservation in separate vials to a maximum duration of the 60 days after adding ampicillin powder @ 1 mg/ml of storage fluid :

1. Deep frozen in normal saline (-20°C),
2. homologous plasma at 4°C and
3. 20 % glycerin at 4°C.

Collection of tendon tissue

The grafts were observed and collected on 1, 7, 15, 30, 45 and 60 days. Frozen tendon grafts were slowly thawed while plasma and glycerin saline preserved tendon grafts were brought to room temperature. Tendon tissue was collected in buffered formalin for histological examination. Heamatoxylin and Eosin and Masson's Trichrome staining procedure (Luna 1968) were used to study the histological architecuture.

Grafts preserved by deep freezing and in plasma at 4° C looked normal up to 2 months, while grafts preserved in pure glycerin turned into a transparent structure, however, in 20% glycerin saline preserved grafts showed no change in normal colour and texture.

Deep frozen tendon grafts showed no degenerative changes (Fig. 1) at any stage up to 60 days and comprised collagen fasciculi with normal tenocytes and is in accordance with the finding of Singh (1993) who also tried deep frozen tendon grafts in dogs. The tendon grafts preserved in homologous plasma at 4°C from day 0 to 60 also showed no degenerative changes grossly as well as histomorphologically (Fig. 2). They were composed of collagen fasciculi and good number of normal tenocytes. Comparatively these grafts looked better than deep frozen grafts on the basis of histological findings.

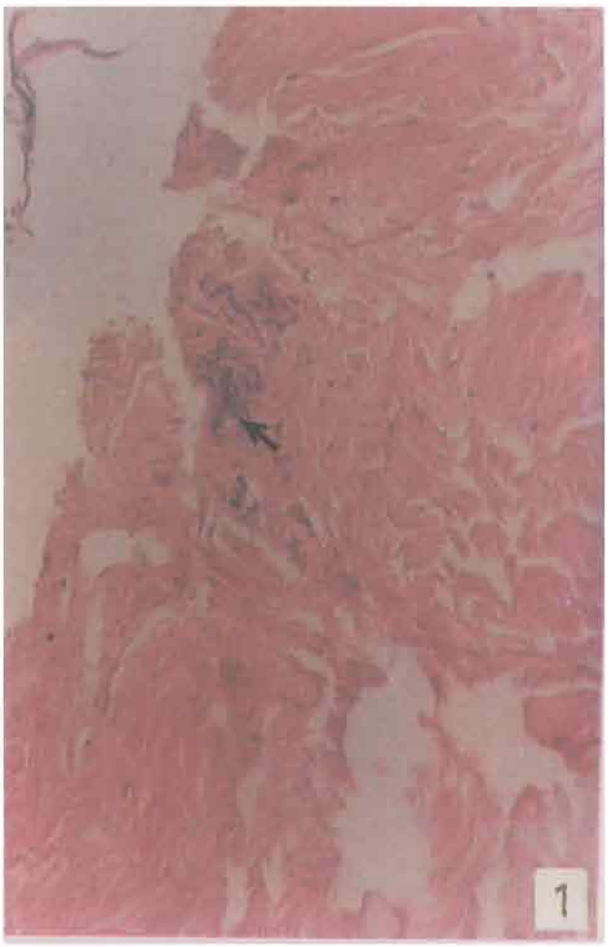
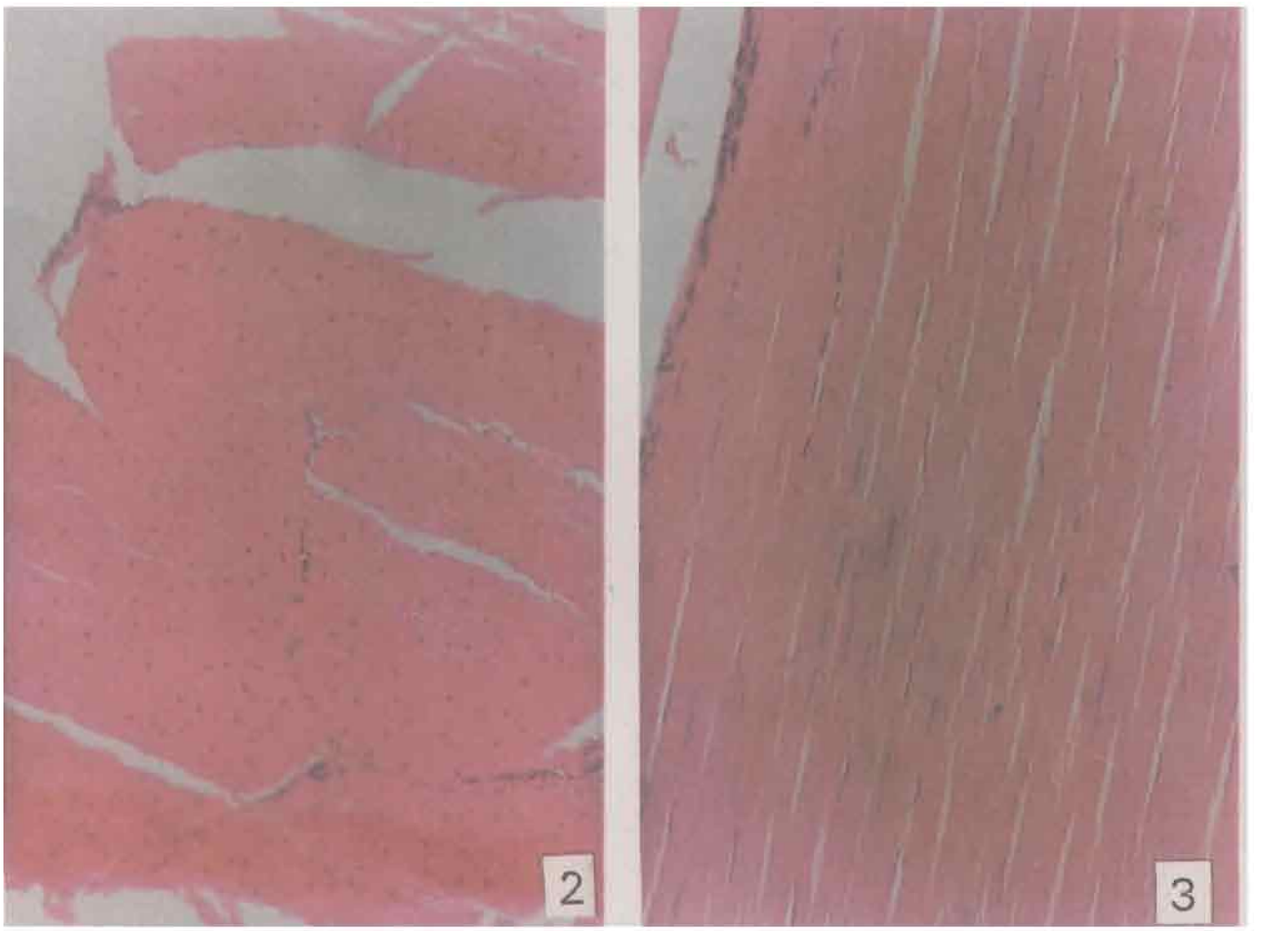


Fig. 1. Section of tendon showing normal tissue with blood vessels (arrow) at 60 days of the storage by deep freezing at -20°C in normal saline. (Haematoxylin & Eosin × 140).

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Figs 2-3. **2.** Section of tendon tissue showing large number nuclei at 60 days of storage in homologous plasma at 4°C (Haematoxylin & Eosin stain × 70.); **3.** Longitudinal section of tendon tissue showing normal tendon structure at 45 days of storage in 20 % glycerin at 4°C (Haematoxylin & Eosin × 140).

The tendon grafts preserved in 20% glycerin saline at 4°C showed near normal histological structure up to 45 days (Fig. 3). However, at 60 days after preservation the number of nuclei of tenocytes started decreasing though few nuclei were still present. Glycerin preserved tendon grafts have been tried with some success in bovines (Dass *et al.* 1987). It is concluded that tendon grafts stored by deep freezing and at 4°C in homologous plasma could be preserved nicely up to 60 days and those in glycerine saline up to 45 days.

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Pharmacokinetics and dosage regimen of sulphadimidine in donkeys following intravenous administration

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Key words : Donkeys, Pharmacokinetics, Sulphadimidine

Sulphadimidine (Indian Immunological) was administered into the jugular vein at the dose rate of 100 mg/kg body weight in 4 healthy donkeys weighing 80-90 kg. Blood samples were collected into heprinized tubes from the contra lateral jugular vein at 5,10 and 15 min and 2,4,6,8,12 and 24 hr after the injection of the drug for preparation of plasma.

The concentration of sulphadimidine was determined spectrophotometrically (Bratton and Marshall 1939). The plasma sulphadimidine concentration plotted on a semi-logrithmic scale as a function of time were analysed separately for each animal and pharmacokinetics and dosage regimen were calculated (Gibaldi and Perrier 1982).

The peak plasma level (187.8±7.88 µg/ml) at 5 min declined rapidly to 135.4± 6.3 µg/ml at 30 min. At 24 hr, the drug concentration was lower than 10 µg/ml. However, the concentration of about 50 µg/ml at 48 hr have been reported for sulphadimidine in cow calves (Srivastava *et al.* 1990). The plasma level of sulphadimidine was reported to follow 2 compartment open model as in buffaloes (Jha 1983) and cow calves (Srivastava *et al.* 1990). The calculated pharmacokinetics parameters of sulphadimidine in donkeys are given in Table 1.

The high values of distribution rate constant (3.88±1.04 / hr) shows that drug is rapidly distributed from central to the peripheral compartment. The high value of apparent volume of distribution Vd (area) (1.41±0.36) and area under plasma concentration curve AUC (868.5±87.4 µg/ml × hr) indicate sulphadimidine is rapidly and fairly distributed in various body fluids and tissues.

The elimination half life of sulphadimidine in donkeys (8.13±1.26) established in present study is longer than that reported in dogs (Nawaz 1980) and sheep (Nawaz and Khan 1979) but it is shorter than that reported in cow calves (Srivastva *et al.* 1990) and buffaloes (Jha 1983). The elimination half-life of sulphadimidine in dogs, sheep, cow calves and buffaloes were 4-6, 3-6, 11-2 and 14.5 hr respectively. The half-life of sulphadimidine in donkeys is shorter than that reported in horses (10-13 hr) (Vree *et al.* 1985). It is due to the higher value of elimination rate constant and total body clearance in donkeys.

Table 1. Pharmacokinetics parameters of sulphadimidine in donkeys following a single intravenous dose of 100 mg/kg body weight (n = 4)

Parameters	Units	Mean±SE
A	µg/ml	154.0±8.36
B	µg/ml	79.7±12.2
oc	/hr	3.88±1.04
t½	hr	0.20±0.04
ß	/hr	0.091±0.014
t½ß	hr	8.13±1.26
AUC	µg/ml × hr	868.5±87.4
K_{21}	/hr	1.364±0.466
K_{12}	/hr	2.341±0.636
Vc	L/kg	0.44±0.02
Vd(area)	L/kg	1.41±0.36
Cl_B	ml/kg/hr	112,3±9.07
T/P	ratio	2.16±0.67
D	mg/kg	101.4±11.7
D'	mg/kg	66.1±12.6

Due to difference in half-life of sulphadimidine, the dosage regimen calculated in horses can not be employed in donkeys. For most of sulfonamides, the minimum inhibitory concentration (MIC) ranged from 0.16 to 64 µg/ml. In the calculation of dosage regimen, 25 µg/ml was taken as MIC of sulphadimidine as this concentration is effective against most of micro-organisms (Nawaz 1980). Based on pharmacokinetic parameters (Table 1) to maintain MIC of 25 µg/ml, the appropriate intravenous dosage regimen for sulphadimidine in donkeys was calculated to be 101.4 mg/kg followed by 66.1 mg/kg at 12 hr interval or under field condition 100 mg/kg followed by 70 mg/kg at 12 hr interval.

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Bulbourethral gland of castrated donkeys : Histoenzymic study

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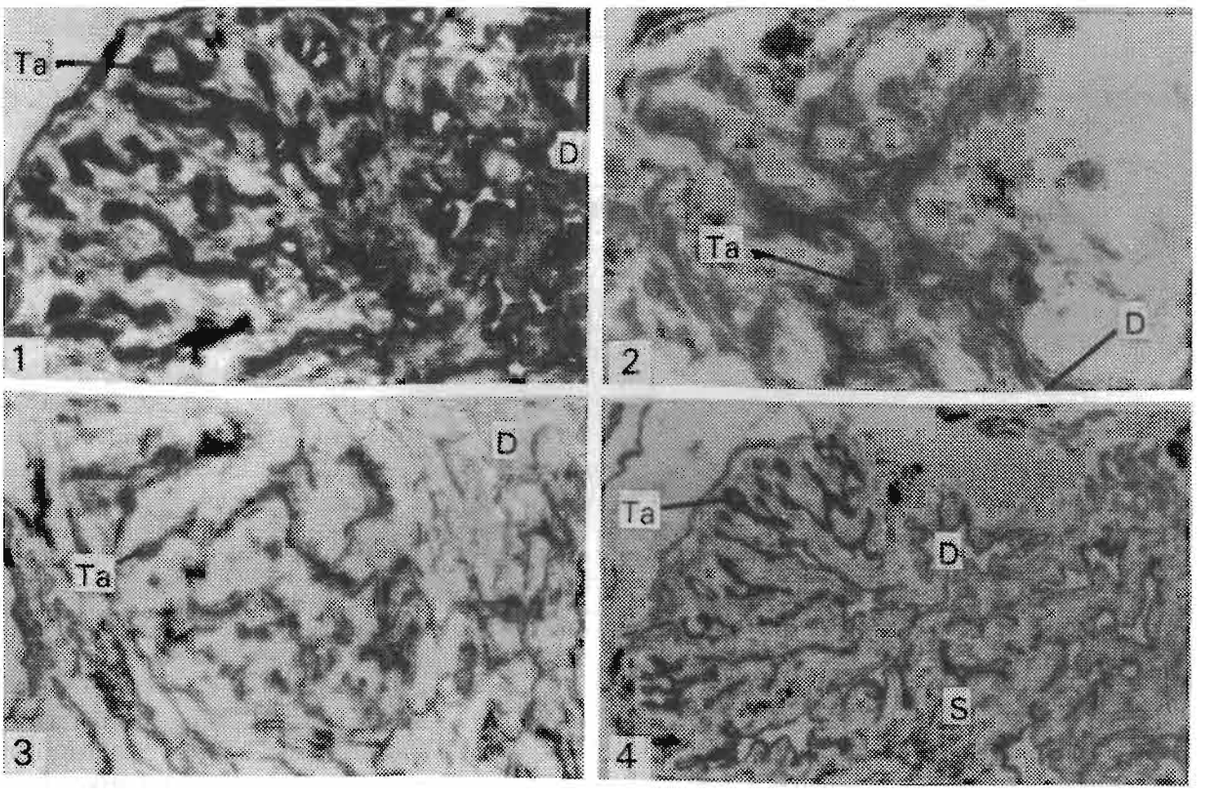
Key words : Donkey, Bulbourethral gland, Castrated donkey

Histoenzymic study was made on bulbourethral gland of 3 adult castrated donkeys to ascertain their enzymic profile relation to secretion. The tissue samples were collected from the bulbourethral gland soon after slaughter of animal and transferred to cryostat at –20° C. The cryostat section (10/μ) were obtained and incubated for various phosphatase and oxidoreductases and also stained by sudan black B (Table 1).

The histoenzymic distribution of phosphatase, oxidoreductase and lipid are summarised in Table 1.

Alkaline phosphatase (AKPase)

Fine granular AKPase activity was only seen at apical border of glandular and ductular epithelium. The activity was much higher in collecting duct as compared to larger ducts. The activity was also seen in the capillaries of stroma and bulbocaverous muscle. AKPase is actively involved in the ionic movement across the cell membrane (Pawar *et al.* 1986) and based on this fact, it appeared that the epithelium of small



Figs 1-4. **1.** Cryostat section of bulbourethral gland of castrated donkey showing : ATPase activity in tubuloalveolar epithelium (Ta) and duct (D); calcium method of ATPase × 140; **2.** SDH activity in alveolar epithelium (Ta) and large duct (D); Nitro BT × 140; **3.** LDH activity tubuloaveloar epithelium (Ta) and duct (D); Nitro BT × 70; **4.** NADPH-diaphorase activity in alveolar epithelium (Ta), small duct (S) and larger duct (D); Nitro BT × 70.

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Table 1. Histoenzymic distribution of phosphatases oxidoreductases and lipid in bulbourethral gland of castrated donkey

Enzymes	Method	Substrate	Reference	Glandular parenchyma	Ductular epithelium		Stroma	Muscle
					Small	Large		
Alkaline phosphatase (AKPase)	Azo dye method (1963)	Napthol ASMX	Barka and Anderson 1963	++	++	+/++	0	0/+
Acid phosphatase (ACPase)	Azo dye method (1963)	Napthol ASTR	Barka and Anderson 1963	+/++	+	0/+	0/+	0/+
Adenosinetri-phosphatase (ATPase)	Calcium activated ATPase	ATP	Chayan *et al.* (1969)	+++	++/+++	+	0/+	++/+++
Succinate dehydrogenase (SDH)	Nitro BT for bound enzymes	Di-Na-succinate	Pearse (1972)	++	+/++	+	0/+	++
Lactic dehydrogenase (LDH)	Nitro BT for bound enzymes	Na-DL-lactate	Pearse (1972)	++	+	+	0/+	+
Reduced nicotinamide adenine dinucleotide diaphorase (NADPH-diaphorase)	Nitro BT for bound enzymes	NADPH	Pearse (1972)	+/++	+/++	+	0/+	++
cytochrome oxidase (CYO)	Butcher's	N-Phenyl-P Phenylediamine 1-Hydroxy-2-napthetic acid	Pearse (1972)	+/++	+/++	+	0/+	+
Monoamino oxidase (MAO)	Nitro BT for bound enzymes	Tryptamine hydrochloride	Pearse (1972)	+/++	+/++	0/+	0/+	+
Acetyle cholinesterase (AChEase)	Acetyl thiocholine iodide	Acetyl thiocholine iodide	EL Badawie and Schenk (1967)	0/+	0/+	0/+	*	*
Lipid	Sudan black B Sudan black in 70% ethnol	13 to 70% ethnol	Chayan *et al.* (1969)	+	+	0/+		*

0-Negative; + - Weak; ++ - Moderate; +++ - Strong. Only in neuromal elements.

duct was physiologically active than the glandular parenchyma.

Acid phosphatase (ACPase)

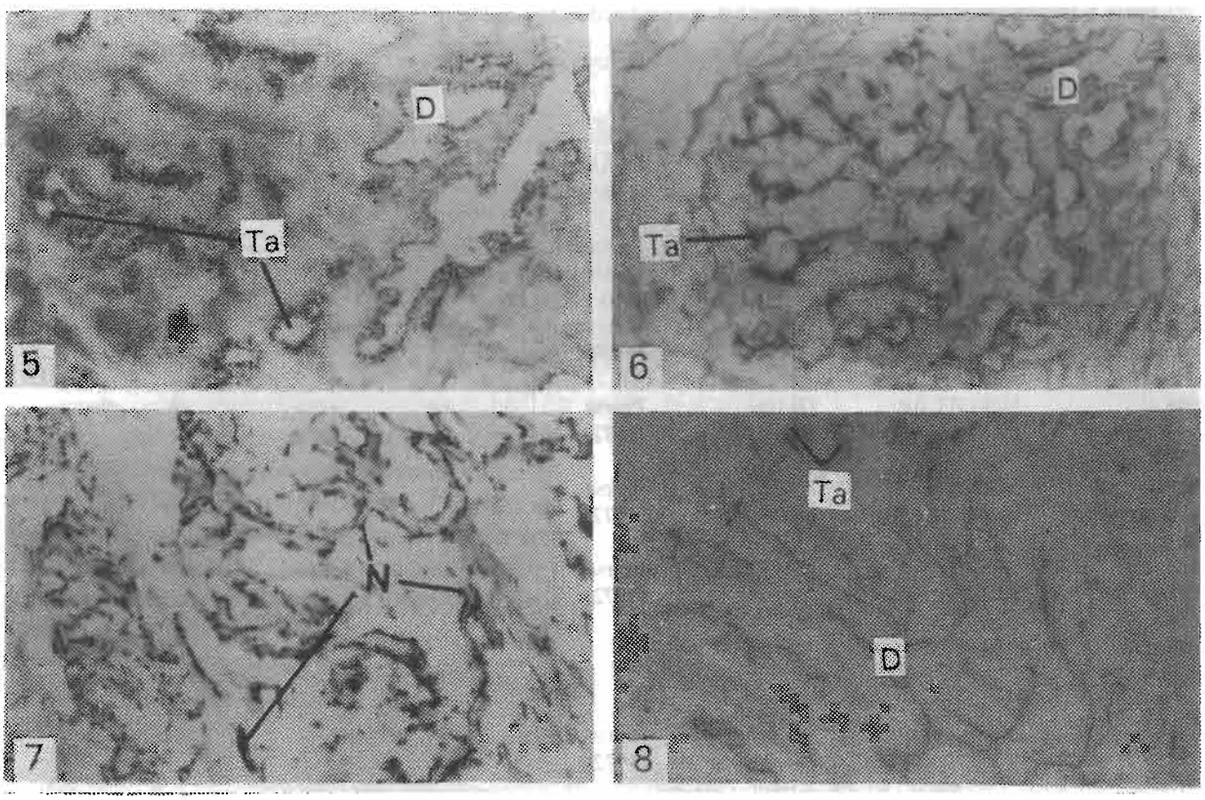
Diffused ACPase activity was perinuclear in the epithelium of alveoli and duct. The end pieces were more reactive than other components of gland for ACPase. It is essentially a lysosomal enzyme (Novikoff 1963) which actively participates in the elaboration of srecetory material in the glandular epithelium. A weak to moderate activity of ACPase in the glandular parenchyma reflects lower secretory activity in the gland.

Adenosine triphosphatase (ATPase)

The tubulo-alveolar epithelium was strongly positive particularly in abluminal border. Activity was much low in larger ducts. The stromal tissue was weakly positive for ATPase, whereas muscle tissue showed moderate to strong reaction. Higher ATPase in the structures reflects better energy utilization in the glandular parenchyma and muscle fibres as reported in the bulbourethral gland of goat (Wrobel 1970).

Oxidoreductases

The activity for SDH, LDH, MAO, CYO and NADPH-diaphorases was uniformly fine granular and perinuclear in distribution. It was either weak to moderate or moderate both in the glandular parenchyma and smaller collecting ducts. The activities were progressively decreased as duct become larger (Figs 2-6). The histoenzymic distribution in the bulbourethral gland of donkey appeared similar in respect of SDH and LDH in the cattle (Stallcup 1969). A low level of these enzymes indicated less synthetical secretory process in the bulbourethral gland of castrated donkeys.



Figs 5-8. 5. Granular monoamino oxidase activity in tubuloaveolar epithelium (Ta) and duct (D); Nitro BT × 140; 6. Cytochrome oxidase activity in tubuloalveolar epithelium (Ta) and duct (D); Butcher method × 70; 7. Acetyle cholinesterase activity in nerve fibre and nerve terminal (N); thiocholine method × 70. 8. Sudanophilic lipid droplets at luminal border of tubuloalveolar epithelium (Ta) and duct (D); Sudan Black B × 70.

Acetyle cholinesterase (AChEase)

Distribution of AChEase in the glandular parenchyma and ductular epithelium was doubtful. It was mainly seen in the neuronal element of stroma and muscle tissues (Fig. 7). Fine nerve terminal could be seen in perialveolar areas as reported in the bulbourethral gland of ram (Pawar *et al.* 1986). The presence of AChEase in nerve fibres indicated cholinergic neurotransmission in the gland.

Lipid

Fine to medium coarse lipid droplet could be seen in the luminal border of the glandular parenchyma and tubular epithelium as reported in bulbourethral gland of ram (Pawar *et al.* 1986). The lipid content progressively decreased with increase in luminal diameter of ducts. In castrated goat, however, the absence of lipids was reported (Gupta and Singh 1982). It was concluded that the tubuloalveolar parenchyma of bulbourethral gland from 3 castrated donkeys showed varied activity for AKPase, ACPase, ATPase, SDH, LDH, NADPH-diaphorase, CYO and MAO. The activity was higher in collecting duct than that in larger ducts. AchEase was better localized in the neuronal elements. Fine sudanophilic lipid droplets were also seen in the glandular and ductular parenchyma.

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Bacterial flora in boar semen and their antibiotic sensitivity

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Key words : Antibiotic, Bacterial flora, Boar-semen

Microbial investigation is useful for semen evaluation because bacteria in semen are associated with diminishing sperm viability and fertility. Semen usually gets contaminated during collection and process of preservation, and may be responsible for reproductive disorders. Limited information is available on bacterial load of boar semen in India (Tamuli *et al.* 1984). The present study was, thus conducted to determine the bacterial load in semen fraction of boar ejaculate, isolation and identification of bacterial flora and antimicrobial sensitivity.

A total of 50 semen samples from 5 Large White Yorkshire boars were studied and bacterial load of samples were estimated by pour plate count method (Cruickshank *et al.* 1975) aseptically. Isolation of bacteria was performed in blood agar and Mac Conkey's agar plates. Identification of bacteria was done following Cowan (1970). The drug sensitivity test *in-vitro* was carried out with standard biodiscs following the paper disc diffusion method (Baur *et al.* 1966).

Table 1. Mean and t-value of bacterial load in pre-sperm and sperm-rich fractions of boar ejaculate

Boar No.	Pre-sperm fraction ($\times 10^6$/ml)	Sperm-rich fraction ($\times 10^5$/ml)	t-value
042	1.10[a] ±0.07	0.338[b] ±0.048	8.90**
043	0.860[a] ±0.017	0.334[b] ±0.041	11.56**
044	0.740[a] ±0.153	0.278[b] ±0.040	2.91*
045	0.820[a] ±0.106	0.290[b] ±0.031	4.77**
047	0.640[a] ±0.092	0.244[b] ±0.033	4.02**
Overall	0.832[a] ±0.054	0.296[b] ±0.017	9.31**

Values bearing different superscript in a row differ significantly; *P<0.05, **P<0.01.

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The average bacterial load in pre-sperm and sperm rich portions were 0.832±0.054 × 10^6 and 0.296±0.017 × 10^5 per millilitre respectively (Table 1). A highly significant difference (P<0.01) in bacterial load of 2 fractions is considered physiological. The average bacterial load in sperm rich fraction, recorded in this study is comparable to the finding to Tanaka *et al.* (1951) in boar semen. However, lower value of bacterial load in sperm rich fraction of boar ejaculate, reported by Waltz *et al.* (1982) might be due to difference in method of study.

Table 2. Antimicrobial sensitivity pattern on gram +ve and gram -ve bacterial isolates

Gram +ve				Gram -ve			
Antibiotics	S	I	R	Antibiotics	S	I	R
Penicillin	3.0 (25.0)	0.0 --	9.0 (75.0)	Strepto-mycin	1.0 (20.0)	0.0 --	4.0 (80.0)
Genta-mycin	12.0 (100.0)	0.0 --	0.0 --	Genta-mycin	5.0 (100.0)	0.0 --	0.0 --
Ampi-cillin	6.0 (50.0)	0.0 --	6.0 (50.0)	Tetra-cycline	2.0 (40.0)	1.0 (20.0)	2.0 (40.0)
Tetra-cycline	5.0 (41.67)	2.0 (16.67)	5.0 (41.67)	Ampi-cillin	4.0 (80.0)	0.0 --	1.0 (20.0)
Co-trim-oxazole	3.0 (25.0)	1.0 (8.33)	8.0 (66.67)	Ceph-alexin	3.0 (60.0)	1.0 (20.0)	1.0 (20.0)
Ceph-alexin	5.0 (41.67)	1.0 (8.33)	6.0 (50.0)	Chloram-phenicol	3.0 (60.0)	1.0 (20.0)	1.0 (20.0)
Cloxa-cillin	0.0 --	0.0 --	0 0 --	Sismo-mycin	5.0 (100.0)	0.0 --	0.0 --

Figure in parenthesis indicates the percentage; S sensitive, I intermediate, R resistnat.

Out of 30 samples, 27 samples (90.0%) were found positive of 7 genra of bacteria gram bacterial isolates of *Bacillus* (25.92), *Micrococcus* (14.81), *Corynebacterium* (14.81), *Staphylococcus* (14.81) and *Streptococcus* (14.81) and *Klebsiella* (11.14%) were identified from 7 samples. The type of bacteria isolated in the present study, was similar to those reported by Sone *et al.* (1982) and Saikia *et al.* (1987). Isolation of *Staphylococcus*, *Escherichia coli* and *Corynebacterium* in the present study is significant as these organisms

Table 3. Antimicrobial sensitivity pattern on bacterial isolates

Isolated bacterial genera	Antibiotics/chemotherapeutic agent										
	P	G	S	T	E	Q	CP	Cl	S	C	SS
Coryne-bacterium (3)	1.0	3.0	2.0	1.0	0.0	1.0	0.0	0.0	--	--	--
Staphy-lococcus (2)	0.0	2.0	1.0	1.0	0.0	0.0	0.0	0.0	--	--	--
Strepto-coccus (1)	1.0	1.0	1.0	1.0	1.0	0.0	0.0	0.0	--	--	--
Bacillus (4)	1.0	4.0	2.0	1.0	2.0	2.0	3.0	0.0	--	--	--
Micro-coccus (2)	0.0	2.0	0.0	1.0	1.0	0.0	1.0	0.0	--	--	--
Escheri-chia coli (3)	--	3.0	3.0	1.0	--	2.0	2.0	--	1.0	2.0	3.0
Klebsiella (2)	--	2.0	2.0	1.0	--	1.0	1.0	--	1.0	1.0	2.0

P, Penicillin; G, gentamycin; A, ampicillin; T, tetracyclin; E, erythromycin; Q, co-trimoxoazole; CP, cephalexin; Cl, cloxacillin; S, streptomycin; C, chloramphenicol; SS, sismomycir.

are regarded abnormal for semen and may reduce the survivability of spermatozoa fertility of sows (Robert 1971).

Antimicrobial sensitivity studies (Table 2) revealed that gram +ve bacterial isolates were highly sensitive to gentamycin (100%) followed by ampicillin, cephalexin, tetracycline, erythromycin, penicillin, and co-trimoxazole (25%). Likewise, gram - ve bacterial isolates were highly sensitive to gentamycin and sismomycin (100%) and least sensitive to streptomycin (20%). Rahman *et al.* (1983) and Saikia *et al.* (1987) also found that bacterial isolates were highly sensitive to gentamycin (100%) and least sensitive to penicillin and streptomycin, commonly used with semen diluents. The increased susceptibility of bacteria towards gentamycin was also reported by Sone *et al.* (1982) and Tamuli *et al.* (1984). Gentamycin does not have any adverse effect on the spermatozoa and the boar semen treated with gentamycin, when inseminated to sows and gilts, they farrowed with an average litter size of 8.4 (Tamuli *et al.* 1984). Ampicillin, cephalexin, tetracycline, erythromycin and co-trimoxazole have shown lesser effect on bacterial isolates in the present study, which corroborates the finding of Waltz *et al.* (1968).

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Electrophoretic study of haemoglobin in rabbits

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Key words : Electrophoresis, Haemoglobin, Rabbits

The present study was undertaken to delineate status of haemoglobin locus and its association with performance traits in exotic and crossbred rabbits. The random bred broiler rabbit population comprising Californian White (19), New Zealand White (39) from CLFRS, Hessaraghatta; New Zealand White (24), Russian Grey Giant (18) and crossbreds (13) from Experimental Animal House unit of UAS; and Angora (24) from Deejay Hatcheries, Bangalore, were utilized under this investigation.

The technique for haemoglobin characterization on Agarose gel electrophoresis was essentially same as described by Teisberg (1970) and Csaikl *et al.* (1980).

Based on the electrophoretic mobility of haemoglobin, it was observed that all breeds of rabbit showed only slow moving band and it was designated as Hb AA by comparing with the standard Hb marker (Fig. 1). The complete absence of Hb BB genotypes either in homozygous or in heterozygous form in current rabbit population led to the contention that Hb B allele was totally absent in rabbit population and thus giving the gene frequency of Hb A allele as a unity.

The present study of monomorphic nature of haemoglobin in rabbit was in accordance with the observation of Bonhomme *et al.* (1981) and Robinson and Osteroff (1983) in the genus Lepus. Thus the situation might be the same as in the hares.Thus, the presence of monomorphic nature of haemoglobin in the rabbit population would limit the scope for further genetic improvement of rabbit population.

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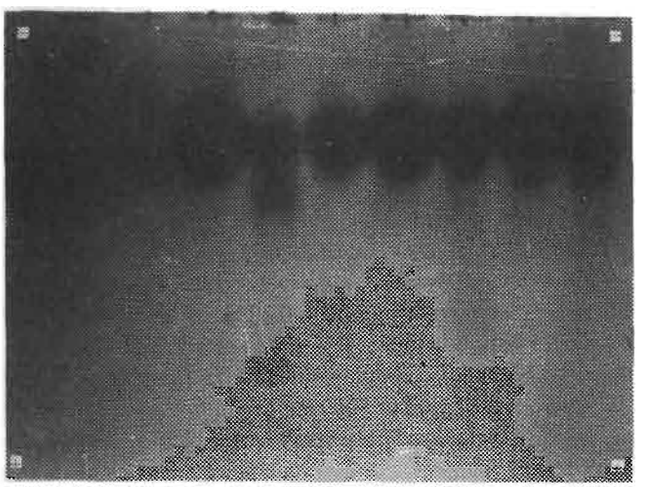


Fig. 1. Electrophoretogram of haemoglobin in rabbits. 1. HbAA: 2. Hb Marker.

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Normal values of some biochemical constituents in blood serum of Iranian one-humped camels (*Camelus dromedarius*)

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Key words : Camel, Blood serum, Biochemical constituents

This paper presents the results of a study undertaken to establish the normal values of biochemical constituents in serum of the Iranian camels.

Blood samples were collected from 317 clinicaly healthy male and female camels of varying ages, located in different parts of Iran for subsequent separation of serum, for determination of total bilirubin, direct and indirect bilirubins, creatinine, urea, uric acid, glucose, cholesterol, triglycerides and blood urea nitrogen (BUN). The samples were collected in venoject tubes early in the morning from the jugular veins of the animals before their feeding. The tubes were placed in ice container and immediately transported to the laboratory, where they were centrifuged at 300 rpm for 15 min to separate the serum. All the aforesaid parameters were determined within 24 hr by using autoanalyser. The data were analysed to determine the mean values of all non-electrolytes. T-test was applied to compare the male and female values. Correlation coefficients of various non-electrolytes with each other and between age and each of these non-electrolytes was determined through regression analysis (Nie *et al.* 1975).

The mean value of total serum bilirubin (Tables 1,2) in camel, regardless of age and sex was close to the values reported by Al-Ani *et al.* (1992). However, the value was lower than the values reported by Al-Ali *et al.* (1988). It may be noted that the number of samples analysed in this investigation was much higher than the number of samples analysed by Al-Ali *et al.* (1988) and Al-Ani *et al.* (1992). A significant difference was observed between males and females of 13 to 36 months age group (Tables 1,2), the values in males being lower than in females. The values of total, direct, and indirect bilirubin significantly decreased with increase in age (Table 1; Fig. 1). However, in the sex-wise values, there was a significant difference only in values of direct bilirubin of males and females ($P<0.05$), the mean values being higher in males than females.

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Sex significantly affected the values of creatinine regardless of the age of animals (Table 2). The males had higher values than the females. The values of creatinine were significantly different between males and females in age groups of 13 to 36 months, 37 to 60 months and 61 to 84 months ($P<0.05$). Regression analysis showed that age of camel significantly affected the serum creatinine values ($P<0.001$) and that values of creatinine decreased with increase in age (Table 1, Fig. 1). These findings are similar to those reported by Al-Ani *et al.* (1992).

The highest values of serum uric acid were observed in the age group of less than 6 months and the lowest in the age group of 61 to 84 months. In general, the values of serum uric acid decreased with increase in age (Table 1; Fig. 1) and the difference was statistically significant ($P<0.001$). The statistical analysis showed that there was no significant difference in the values of serum uric acid between the male and female camels regardless of their age (Table 2). The mean uric acid value obtained in this investigation was higher than the values reported by Al-Ali *et al.* (1987). This may be due to the differences in technique of uric acid estimation or some other factors.

The highest and lowest values of serum glucose, regardless of the sex of camels, were observed in the age groups of less than 6 months and 85 to 108 months respectively. The increase in age led to decrease in the serum glucose levels (Table 1; Fig. 1) and the difference was statistically significant ($P<0.001$). The regression analysis showed that 17.22% variation in the serum glucose levels was due to age. The difference between the mean values of serum glucose in males and females, regardless of age, was statistically significant ($P<0.05$). The values in the male were higher than the female (Table 2). Further, the difference between the values in males and females of age groups 6 to 12 months, 37 to 60 months, 61 to 84 months and 85 to 108 months, was statistically significant ($P<0.05$).

The mean general serum glucose value of our camels was similar to that reported by Soliman and Shaker (1966), but was higher than that reported by Al-Ani *et al.* (1992). In the levels

Table 1. Mean * values of blood serum non-electrolytes of Iranian camels at different ages regardless of sex

Non-electrolytes	Age in months							Overall mean values (317)
	<6 (13)**	6-12 (18)	13-36 (51)	37-60 (41)	61-84 (51)	85-108 (43)	>109 (100)	
Total bilirubin (mg/dl)	0.37±0.6	0.54±0.08	0.39±0.04	0.26±0.03	0.30±0.04	0.23±0.02	0.21±0.02	0.29±0.01
Direct bilirubin (mg/dl)	0.29±0.05	0.31±0.08	0.19±0.03	0.11±0.01	0.13±0.01	0.11±0.07	0.10±0.01	0.14±0.01
Indirect bilirubin (mg/dl)	00.08±0.04	0.23±0.05	0.21±0.03	0.15±0.02	0.17±0.03	0.11±0.02	0.10±0.01	0.14±0.01
Creatinine (mg/dl)	1.13±0.04	0.39±0.09	1.06±0.03	1.04±0.04	1.02±0.03	1.06±0.074	0.93±0.05	1.03±0.02
Uric acid (mg/dl)	0.07±0.07	0.38±0.06	0.46±0.04	0.38±0.04	0.30±0.03	0.37±0.04	0.33±0.03	0.37±0.01
Glucose (mg/dl)	149.46±5.59	11.17±5.44	88±3.96	82.95±4.15	75±3.61	68.67±3.59	73.02±3.63	81.60±10.74
BUN (mg/dl)	19.08±1.25	14.67±1.25	14.96±0.65	19.22±0.98	17.2±0.88	16.72±0.67	14.68±0.73	16.10±0.31
Cholestrol (mg/dl)	38.92±4.02	56.28±5.86	32.47±1.88	27.02±2.31	31±2.28	29.35±2.04	35.41±2.36	33.57±2.36
Urea (mg/dl)	40.82±2.67	31.81±2.67	32.02±1.40	4.13±2.10	36.80±1.89	35.79±1.44	31.38±1.55	34.53±0.67
Triglceride (mg/dl)	56.46±5.33	46.67±5.71	42.46±2.43	28.24±2.07	28.65±1.89	22.40±1.87	24.64±1.62	30.85±0.96

*:(X±S.E); ** Number of anjmals.

Table 3. Correlation coefficients between age and non-electrolytes in Iranian dromedary camels

Parameter	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Indirect bilirubin (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)	Glucose (mg/dl)	BUN (mg/dl)	Urea (mg/dl)	Cholestrol (mg/dl)	Triglyceride (mg/dl)
Total bilirubin (mg/dl)	1.00	.6763**	.6086**	.1882**	.2915*	.2673**	.0743	.0869	.1700**	.2797**
Direct bilirubin (mg/dl)	.6763**	1.00	.1127	.1941**	.4474**	.2421**	.1076	.1159	.1880**	.3585**
Indirect bilirubin (mg/dl)	.6086**	.1127	1.00	.0776	.0497	.2696**	.0177	.0310	.3365	.1693**
creatinine (mg/dl)	.1882**	.1941*	.0776	1.00	.1950**	.1915**	.0112	.0086	.2246**	.1674*
Uric acid (mg/dl)	.2915**	.4474**	.0497	.1950**	1.00	.1729*	.1493*	.1468*	.0997	.3574**
Glucose (mg/dl)	.2673**	.2421**	.2696**	.1915**	.1729*	1.00	.1264	.1263	.002	.2818**
BUN (mg/dl)	.0743**	.1076	.0177	.0112	.01493*	.1264	1.00	.9926*	.2434*	.2237*
Urea (mg/dl)	.0869	.1159	.310	.0086	.1468*	.1263	.9926*	1.00	-.2352**	.2397**
Cholestrol (mg/dl)	.1700**	.1880**	.0065	.2246**	.997	.0002	-.2434**	-.2352**	1.00	1713**
Triglyceride (mg/dl)	.2979**	.3585**	.1693*	.1674*	.3574**	.2818**	.2237**	.2397**	.1713*	1.00
Age (Months)	-.3428**	-.3387**	-.2911**	-.3418--	-.2249**	-.4155**	-.1348*	-.1462*	-.0707	-.4918**

*P<0.01, **P<0.001.

Table 2. Mean * Values of blood serum non-electrolytes of male and female Iranian camels of different ages

Non-electrolytes	Age in months														Overall mean	
	<6		6-12		13-36		37-60		61-84		85-108		>109			
	M (8)**	F (5)	M (13)	F (5)	M (34)	F (17)	M (17)	F (24)	M (14)	F (37)	M (18)	F (25)	M (12)	F (88)	M (116)	F (201)
Total bilirubin (mg/dl)	0.33 ±0.07	0.45 ±0.11	0.49 ±0.08	0.66 ±0.22	0.28 ±0.04	0.62 ±0.7	0.33 ±0.06	0.22 ±0.02	0.37 ±0.07	0.28 ±0.04	0.23 ±0.03	0.22 ±0.03	0.24 ±0.05	0.21 ±0.02	0.31 ±0.02	0.27 ±0.02
Direct bilirubin (mg/dl)	0.23 ±0.04	0.38 ±0.11	0.26 ±0.7	0.42 ±0.19	0.18 ±0.03	0.20 ±0.05	0.12 ±0.02	0.11 ±0.01	0.17 ±0.03	0.11 ±0.01	0.13 ±0.03	0.10 ±0.01	0.10 ±0.01	0.10 ±0.01	0.17 ±0.02	0.13 ±0.01
Indirect bilirubin (mg/dl)	0.09 ±0.07	0.07 ±0.02	0.23 ±0.05	0.25 ±0.06	0.09 ±0.02	0.42 ±0.06	0.20 ±0.05	0.11 ±0.02	0.20 ±0.05	0.16 ±0.03	0.10 ±0.02	0.12 ±0.03	0.11 ±0.02	0.11 ±0.02	0.14 ±0.02	0.15 ±0.01
Creatinine (mg/dl)	1.14 ±0.05	1.11 ±0.5	1.39 ±0.10	1.35 ±0.16	1.6 ±0.03	0.97 ±0.05	1.14 ±0.05	0.96 ±0.05	1.21 ±0.04	0.95 ±0.04	1.14 ±0.05	1 ±0.05	1.09 ±0.14	0.94 ±0.05	1.17 ±0.02	0.95 ±0.02
Uric acid (mg/dl)	0.65 ±0.10	0.79 ±0.08	0.42 ±0.07	0.27 ±0.04	0.42 ±0.05	0.54 ±0.07	0.31 ±0.06	0.42 ±0.06	0.21 ±0.02	0.33 ±0.03	0.42 ±0.06	0.34 ±0.04	0.31 ±0.04	0.33 ±0.04	0.38 ±0.02	0.37 ±0.02
Glucose (mg/dl)	142 ±7.36	161.40 ±5.84	117.92 ±5.89	93.60 ±8.65	85.65 ±4.37	92.71 ±8.14	94.56 ±5.43	74.50 ±5.41	87 ±6.34	70.46 ±4.16	78.56 ±5.37	61.56 ±4.35	74.36 ±5.55	73.60 ±4.03	91.88 ±2.74	75.67 ±2.13
BUN (mg/dl)	18.75 ±0.77	19.60 ±3.22	16.46 ±1.33	10 ±1.58	14.03 ±0.78	16.82 ±1.08	17.76 ±1.89	20.25 ±0.99	18.86 ±1.51	16.57 ±1.07	18.39 ±1	15.52 ±0.84	12.50 ±1.28	15.25 ±0.80	16.23 ±0.51	15.97 ±0.40
Cholestrol (mg/dl)	39 ±5.65	38.80 ±6.03	59.92 ±7.52	46.80 ±7.28	35.85 ±2.18	25.71 ±3.06	27.76 ±2.02	26.50 ±3.60	27 ±2.02	32.51 ±3.02	26.61 ±2.33	31.32 ±3.05	37.27 ±4.63	35.36 ±2.73	35.14 ±1.59	32.67 ±1.17
Urea (mg/dl)	40.13 ±1.66	41.94 ±6.89	35.22 ±2.856	26.54 ±5.69	30.05 ±1.67	36 ±2.30	38.02 ±4.05	43.34 ±2.12	40.35 ±3.23	35.45 ±2.29	39.35 ±2.13	33.22 ±1.80	27.63 ±2.68	32.62 ±1.61	34.91 ±1.09	34.30 ±0.85
Triglyceride (mg/dl)	64.25 ±7.03	44 ±4.51	50.53 ±6.70	36.6 ±10.66	43.94 ±3.27	41 ±3.31	22.41 ±1.24	32.38 ±3.16	24 ±2.38	30.41 ±2.40	25.56 ±2.75	20.12 ±2.49	23.41 ±4.88	25.01 ±1.62	35.66 ±1.90	28.07 ±0.99

* X± S E; ** Number of animals; M male; F female.

of serum glucose variations due to lactation, feeding, season etc. in sheep and cattle were reported (Sykes and Field 1974, Tasker 1978) and these factors are also likely to operate in camels.

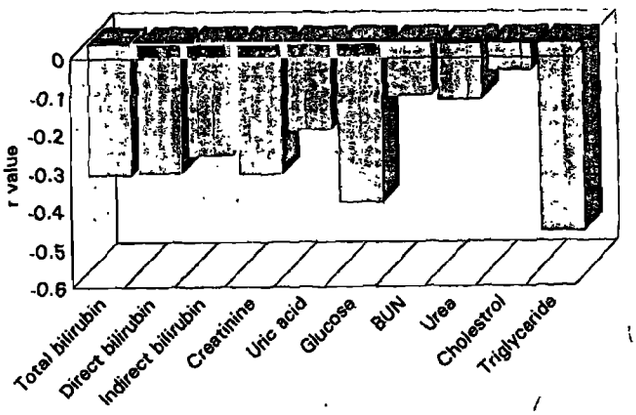


Fig. 1. Correlation coefficients between age and non-electrolytes in Iranian Dromedary camels.

The mean values of BUN and urea in camels regardless of age and sex, were similar to those reported by Kouider *et al.* (1988), Show *et al.* (1988) and Maghrabi (1993). Statistical analysis showed that there was no significant difference in the values of BUN and urea in males and females, regardless of age (Table 2). However, there was significant difference in male and female values in age groups 13 to 30 months, 85 to 108 months, and more than 109 months ($P<0.05$). The urea levels also showed a significant difference in the age group 6 to 12 months ($P<0.05$). Regression analysis showed that age affects the BUN and urea levels in camels ($P<0.001$) and with increase in age these 2 parameters decrease (Table 2; Fig 1).

The general mean values of serum cholesterol in males and females, regardless of age, did not show a significant difference (Table 2). However, there was a statistically significant difference in male and female values of the age group 13 to 36 months ($P<0.05$). The regression analysis showed that the age did not affect the serum cholesterol values in camel (Table 3). The general mean value of our study was similar to the values reported by Al-Ali *et al.* (1988) and Al-Ani *et al.* (1992).

The highest and lowest values of serum triglycerides, regardless of sex of animals, were observed in age groups of less than 6 months and 85 to 108 months respectively (Table 1). Regression analysis of data showed that age affected the triglyceride levels and that with increase in age the levels decreased significantly ($P<0.001$). It further showed that 24.1% of the variations in triglyceride levels were due to age (Table 3). There was a statistically significant difference in the values of serum triglycerides in males and females, regardless of age. The mean value of males (35.66±1.90) mg/dl) was higher than the females (28.07±0.99 mg/dl). References on triglyceride levels in camel are very scarce. Show *et al.* (1988) reported a mean value of 1.09±0.32 mmol/litre in 5 to 6-year-old camels which is at variance to the results of present study (30.85±0.96 mg/dl).

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Effect of supplementing urea molasses blocks in the ration of buffaloes on nutrient utilization and milk production

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ABSTRACT

Lactating buffaloes (25) in their mid lactation yielding 7-8 litres of milk were divided into 5 groups of 5 each and were allotted to 5 treatments, viz. control, and treatments T-1, T-2, T-3 and T-4. The buffaloes were offered wheat straw (W.S) *ad lib.* + green berseem + 3-4 kg concentrate mixture in control groups, while 20% of the concentrate mixture was replaced by urea molasses mineral block (UMB) containing cottonseed-cake, deoiled mustard-cake, guar-meal and deoiled groundnut-cake as varied degradable protein sources in T-1, T-2, T-3 and T-4 groups respectively. The voluntary DM intake (kg/100 kg BW) was recorded as 2.97, 2.95, 3.05, 2.88, and 3.04 in the control, T-1, T-2, T-3 and T-4 groups respectively. The curtailment of 20% concentrate mixture in T-1, T-2, T-3 and T-4 groups was well compensated by consuming 670, 653, 673 and 633 g of UMB respectively. The digestibility of various nutrients was similar in all the treatment groups. Throughout the experimental period of 84 days, all the lactating buffaloes maintained their body weights. The quality and quantity of milk produced remained unaffected in all the treatments due to the substitution of concentrate mixture with UMB. It is concluded that by supplementing UMB *ad lib.* along with wheat straw as basal roughage to the milch buffaloes yielding 7-8 litres of milk, in their mid-lactation, 20% of the concentrate mixture can be saved.

Key words : Buffaloes, Milk production, Nutrient utilization, UMB, Urea molasses blocks

Experiments and field trials reported in India, on urea molasses mineral block feeding, have shown good response on animal production in terms of milk yield and growth rate. In addition to this it reduced the cost of production by reducing concentrate requirement (Kunju 1986). The present study was undertaken to see the effect of supplementing traditional protein cakes through UMB to lactating buffaloes when wheat straw based rations are offered, on nutrient utilization and milk production in their mid-lactation.

MATERIALS AND METHODS

Animals

Lactating buffaloes (25) yielding 7-8 litres of milk in their mid-stage of lactation were divided into 5 groups on the basis of their body weight, lactation, number and milk yield. All the experimental animals were individually stall fed for 84 days as follows :

Control : Wheat straw *ad lib.* + 30 kg green berseem + concentrate mixture (Kearl 1982)

Treatment-1 (T-1) : Wheat straw *ad lib.* + 30 kg green berseem +20% less concentrate mixture + urea molasses mineral block (UMB) containing 15% cottonseed-cake (CSC)

Treatment-2 (T-2) : Wheat straw *ad lib.* + 30 kg green berseem + 20% less concentrate mixture + UMB containing 15% deoiled mustard-cake (MC)

Treatment-3 (T-3) : Wheat straw *ad lib.* + 30 kg green berseem + 20% less concentrate mixture + UMB containing 15% guar-meal (GM)

Treatment-4 (T-4) : Wheat straw *ad lib.* + 30 kg green berseem + 20% less concentrate mixture + UMB containing 15% groundnut-cake (GNC)

At the end of the experiment a digestion trial was conducted for 7 days. The milk samples were also collected for 3 consecutive days from each buffalo, and analysed for fat, protein, total solids and SNF. The concentrate mixture constituted 40 parts wheat, 27 parts deoiled rice polish, 30 parts groundnut-cake, 2 parts mineral mixture and 1 part common salt.

Preparation of UMB

Urea molasses mineral blocks (UMB) were prepared by boiling 1.5 kg urea alongwith 4.5 kg molasses in a iron con-

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tainer for 30 min. While this mixture was hot, 1.5 kg mineral mixture, 1.5 kg groundnut-cake and 1.0 kg salt was mixed in it thoroughly. Whole of this mixture was allowed to solidify at room temperature in the container itself. The individual blocks prepared by adding different cakes were offered to the animals in the containers itself throughout the experiment.

Table 1. Chemical composition (per cent) on DM basis of feed ingredients and urea molasses blocks (UMB) fed to lactating buffaloes

Particulars	Feed ingredients		
	Concentrate mixture	Wheat straw	Berseem
Organic matter	90.05	88.75	87.75
Dry matter	91.00	95.70	94.50
Crude protein	19.95	2.50	19.65
Crude fibre	8.05	40.05	21.10
Ether extract	4.10	2.05	2.35
Nitrogen-free extract	57.95	44.15	44.65
NDF	25.70	75.23	45.49
ADF	12.70	50.84	24.32
Hemicellulose	13.00	24.39	21.17
In-vitro OMD	86.90	44.70	69.30
GE Kcal/g diet	4.46	4.38	4.30

Urea molasses blocks (UMB)

Sl. No.	UMB	CP	CF	EE	Total ash	NFE	P % %	Ca	Cu ppm	Mn	Zn
1.	Block-1 CSC	42.5	2.50	0.95	24.46	29.59	2.97	5.77	9.93	46.15	217.95
2.	Block-2 MC	40.8	3.00	1.04	23.48	31.68	2.84	4.55	12.2	44.41	145.22
3.	Block-3 GM	45.4	2.55	1.15	21.45	29.45	2.19	4.12	9.22	42.43	171.09
4.	Block-4 GNC	48.3	1.75	1.05	20.24	28.56	3.15	567	10.3	62.09	233.58

Analysis

Samples of feed, faeces and the residue left for different proximate principles were analysed (AOAC 1985). The *in vitro* organic matter digestibility of different samples of concentrate mixture, wheat straw and berseem were determined periodically (Tilley and Terry 1963). The gross energy of the pooled samples of feed, faeces and residues left were determined. Milk fat was determined by Gerber's method. The cell wall constituents of different samples of feed were determined (Goering and VanSoest 1970). The statistical analysis of the data was done (Snedecor and Cochran 1968). The macro-and micro-elements in various UMB were determined using Atomic Absorption Spectrophotometer.

RESULTS AND DISCUSSION

Chemical composition

The chemical composition of concentrate mixture, berseem forage, wheat straw and various UMB is presented in Table 1. The *in-vitro* organic matter digestibility of berseem forage was 69.3%. In concentrate mixture and berseem forage CP was almost equal, whereas CP of various UMB'S were between 40.8 and 48.3%. CF content of the blocks was between 1.75 to 3.0%.

Apparent digestibility

The digestibilities of DM, OM, CP, CF and NFE were similar in all the experimental groups (Table 2) in comparison to control, indicating uniformity of various feed ingredients consumed by these buffaloes. Supplementing (600-700 g) UMB to these groups has added additional nitrogen and energy alongwith all the minerals required. About 250-300 g of CP was ingested from these blocks alongwith minerals. Chauhan *et al.* (1995) observed similar results.

Voluntary DM intake and body weight changes

The voluntary DM intake differed nonsignificantly among each other (Table 3). The corresponding values of intake g/day of various mineral blocks were 670, 653, 673 and 633 g, respectively, in the groups T_1 to T_4.

Similar results were observed in our laboratory when these blocks were offered to the lactating buffaloes in their early lactation with oat silage prepared at milk stage *ad lib.* as basal roughage (Chauhan *et al.* 1994). However, intake of these blocks was higher as compared to the blocks prepared by NDDB (Kunju 1986). Since these blocks were prepared in iron containers without the use of any binder, so this may be the reason of their higher intake.

Milk yield and composition

Milk yield and composition during 84 days milk trial in various treatment groups v/s control remained unaffected and the differences in overall milk yield during the mid-lactation period was statistically ($P>0.05$) nonsignificant (Table 3). As the digestibility of organic matter and other proximate principles was similar in these experimental groups and reduction of 20% concentrate mixture in the treatment groups was well compensated with the energy, nitrogen and minerals supplied

through these blocks, hence their milk yield remained almost

Table 2. Apparent digestibility (per cent) of various proximate principles

Particulars	Control	T-1	T-2	T-3	T-4
Dry matter	70.49	68.44	69.37	69.71	70.73
	±1.10	±1.28	±0.96	±0.90	±0.10
Organic matter	71.09	70.84	71.82	70.05	71.44
	±1.02	±1.40	±0.80	±0.90	±0.70
Crude protein	81.16	77.86	80.46	80.72	82.28
	±1.11	±0.70	±0.90	±1.20	±1.18
Crude fibre	67.73	65.88	66.64	65.59	66.80
	±1.20	±0.84	±0.88	±0.70	±1.30
Ether extract	82.32	81.85	84.95	82.75	84.08
	±0.07	±0.88	±0.90	±1.20	±0.11
Nitrogen-free extract	71.71	69.44	70.27	70.85	70.67
	±0.11	±1.20	±0.75	±0.65	±1.80
Energy	68.80	69.07	68.70	69.40	70.05
	±1.07	±0.80	±1.01	±0.85	±1.01

The results between various treatments were statistically ($P<0.05$) nonsignificant.

Table 3. Voluntary DM intake milk yield and composition in lactating buffaloes fed different UMB

Particulars	Control	T-1	T-2	T-3	T-4
Voluntary DM intake (kg/animal/day)					
Wheat straw (kg)	4.54± 0.25	4.70± 0.70	4.79± 0.64	4.270± 0.77	4.64± 0.80
Berseem hay (kg)	6.48	6.48	6.48	6.48	6.48
Concentrate mixture (kg)	3.43	2.83	3.07	2.90	3.43
UMB (kg)	0.00	0.670	0.653	0.673	0.633
Total DM intake (kg)	14.45	14.68	14.99	14.32	15.18
DM intake kg/100 kg BW	2.97± 0.20	2.95± 0.02	3.05± 0.11	2.88± 0.08	3.04± 0.02
Milk yield (kg/animal)					
Average milk yield (kg/animal/day)	6.90± 0.13	7.30± 0.09	7.00± 0.05	7.67± 0.11	7.20± 0.07
Total milk yield (kg) during 84 days/animal	579.60	613.20	588.00	644.28	604.80
Milk composition (%)					
Protein	4.08	4.18	3.99	4.08	3.95
Casein	3.30	3.18	3.19	3.33	3.28
Fat	7.55	7.90	7.08	7.77	7.62
Total solids	16.70	15.92	15.95	16.12	16.08
S N F	9.15	8.02	8.87	8.35	8.48

(a) Each value is the average of 6 fortnights analysis done in triplicate.

(b) Results between treatments remained statistically non-significant.

Table 4. Variable cost of milk production during 84 days milk trial per animal

Particulars	Control	T-1	T-2	T-3	T-4
Total feeds consumed (q/animal)					
Wheat straw	3.81	3.95	4.02	3.59	3.90
Concentrate	2.88	2.38	2.58	2.44	2.88
Berseem hay	5.44	5.44	5.44	5.44	5.44
UMB	--	0.560	0.548	0.565	0.532
Total cost in Rs (item-wise)/animal					
Labour charges for milking, cleanliness of sheds and animal, and preparations of UMB	700	700	700	700	700
UMB	--	112	110	113	106
Wheat straw	381	395	402	359	390
Concentrate mixture	864	714	774	732	864
Berseem	544	544	544	544	544
Miscellaneous charges for medicines etc.	200	200	200	200	200
Total cost (Rs)	2689	2665	2730	2648	2804
Total milk/animal during 84 days	579.60	613.20	588.00	644.30	604.80
Cost of 1 kg milk production	3.90	3.65	3.90	3.49	3.89

Cost of berseem hay, wheat straw, concentrate mixture and UMB per quintal were Rs 100, 100, 300 and 500, respectively.

similar. However, increasing trend in milk yield was observed due to the supplementation of partly degradable protein cakes through various mineral blocks.

Cost of milk production

The variable cost which includes feeding cost (Table 4) showed a reduction of 6-11% in cost of milk production due to the supplementation of these mineral blocks in T-1 and T-3 groups, respectively. Similar results were obtained in the early lactation study with these buffaloes in which about 5-8% of the cost of milk produced/animal was reduced.

It may be inferred from this study that by supplementing mineral blocks (UMB) *ad lib.* to the lactating buffaloes yielding 7-8 of milk in their mid-lactation 20% of the concentrate mixture can be curtailed without affecting milk yield, composition body weights and utilization of nutrients, when wheat straw is offered as basal roughage *ad lib.* alongwith 25-30 kg available green fodder.

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Effect of guineacorn/palm kernel-meal based diet with niacin supplementation on the performance of broilers

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ABSTRACT

Commercial broiler chicks, 350 day-old, were randomly assigned to duplicate floor pens with 25 chicks each. They were fed a guineacorn-palm kernel-meal based diet supplemented with 0.0, 7.5, 15.0, 22.5, 30.0, 37.5 and 45.0 mg niacin/kg feed for 42 days. Significantly poorer live-weight gain, feed intake and apparent utilization of nitrogen metabolizable energy, calcium and phosphorus, and a higher incidence of dermatitis and leg bone deformities in broilers on niacin-unsupplemented diets showed a deficiency of niacin in the basal diet which were all reversed with addition of 30.0 mg niacin/kg feed.

Key words : Broilers, Guineacorn, Palm kernal-meal, Niacin, Supplementation

High cost and inadequate supply of groundnut-cake have led to an increased use of palm kernel-meal in poultry diet. However, in palm kernel-meal-based rations the birds developed pellagra-like symptoms attributed to a deficiency of niacin in guineacorn-based diets (Filho *et al.* 1983) besides a modification of niacin requirement in palm kernel-meal-based diets (Fisher *et al.* 1955). This paper describes the effect of supplemental niacin to guineacorn-palm kernel meal-based diets with a view to improve broiler performance.

MATERIALS AND METHODS

Commercial broiler chicks 350 day-old were randomly assigned to 7 supplemental niacin groups (0.0, 7.5, 15.0, 22.5, 30.0, 37.5 and 45.0 mg/kg feed) each level with 2 replicates consisting of 25 birds each. Practical experimental diets based on guineacorn and palm kernel-meal were formulated (Table 1), analysed for niacin content by the method of Association of Vitamin Chemists, Inc. (1966) and then supplemented with graded levels of the vitamin. Chicks were raised in 14 floor pens, each of 5.5m^2 floor area and containing dry wood shavings litter, two 4-litre plastic drinkers, a through feeder and a 200-W tungsten filament lamp. Throughout the period of experimentation (1 to 42 days), the birds were offered the test diets *ad lib.*

Individual body weights, feed intake, per cent incidence of dermatitis and of leg-bone deformities (twisted legs, perosis, crippled) were recorded at weekly intervals during the 6 week test period.

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During the last 3 weeks of the experiment, a metabolic study was conducted on 4 replicate samples of experimental chicks randomly selected from each group at the beginning of the fourth week. Following the total collection procedure, excreta were collected daily from each treatment group on 14 successive days during the fifth and sixth week. Feed and excreta were analysed for nitrogen and phosphorus (AOAC 1980) and calcium (Perkin-Elmer Inc. 1973). Gross energy values were determined with a ballistic bomb calorimeter and apparent metabolizable energy values of diets were calculated. Apparent retention of nitrogen, phosphorus and calcium were calculated as the difference between the amount of the constituent in the diets and excreta samples collected.

At the end of the experiment, 4 replicate samples of birds were selected from each treatment group in the floor pens, starved for 6 hr weighed, slaughtered and dressed for evaluation of dressing percentage. The abdominal fat pad was carefully excised and weighed. Bones in the carcasses were carefully removed and the edible meat was separated. Total edible meat and total bone from each of the carcasses were weighed and the meat to bone ratio was calculated. Meat and bones were also expressed as percentage of carcass weights.

The data obtained were subjected to analysis of variance (Steel and Torrie 1960) and significantly different treatment means were compared using the multiple range test of Duncan (1955).

RESULTS

Feed utilization and health performance

Weight gain and feed intake were significantly affected by the dietary supplemental niacin but feed efficiency was not

(Table 2). Birds on up to 22.5 mg supplemental niacin/kg feed consumed significantly less feed, had poorer live-weight gain and weighed less at 42 days than those given higher supplemental niacin levels.

Appearance of dermatitis and leg deformities in the birds were also significantly influenced by the dietary treatments. In some of the affected birds, vision was impaired because the edges of the eyelids became granular and contracted. Also, there was a production of viscous exudate in the affected birds. Skin lesions of varying degree appeared around the corners of the mouth and the nostrils. Dermal lesions also occurred on the feet and toes with pronounced haemorrhagic fissures on the bottom of the feet to the extent that birds found it difficult to stand or walk. Other leg deformities observed included bowing and or twisting of leg bone and enlargement of tibiotarsal joints. All cases of skin lesions and leg deformities recorded developed within the 4th and 16th day of the feeding trial. Results presented in Table 2 niacin/kg indicated that supplemental 15.0 mg niacin/kg feed and 22.5 mg niacin/kg feed prevented incidence of dermatitis and leg deformities respectively.

Table 1. Composition of practical diet supplemented with graded levels of niacin

Constituent	Inclusion level (g/kg)
Guineacorn	505
Palm kernel-meal	200
Blood-meal	72
Fish-meal	52
Wheat offal	128
Oyster shell	10
Bone-meal	20
Vitamin/mineral premix*	1
Salt (NaCl)	2
Palm kernel oil	10
Chemical composition (analysed)	
Niacin (mg/kg)	14.8
Gross energy (Kcal/kg)	3037.9
Crude protein (%)	21.7
Calcium (%)	1.18
Phosphorus (%)	0.80
Tryptophan, calculated (%)	0.23

*Vitamin/mineral premix supplied the following vitamins and mineral elements per kg of feed : Vit. A, 1200 IU; vit. D, 2300 IU, Vit. E, 10 IU; menadione sodium bisulphite (vit. K), 1.5 mg; vitamin B_1 2.5 mg; vit. B_2 5 mg; choline chloride, 500 mg; calcium D-pantothenate, 10mg; vit. B_6, 4mg; vit. B_{12}, 0.02 mg; biotin, 0.2 mg; iron, 50 mg; manganese, 150 mg; copper 2.5 mg; zinc, 45 mg; cobalt, 0.2 mg; selenium, 0.08 mg; iodine, 1.4 mg.

Carcass characteristics

All the parameters of carcass characteristics (Table 2) except abdominal fat were markedly influenced by dietary supplemental niacin. Supplementation of niacin at 22.5 mg/kg diet seemed to be adequate for dressing percentage, both meat and bone expressed as per cent of carcass weight and meat : bone ratio, higher level of supplementation (i.e. 30.0 mg/kg diet) appeared to be needed for carcass weight, total edible meat, total bone weight and abdominal fat expressed as per cent of carcass weight.

Nutrient utilization

All the nutrients examined were significantly affected by dietary treatments, where supplemental 22.5 mg niacin/kg feed appeared to be adequate for metabolizable energy and calcium utilization (Table 3). However, this supplemental niacin level seemed marginal for nitrogen retention, but was inadequate for phosphorus utilization.

DISCUSSION

The niacin requirement of birds and/or levels of supplementation vary widely due to the types of ingredients used in diets, niacin bioavailability in diets and tryptophan content of diets. In this study, practical ration based on guineacorn and palm kernel-meal and containing 0.23% tryptophan (Table 1) constituted the test diet that was supplemented with graded levels of niacin. Feed utilization results indicated that a minimum of 30.0 mg added niacin/kg feed was needed for satisfactory feed consumption and growth rate. Czarnecki *et al.* (1983) and Waldroup *et al.* (1985) concluded that the birds required supplementary niacin ranging from 30-100 mg/kg. Working with purified diets, with adequate tryptophan levels, Childs *et al.* (1952), Patterson (1956) and NRC (1984) reported a lower niacin requirement of 18-33 mg/kg. The marked reduction in feed intake of birds fed diets supplemented with 0-22.5 mg niacin/kg might be due to reduction in free movement of the birds due to bowing and/or twisting of the leg bone and the dermal lesions developed in the feet.

Skin lesions and leg deformities characteristic of niacin deficiency in young chickens (Summers *et al.* 1984, Gries and Scott 1972, Cook *et al.* 1984, Leeson 1988) developed distinctly in birds given lower levels of the supplemental niacin. The results indicated that while 22.5mg niacin/kg was the minimum level needed to prevent incidence of dermatitis, a higher supplemental level of 30.0 mg niacin/kg was required for normal leg bone development.

Information is lacking on the niacin requirement for carcass characteristics such as those examined in this study excepting abdominal fat pad. The nonsignificant effect of supplemental niacin on abdominal fat pad *per se* is in agreement with the observation of Waldroup *et al.* (1984). However, the result of this study indicated the ability of higher levels of supplemental niacin (30.0-45.0 mg/kg) to significantly lower abdominal fat pad which is a desirable feature from the con-

Table 2. Growth, feed efficiency, health performance and carcass characteristics of broilers fed graded levels of niacin from 1-42 days of age

Parameter	Supplemental niacin (mg/kg feed) 0.0	7.5	15.0	22.5	30.0	37.5	45.0	±SEM**
			Feed utilization					
Live weight at 42 days (g)	1074.0b*	1120.8b	1150.1b	1183.3b	1540.9a	1549.1a	1528.0a	101.95
Live weight gain (g/bird-day)	24.6b	25.7b	26.4b	27.2b	35.7a	35.9a	35.4a	2.43
Feed intake (g/bird-day)	82.1b	84.8b	84.2b	91.7b	110.6a	112.1a	110.4a	6.23
Feed efficiency (g gain/g feed)	0.30	0.30	0.30	0.30	0.32	0.32	0.32	4.95E-03
			Health performance					
Incidence of dermatitis (%)	8a	4b	0c	0c	0c	0c	0c	1.46
Incidence of leg deformities (%)	6a	2b	2b	0c	0c	0c	0c	1.03
			Carcass characteristics					
Carcass weight (g)	654.1c*	687.1c	694.7c	824.8b	1081.7a	1072.0a	1051.3a	90.88
Dressing percentage	60.9b	61.3b	60.4b	69.7a	70.2a	69.2a	68.8a	2.14
Abdominal fat (g)	23.8	24.9	24.09	25.2	24.6	25.0	24.8	0.24
Abdominal fat (% carcass weight)	3.6a	3.6a	3.5a	3.1a	2.3b	2.3b	2.4b	0.89
Total edible meat (g)	412.7c	442.5c	442.5c	570.8b	744.2a	739.7a	719.1a	70.12
Meat (%carcass weight)	63.1b	64.4b	63.7b	69.2a	68.8a	69.0a	68.4a	1.28
Total bone (g)	217.6b	219.7b	228.2b	228.8b	312.9a	307.3a	307.4a	21.28
Bone (% carcass weight)	33.3a	32.0a	32.8a	27.7b	28.9b	28.7b	29.2b	1.05
Meat : bone ratio	1.90b	2.01b	1.94b	2.49a	2.38a	2.41a	2.34a	0.12

*Mean values in a row followed by different subscripts are significantly different at P<0.05.

**SEM, standard error of the mean.

Table 3. Effect of supplemental niacin on apparent nitrogen, calcium and phosphorus retention and metabolizable energy values

Supplemental niacin (mg/kg feed)	Nitrogen retention (%)	Calcium retention (%)	Phosphorus retention (%)	Metabolizable energy (Kcal/kg)
0.0	54.1b*	65.2b	53.2c	2732.0b
7.5	52.6b	64.0b	52.6c	2770.6b
15.0	53.7b	66.1b	54.8c	2752.3b
22.5	60.4ab	72.6a	68.2b	2816.1a
30.0	67.6a	74.2a	73.5a	2858.7a
37.5	68.2a	73.4a	74.1a	2846.5a
45.0	68.4a	74.5a	72.7a	2849.6a
±SEM**	3.39	2.16	4.69	23.90

*Mean values in column followed by different subscripts are significantly different at P<0.05.

**SEM, Standard error of the mean.

sumers' and processors' points of view. Based on all considerations, it appeared that a minimum of 30.0 mg niacin/kg supplemented to feed was needed for good carcass characteristics.

The physiological function of niacin in chickens not only affects energy metabolism but also utilization of other nutrients as well (Lockhart *et al.* 1966). Poor utilization of calcium and phosphorus, which are required for normal bone formation and egg production was recorded in chicken deficient in niacin (LONZA 1984). In support of earlier reports, marked improvement in apparent utilization of nitrogen, metabolizable energy, calcium and phosphorus was observed in experimental birds at higher levels of supplemental niacin. The results also tended to suggest that a supplement of 30.0 mg niacin/kg was needed for optimum utilization of the nutrients.

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Effect of dietary supplementation of different strains of *Streptococcus lactis* on the biological performance of broilers

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ABSTRACT

The dietary supplementations (6.9×10^8 cell/kg feed) of 4 strains (3 from HPKV, Palampur and 1 from CFTRI, Mysore) of *Streptococcus lactis* were undertaken to study the biological performance of *Starbro* broilers up to 6 weeks of age. S1 strain procured from the CFTRI, Mysore, was the most effective because it showed significantly ($P<0.05$) higher broiler, live-weight gain coupled with efficient feed efficiency ratio, and minimum chick mortality during both starter and finisher phases,. There were also nonsignificant variations in dressing percentage, duodenal pH and duodenal thickness with this strain as compared to other treatments including control.

Key words : Biological performance, Broiler, Probiotics diet, *Streptococcus lactis* strains

Probiotic preparations may consist of a single strain or more and even up to 8 strains and the multiple strains are active against a wider range of conditions and in a wider range of animal species (Fuller 1988). On the basis of this information, the present investigation was undertaken to study the biological effect of *Streptococcus lactis* from different sources on the performance of broilers up to 6 weeks of age.

MATERIALS AND METHODS

One-week-old, 240 commercial broiler chicks were distributed at random for 6 treatment in 12 compartments of thermostatically controlled battery brooders having 40 chicks treatment with 2 replicates of 20 chicks in each with comparable mean body weights. The details of the treatments including the sources of *S. lactis* and their concentration are given in Table 1.

Bacterial cultures (1.0 ml) 6.9×10^8 cells/ml was diluted with 100 ml distilled water and mixed with 1 kg feed of particular treatment before feeding. Everyday fresh mixing of the culture was done. The standard management practices were followed for rearing the chicks in the battery brooders up to 4 weeks of age (starter phase) and thereafter on deep litter up to 6 weeks of age (finisher phase). Feed and water were provided *ad lib.* During the respective starter and finisher phases, the experimental stock was fed starter and finisher diets (Table 2) respectively.

Table 1. Experimental plan

Treatment	Treatment code	Microbial conc. per g of feed
Control	S0 --	--
Culture medium	S0 (M) --	--
S. lactis	S1 CFTRI, Mysore	6.9×10^5
S. lactis	S2 HPKV, Palampur	6.9×10^5
S. lactis	S3 HPKV, Palampur	6.9×10^5
S. lactis	S4 HPKV, Palampur	6.9×10^5

The chicks were weighed at weekly intervals and gain in weight (GIW), feed consumption and feed efficiency ratio (FER) were calculated. At the end of sixth week of age, all the experimental broilers were sacrificed to record the dressing percentage and pH of the different parts of gastrointestinal tract and duodenal thickness. The pH of the gastrointestinal contents was recorded by dissolving 5 g ingesta each from crop and duodenum, in 100 ml of hot distilled water and bringing the temperature to about 20°C. The proximate composition, calcium (AOAC 1985), phosphorus (Parks and Dunn 1963) and fibre composition (Goering and Van Soest 1970) were determined in feeds and droppings.

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RESULTS AND DISCUSSION

Growth response, feed efficiency ratio and mortality

During the starter phase (Table 3) significantly higher live-weight gain (GIW) was recorded in chicks fed diet containing *S. lactis* HPKV S3 strain as compared to both the controls. The differences in weight gains due to CFTRI S1 or HPKV S2 and HPKV S3 strains were not significant, which showed that these were comparable in growth promotions in starters. The feed efficiency ratios (FER) were significantly ($P<0.05$) better in HPKV S1 and HPKV S2 strains as compared to controls as well as other treatments. The mortality % was 3.0, 3.0 and 11.7 under S2, S3 and control treatments, respectively, while nil in other treatments. The low mortality in treatment groups may be due to the production of their antibiotic metabolites like nicin and diplococcin (Fuller 1988), because *Streptococcus faecium* suppress the *in-vitro* growth of hemolytic *E. coli* (Krecov and Pujic 1975) and decreased its caecal contents count by 50% (Kumprecht *et al.* 1994). These results indicated that CFTRI S1 and HPKV S3 strains are promising in producing higher weight gains and better feed efficiency than the control because of their antibiotic metabolites.

During the finisher phase (Table 3), the highest gain in live-weight was recorded with the CFTRI S1 strain are comparable to all other strains and the control. Feed efficiency ratios were comparable in groups of broilers fed control diet and diets supplemented with CFTRI S1, HPKV S2 and HPKV S4 strains and were also significantly ($P<0.05$) better than those of culture medium (SOM) and HPKV S3 strain fed groups. The poorest FER of HPKV S3 strain was due to the higher feed intake. The significant differences in the FER's of control and culture medium fed groups of broilers may be due to the biological fluctuations of the stock. These results thus showed that CFTRI S1 strain was biologically superior and maintained its superiority right from the starter phase over its other counterparts as well as controls apparently due to its more effective antibiotic metabolites and growth stimulatory effects.

Table 2. Composition of experimental diets

Ingredients (%)	Broiler starter mash	Broiler finisher mash
Maize	22.5	30.0
Wheat	22.5	28.0
Groundnut-cake	5.0	5.0
Sunflower-cake	5.0	2.5
Sesame-cake	5.0	2.5
Soyaflakes	25.0	19.0
Fish-meal	5.0	5.0
Molasses	6.0	4.0
*Premix	4.0	4.0

* i. Intramix : 1.5 g (Vit. A, 5.0 lac. D3, 1.0 lac I/g)
ii Rovibee : 25 g (B1, 4 mg, niacin, 60 mg, B6, 8 mg, B12 40 microgram, E, 40 mg, calcium pantothenate 40 mg/g).
iii Choline chloride (50%0 : 50 g.
iv Trace minerals : 100 g (ferrous oxide 2 g, dicalcium phosphate 54 g, copper sulphate 2 g, manganese sulphate 25 g, zinc sulphate 6 g, ferrous sulphate 10 g, potassium iodide+sodium thiosulphate 250+750 mg)
v Marble powder : 2.000 kg
vi Dicalcium phosphate : 1.75 kg.

27.5 g maize flour was used as a carrier

The overall broiler growth (one to 6 weeks of age) performance (Table 3) under different treatments showed the highest gain in weight ($P<0.05$) with the CFTRI S1 strain which was 5.81 and 4.64% higher than those of control (SO) and culture medium (SOM) fed groups respectively. The feed efficiency ratio in the broilers group fed this strain was matching to those of control (SO), HPKV S2 and HPKV S4 strains fed groups because the differences amongst them were nonsignificant but in comparison to other treatments their

Table 3. Gain in weight, feed efficiency ratio, mortality per cent of experimental broilers

Treatment	Starter phase			Finisher phase			Overall		
	GIW (g)	*FER	Mortality (%)	GIW (g)	*FER	Mortality (%)	GIW (g)	*FER	Mortality (%)
Control	$514.9^{c}\pm1.7$	45.4^{c}	11.7	$664.4^{b}\pm4.8$	35.0^{a}	17.6	$1179.4^{b}\pm6.6$	39.0^{a}	29.3
Culture medium	$530.2^{b}\pm16.7$	45.5^{c}	--	$662.3^{b}\pm1.3$	34.4^{b}	11.8	$1192.5^{b}\pm18.0$	37.9^{b}	11.8
CFTRI S1	$534.9^{ab}\pm7.2$	47.5^{ab}	--	$713.0^{a}\pm18.3$	34.8^{a}	2.9	$1247.9^{a}\pm25.6$	39.3^{a}	2.9
HPKV S2	$537.7^{ab}\pm8.8$	48.6^{a}	3.0	$668.5^{b}\pm13.0$	34.6^{a}	5.9	$1208.2^{b}\pm21.8$	39.7^{a}	8.9
HPKV S3	$546.6^{a}\pm3.6$	47.0^{b}	3.0	$589.7^{d}\pm3.4$	31.7^{c}	2.9	$1136.4^{c}\pm7.0$	37.6^{b}	5.7
HPKV S4	$491.3^{d}\pm3.8$	44.9^{c}	--	$642.8^{c}\pm3.7$	35.3^{a}	19.3	$1134.2^{c}\pm9.6$	38.9^{a}	19.3

Figures bearing different superscripts among columns are significantly ($P<0.05$) different among each other.

$$\text{*FER} : \frac{\text{Gain in weight (g)}}{\text{Feed intake (g)}} \times 100$$

Table 4. Digestibility and balance of nutrients in broiler chicks fed diets supplemented with microbes

Particulars	Treatment					
	Control	Culture medium	CFTRI S1	HPKV S2	HPKV S3	HPKV S4
Digestibility coefficients (%)						
DM	65.5^{b}±2.1	46.5^{ab}±0.8	67.9^{a}±1.0	67.1^{a}±1.6	67.2^{a}±0.5	62.7^{c}±1.1
CP	42.9^{b}±0.8	42.5^{b}±5.4	44.1^{a}±0.7	41.1^{c}0.3	39.7^{c}±2.1	42.9^{b}±2.9
EE	72.1^{c}±1.1	76.7^{a}±2.2	76.7^{a}±3.1	76.8^{a}±0.4	72.3^{c}±4.1	75.9^{b}±3.0
ADF	70.7^{b}±3.0	66.9^{c}±5.0	75.8^{a}±5.0	67.7^{c}±6.5	66.9^{c}±1.2	63.0^{a}±3.3
NDF	79.9^{b}±0.3	80.6^{a}±2.5	83.7^{a}±0.4	78.5^{b}±1.5	77.9^{b}±3.9	81.1^{a}±1.1
Cellulose	46.1^{a}±0.3	42.8^{b}±3.5	48.9^{a}±1.0	40.1^{b}±5.6	42.8^{b}±7.1	42.7^{a}±1.8
Hemicellulose	77.5^{b}±2.2	77.3^{b}±1.8	79.0^{a}±3.5	77.1^{b}±5.9	82.4^{a}±2.3	81.5^{a}±4.9
Balance of nutrients (% of intake)						
N	55.3^{b}±1.5	54.9^{b}±1.1	57.5^{a}±1.4	57.4^{a}±0.1	56.8^{a}±1.8	54.5^{b}±3.1
Ca	36.0^{a}±1.4	32.3^{b}±2.6	36.9^{a}±1.5	31.2^{b}±1.0	28.2^{c}±1.0	33.9^{b}±2.1
P	65.9^{a}±72.1	63.4^{b}±1.8	65.5^{a}±2.2	61.5^{b}±1.4	60.6^{c}±1.4	62.9^{b}±2.1

Figures bearing different superscripts among rows are significantly (P<0.05) different from each other.

Table 5. Dressing percentage and gut pH of the broilers under different treatments

Treatment	Dressing (%)	Crop pH	Duodenal pH	Duodenal thickness (nm)
Control	63.9±0.9	5.55^{a}±0.21	6.00±0.28	0.056±0.05
Culture medium	62.2±0.7	5.27^{a}±0.60	6.22±0.10	0.062±0.00
CFTRI S1	62.9±0.4	5.25^{a}±0.21	6.40±0.00	0.053±0.00
HPKV S2	64.9±0.0	4.87^{b}±0.10	6.15±0.21	0.061±0.00
HPKV S3	64.9±0.5	5.25^{a}±0.21	6.10±0.14	0.049±0.00
HPKV S4	64.6±0.8	4.90^{b}±0.07	6.30±0.14	0.046±0.00

Figures bearing different superscripts among columns are significantly (P<0.05) different from each other.

differences were statistically significant (P<0.05). There was also lowest (2.9%) mortality in the group of broilers fed this strain. In control group the highest mortality (29.3%) may be due to the establishment of pathogenic microbes because no antibiotics was fed to the stock during the experiment.

Digestibility of nutrients

The differences in the digestibility of various nutrients excepting hemicellulose (Table 4) during the end of starter phase were statistically (P<0.05) but conspicuously higher in CFTRI S1 strain fed group of broilers as compared to that of control group (SO). It may be because of the stimulatory effect of this strain to boost the carboxymethyl cellulase activity in the gut of the broilers leading to significantly (P<0.05) highest digestibilities of ADF and NDF fractions of diet, as the supplementation of *Streptococcus faecium* C-68 to the broiler diets increased the activity of this enzyme in caecal contents (Kumprecht *et al.* 1994). The positive balance of nitrogen (Table 4) was also statistically (P<0.05) higher in CFTRI S1 treatment as compared to the control groups while Ca and P retentions were almost same as those of controls. These results correlate positively with the better growth performance of CFTRI S1 strain treatment as compared to its other counterparts.

Dressing percentage and gut pH

The differences for the dressing percentage (with skins) among different treatments were statistically nonsignificant (Table 5). Significantly (P<0.05) lower crop pH was recorded in HPKV S2 and HPKV S4 strains fed treatments as compared to all other treatments including controls. However, lower pH with probiotics administration was reported by Lyons (1987). The differences in the duodenal pH and duodenal thickness amongst different treatments were also statistically nonsignificant.

The perusal of the overall results indicate that S1 strain procured from the CFTRI, Mysore, was the most promising out of the 4 strains tried for better broiler production because of significantly (P<0.05) higher broiler live-weight gains coupled with efficient FER, minimum chick mortality and nonsignificant variations with respect to dressing percentage, gut pH and duodenal thickness as compared to all other treatments including control.

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Comparative performance and draughtability of buffaloes and cows during summer and winter

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ABSTRACT

To determine suitability of the female buffaloes and cows for work, 10 crossbred cows and 10 female buffaloes, which included 5 dry non-pregnant (DNP) and 5 lactating non-pregnant (LNP), were used in pairs for ploughing during summer and winter. The ploughing was carried out in loam soil with a wooden plough and traditional double neck yoke. The average width and depth of furrow cut by plough was 18 and 21 cm respectively. The speed of cows was more than buffaloes. The average working time was longer during winter than summer in both the species. The average horse power (hp) developed by cows was 0.54±0.05 and 0.52±0.12 and by buffaloes 0.47±0.04 and 0.47±0.12 during summer and winter respectively. The study concluded that buffaloes and cows may be used for light to moderate work without any adverse effect on body functions.

Key words : Buffaloes, Cows, Draughtability, Seasonal effect on draughtability

The role of draught animal power (DAP) on Indian farms is decreasing due to partial replacement of animal power by electrical motors and petroleum run vehicles. The scarcity in the power for agriculture has been highlighted and greater gaps in demand and supply indicated (Moulik *et al.* 1991). At present the contribution of females to farm power is about 6.25% in Indian Agriculture (Sharma 1982). The traditional animal husbandry and agriculture system is being replaced by more intensive scientific animal husbandry practices and this has made more impact on the females. Due to economic considerations and scarcity of feed and fodders, low producing farm animals have been replaced by more profitable animals. In this process the less productive females particularly dry non-pregnant (DNP) and lactating non-pregnant (LNP) animals are ignored. To examine possibility of use of non-productive animals both buffaloes and cows (DNP and LNP) were subjected to ploughing work and their work capacity was measured.

MATERIALS AND METHODS

Apparently healthy crossbred cows (10) and female buffaloes (10) were used for the experiment, and among these 5 animals, were dry non-pregnant (DNP) and other 5 were lactating non-pregnant (LNP). The experiment was carried out on both groups during summer and winter. The climatic elements i.e. minimum/maximum temperature was (7.47°-20.9°C) during winter and during summer (21.3°-36.3°C). The relative humidity (RH %) was 92/67 during summer and winter respectively. All the experimental animals were maintained under uniform conditions of feeding and management and they were given concentrate mixture. DNP animals were given concentrate @ 2 kg/day and lactating animals were given 1 kg of concentrate/2.5 kg milk production and maintenance ration was also given. The composition of concentrate mixture was : groundnut-cake 35%, barley/maize, 2.8%, wheat bran 34%, mineral mixture 2%; and common salt 1%.

The animals were trained for 2-3 months before actual ploughing work was carried out. The ploughing was carried out in loam soil with moisture content 20-30% (24.82±5.1). Animals were subjected to work for 2 hr continuously or till fatigue onset whichever was earlier. Work was discontinued if either of animals exhibited fatigue symptom. Physiological reactions, viz. respiration rate (flank movements), rectal temperature (clinical thermometer) and pulse rate (coccygeal artery) were recorded and fatigue was assessed (Upadhyay and Madan 1985). A 2 hr rest was given to animals and recovery was monitored. After the rest animals were again subjected to ploughing work. The speed of ploughing was measured on a speedometer aligned with the plough (Fig. 1). The horse power was calculated from the corrected draught (kg) and angle of pull. The data were subjected to statistical analysis for finding out variations due to different effect (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

The variation observed in speed of work was primarily due to differences in body size, breed, and moisture content of soil. The speed of work (Tables 1, 2) decrease as the duration

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Table 1. Power output of DNP and LNP buffaloes during summer and winter seasons

Parameters	Animals I	II	III	IV	V	Average
Summer (dry non-pregnant)						
Cos Ø	0.90	0.89	0.88	0.90	0.90	0.89±0.01
Correct draught (kg)	22.6	22.3	22.1	22.4	22.4	22.4±0.01
Working time (min)	53.0	45.0	50.0	53.0	58.0	52.0±0.02
Area ploughed (m^2/hr)	420	331	453	407	570	436±50
Speed (m/min)	54	43	59	54	50	52±3.6
HP output	0.59	0.41	0.55	0.40	0.49	0.49±0.04
Winter						
Working time (min)	50.0	50.0	49.0	51.0	51.0	50.0±0.04
Area ploughed (sqm/hr)	342	370	325	298	370	341±13
Speed (m/min)	51	51	42	42	51	47±2.0
HP output	0.48	0.49	0.40	0.37	0.48	0.44±0.22
Summer (lactating non-pregnant)						
Cos Ø	0.88	0.90	0.88	0.90	0.88	0.89±0.01
Corrected draught (kg)	22.2	22.6	22.0	22.4	22.1	22.3±0.1
Working time (min)	53.0	43.0	45.0	43.0	45.0	46.0±2.0
Area ploughed (m^2/hr)	363	355	335	360	453	373±20
Speed (m/min)	51	50	48	45	50	49±0.01
HP output	0.39	0.45	0.40	0.45	0.55	0.45±0.03
Winter						
Working time (min)	58.0	61.0	58.0	61.0	61.0	60.0±0.1
Area ploughed (m^2/hr)	445	387	437	387	387	408±13
Speed (m/min)	53	47	53	47	47	49±1.0
HP output	0.52	0.48	0.50	0.47	0.48	0.49±0.01

tion of work prolonged. The decrease in speed may be also due to decrease in work strength and fatigue onset. Decrease in speed due to various effects has also been reported by Maurya and Devadattam (1982) in crossbred bullocks. Upadhyay (1982) also observed a decrease in the speed of animals at work in relation to the time and duration of continuous work during different seasons.

The horse power developed by crossbred cows (Table 2) was comparatively more than buffaloes (Table 1). The horse power developed by the females during present study was higher than values reported by Bhosrekar and Mangurkar (1990) in crossbred bullocks and non-descript animals. The present study was carried out on female cows and buffaloes and parallel information on such animals is scanty therefore direct comparsions could not be made. The horse power developed by DNP and LNP buffaloes and cows during summer and winter are also in close agreement with the values reported by Maurya and Devadattam (1982) for bullocks. The horse power developed by crossbred and Hariana bullocks under various working conditions has been reported to be 0.5 to 0.9 HP. Horse power developed by DNP and LNP animals varied significantly ($P<0.05$) without any seasonal influence (Upadhyay 1982).

The area covered during ploughing was more by cows (Table 2) than buffaloes (Table 1) during summer and winter. These values are comparable with the values reported by

Table 2. Power output of DNP and LNP cows during summer and winter

Parameters	Animals I	II	III	IV	V	Average
Summer (dry non-pregnant)						
Cos Ø	0.90	0.90	0.89	0.90	0.89	0.90±0.01
Corrected draught (kg)	22.5	22.1	22.3	22.6	22.3	22.4±0.08
Working time (min)	23.0	34.0	29.0	30.0	29.0	29.0±0.03
Area ploughed (m^2/hr)	481	661	730	543	500	483±47
Speed (m/min)	50	60	74	43	58	57±5.0
HP output	0.61	0.60	0.69	0.49	0.44	0.57±0.04
Winter						
Working time (min)	54.0	54.0	58.0	58.0	58.0	56.0±0.01
Area ploughed (m^2/hr)	431	431	360	360	430	403±18
Speed (m/min)	54	54	44	44	54	50±2.2
HP output	0.53	0.52	0.44	0.44	0.53	0.49±0.21
Summer (lactating non-pregnant)						
Cos Ø	0.90	0.90	0.91	0.84	0.90	0.90±0.01
Corrected draught (kg)	22.4	22.6	22.8	22.1	22.6	22.5±0.1
Working time (min)	26.0	34.0	38.0	31.0	33.0	32.0±0.02
Area ploughed (m^2/hr)	570	325	492	462	286	427±53
Speed (m/min)	58	42	53	52	39	49±3.5
HP output	0.70	0.40	0.53	0.56	0.38	0.51±0.1
Winter						
Working time (min)	48.0	45.0	53.0	45.0	53.0	49.0±0.02
Area ploughed (m^2/hr)	442	505	403	442	487	456±18
Speed (m/min)	51	66	50	51	66	51±3.8
HP output	0.55	0.62	0.50	0.57	0.50	0.55±0.02

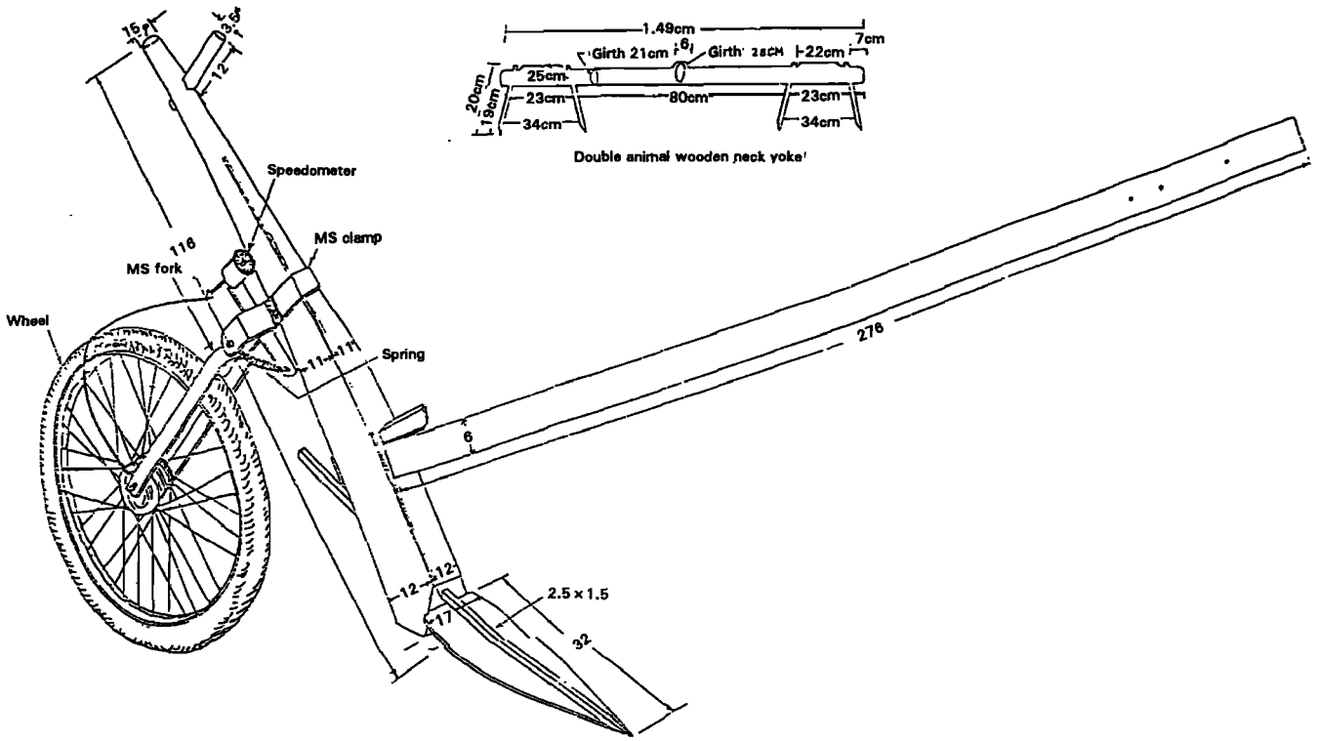


Fig. 1. Wooden plough aligned with speedometer wheel (all dimensions in cm).

Bhosrekar and Mangurkar (1990) in crossbred and local non-descript bullocks. The area ploughed by these animals differed significantly ($P<0.01$) between the species and interaction of season × group.

During winter the physiological reactions i.e. respiration rate, pulse rate and rectal temperature were lower than summer. The recovery in the physiological parameters was faster during winter than summer. The physiological reactions remained at a higher level even after 2 hr of rest. The rise in rectal temperature with progression of work was more during summer and affected the work capacity of the animals. This could be due to higher heat load and their limited sweating capacity and less number of sweat glands per unit area of skin. The animals were unable to loose heat of work which affected the duration of work performed and animals fatigued at the end of work.

Average milk yield of buffaloes were 4.6±0.4 and 4.2±0.3 kg/day during summer and winter respectively, and the respective values for cows were 5.7±0.5 and 4.2±0.5 kg/day. During work the milk yield decreased in all the animals and did not reach to prework level even 2 weeks of rest after work. Rizwan-ul-Muqtadir *et al.* (1975) also reported decline in the milk yield of working animals. In this study the drop in the milk yield was attributed to a combined effect of environmental temperature, stage of lactation and work.

Female of bovines because of their physiological and configurational differences do not have capacity to sustain hard work for longer duration on their counter part males have and therefore are not able to exert fully for work, but feeding improve their capacity for sustained work. The present investigation revealed that females of cattle and buffaloes may be used for moderate work and during early hours of the day during summer. Females are able to produce a maximum pull of about of 8-10% of their body weight on sustained basis, which may be utilized for optimizing production for efficient utilization under Indian farming system wherever farm power is deficient.

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Exercise induced changes in cutaneous and pulmonary water loss in buffaloes

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ABSTRACT

Skin and pulmonary evaporative losses and the changes induced due to exercise in hot humid conditions were measured in young buffaloes. Water loss from skin and pulmonary system at rest was 492±57.90 m^{-2} hr^{-1} and 72.43 ±6.18 g m^{-2} hr^{-1} respectively. The corresponding increase after 1 hr exercise on treadmill was 54% and 60%. Evaporative losses increase with exercise. The exercise increased evaporative losses and loss through the skin remained higher than respiratory water loss. Sweating in buffaloes was more closely controlled by skin temperature than by ambient temperature. Tidal volume, dead space ventilation and RQ decreased after exercise and pulmonary ventilation, volume of oxygen consumed and alveolar ventilation increased after 1 hr of exercise.

Key words : Buffaloes, Cutaneous water loss, Exercise, Pulmonary water loss, Water loss

Buffaloes in summer maintain thermal equilibrium chiefly by instinctive behavioural preference for wallowing (Mullick 1964). The number of sweat glands in buffaloes is 160/cm^2 (Nair and Benzamin 1963) and the size of glands (0.247 cm^2) in buffalo is about twice the size of cattle (0.124 cm^2) and glandular surface, 1.07 in buffaloes is 1/3rd of cattle (Hafez 1955). Skin thickness being 6.5-6.8 mm in adult males help in reducing the effects of thermal radiation and minimize water loss (Cockrill 1974). The understanding on the subject of thermoregulation and the role of skin in evaporative heat loss mechanism is precisely not known. In this study therefore an attempt was made to measure skin and pulmonary evaporative losses in Murrah buffaloes during treadmill exercise in summer.

MATERIALS AND METHODS

Apparently healthy Murrah buffalo males (7) with a mean body weight of 220±5 kg were selected for treadmill experiments. During the experimental period the animals were maintained on green fodder (berseem) and wheat *bhusa ad lib.* except during exercise on treadmill. Animals were exercised on a treadmill when ambient conditions were hot humid (Table 1). Each animal before subjecting to actual experimental protocol of treadmill run was trained for walking on the treadmill and to the speed of treadmill, i.e. 5 km/hr on the day of experiment, individual animal was observed for pre-work resting values after a rest period of 20-30 min. After recording resting values in standing position animals were subjected to exercise at a speed of 5 km/hr continuously for 1 hr till a mechanical break was applied to stop treadmill system.

Rectal temperature (clinical thermometer) and respiration rate (flank movement) and heart rate was calculated from ECG recorder (Updhyay and Madan 1985). Ventilation volume and tidal volume were measured on a spirometer (Upadhyay and Madan 1985). Evaporative loss from pulmonary system was recorded by ventilated mask method and skin evaporative loss using a ventilated capsule, with YSI thermistors attached to record DBT and WBT of ingoing and outgoing air and losses were calculated (McLean 1963).

Body surface area (m^2) of buffaloes was calculated from body weight of (kg) animals $SA=0.15\ W^{0.56}$ (Brody 1945). The temperature humidity index (THI) was calculated using the formula recorded by McDowell (1972).

The expired air was analysed for alveolar oxygen and CO_2 by OM-11 and LB-2 analysers. The respiratory alveolar and dead space ventilation of individual animal was calculated as per the method outlined in "Handbook of Respiration." RQ was calculated from expired air collected (Yousef 1984) and metabolic heat production was calculated assuming 1 lit of O_2 = 20.46 Cal (McLean 1972).

Thermal balance was calculated on the basis of total evaporation from sweating and respiration (E_t), metabolic heat production (M), and heat storage (S_t) and then rate of environmental heat gain at the skin (Hs) was calculated Hs (W/ma^2) = E_t-M+S_t (Virginia Finch *et al.* 1984).

Heat storage was estimated from changes in rectal and mean skin temperature at the beginning and end of 1 hr tread-

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mill exercise, in the ratio 0.8 : 0.2 (Burton and Edholm 1955). An estimate of heat storage was made by multiplying the weighed changes in temperatures for 1 hr period by the specific heat of tissues (3.47 KJ $Kg^{-1}°C$) and the weight of the animal (kg). The components of the thermal balance were expressed per unit surface area (Virginia Finch 1985).

The results were analysed statistically using t test. (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

Pre-exercise values for rectal temperature, respiration rate and heart rate after 1 hr of treadmill exercise increased (Table 2). The skin temperature increased significantly ($P<0.01$). Relationship of air temperature with rectal temperature and skin temperature has been presented in Fig. 1.

At prework mean skin water loss from skin increased due to treadmill exercise ($P<0.01$). Thereby indicating that skin evaporative losses increased by more than 50% due to exercise. The pulmonary water loss increased after exercise. Increase in pulmonary water loss was 60% due to exercise effect. This indicates that water loss through both these channels increased proportionately.

Heat stored (H_s) during exposure to solar radiation was positive, i.e. net heat exchange was inward from the environment to the skin during present experiment. After 1 hr treadmill exercise H_s was 2646.34±274.22 W/m^2 (Table 3).

Tidal volume decreased ($P<0.01$) after exercise (Table 2). The decrease in tidal volume is generally considered to limit the increased ventilation on the respiratory surfaces (Breazile 1971). Uptake of oxygen (VO_2) during rest in buffaloes was 3.18±1.08 ml kg^{-1} b wt and 1 hr of treadmill exercise increases oxygen uptake. Pulmonary ventilation increased significantly ($P<0.01$). Alveloar ventilation increased and dead space ventilation decreased due to exercise (Table 2) and RQ registered a decline after exercise. The resting oxygen consumption values were within the range reported by Rao and Mulick (1963) for buffaloes.

Behavioural symptoms exhibited by animals during exercise were profuse salivation, open mouth panting, tongue protrusion and general restlessness which was similar to the observations made by Upadhyay and Rao (1985) in buffaloes.

Assuming the sweat gland surface per unit of skin area in cattle is at least three times that of buffaloes (Hafez *et al.* 1955), it seems that under thermal stress conditions the sweat glands of buffaloes are nearly as functional as those of cattle after being exposed to hot conditions (Joshi *et al.* 1968). During present investigation skin water loss increased due to exercise effect in buffaloes. Evaporative losses increase with exercise and proportional contribution of sweating was more than 80% and pulmonary losses contributed remaining evaporative losses. Since in hot environments sweating is the main avenue of evaporative heat loss in buffaloes in cattle (Brody 1956), it is reasonable to infer, especially from the observations of Joshi *et al.* (1968) and also from the temperature and humidity conditions at which the experiments were performed, that the sweat glands were the main source of the moisture loss from the skin. The complementary nature of sweating and pulmonary losses was also reported in large animals (Brody 1945, McLean 1963 b). The role of respiratory vaporization at high ambient temperature declines (McDowell 1972) and in buffaloes particularly who lack in effective thermolytic system, the heat loss through skin vaporization is augmented and contribution of pulmonary system nearly remains unchanged.

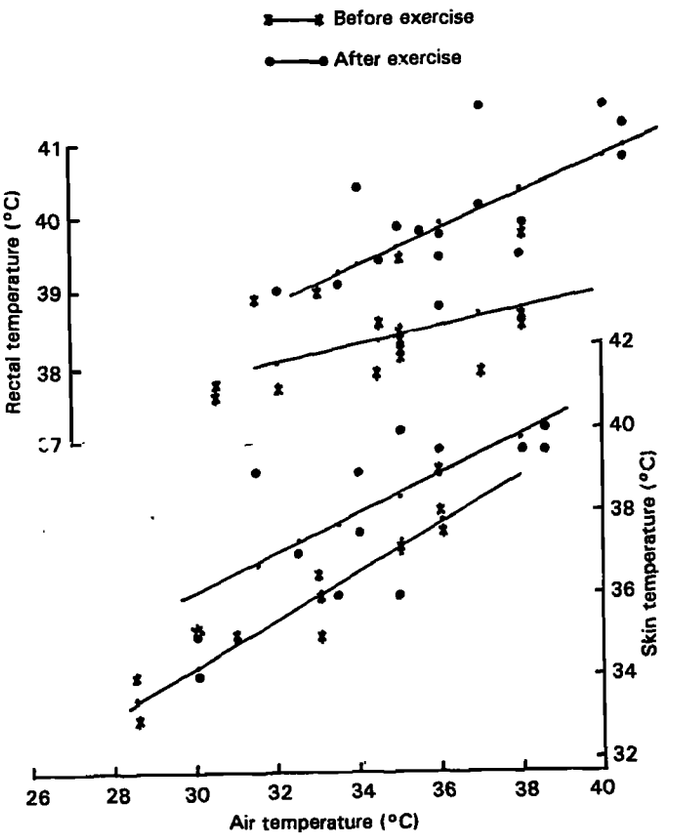


Fig. 1. Relationship of air temperature with rectal temperature and skin temperature before and after exercise in buffaloes.

In our experiment, it was observed that the skin evaporation measured on different days was more closely related to skin temperature than to rectal temperature. Sweating was observed to be different on different days when ambient temperature and humidity varied day to day. The rate of sweating was related to the skin temperatures. In our experimental animals the water loss by sweating was 799.4 and 681.2 g m^{-2} hr^{-1} on 12 different days at the same rectal temperature of 39.4°C respectively. Therefore variations observed in water loss at the same rectal temperature are attributed to the differences in skin temperature. Thus this study revealed that sweating in buffaloes is more closely controlled by peripheral temperature (skin temperature) than by internal body temperature (Fig. 2). The rate of sweating was negatively correlated to

temperature (Table 4). Body heat content necessarily do not continue to initiate thermolytic process particularly in the open environment when thermal changes in skin receptors are activated to greater levels and keep on changing as per need (Kuno 1934).

Studies on human beings support the observations recorded in buffaloes during present investigation that high body temperature alone cannot be the cause of sweating in

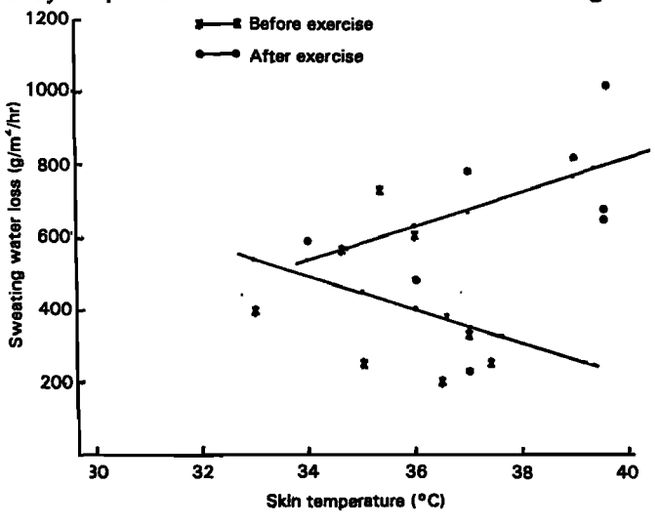


Fig. 2. Relationship between skin temperature and sweating heat loss before and after exercise in buffaloes.

man (Kuno 1934) and high rectal temperature changes may not evoke the evaporative losses to the same extent as the skin temperature changes. The skin temperature presumably set up reflex discharge of cord or brain stem neurons to induce sweating. Most likely similar mechanism(s) operate in large animals and buffaloes. Therefore it appears that sweating is initiated by the changes other than body heat and in this process skin receptors play an important role in buffaloes.

Table 1. Ambient conditions during experimental period

Environmental variations	Before exercise	After exercise
Dry bulb temperature (°C)	32.5±0.62	34.4±0.63
Wet bulb temperature (°C)	24.8±0.38	28.5±0.42
Globe thermometer temperature (°C)	39.8±0.51	44.5±0.92
Environmental humidity (%)	75±1.24	72±1.21

The pre-exercise values on pulmonary volumes and ventilation were within normal limits of buffaloes. Alveloar ventilation at pre-work stage increased 1 hr exercise. In ox, ventilation has been reported to be 24.4 lit min^{-1} (Hales and Findlay 1968). The pulmonary ventilation and tidal air in buffalo during summer has been reported to be 37 lit min^{-1} and 1.7 lit min^{-1} respectively (Mullick 1964).

Table 2. Physiological changes due to exercise in buffaloes

Physiological parameters	Before exercise	After exercise
Rectal temperature (°C)	38.3±0.28	40.0±0.38**
Respiration rate (min^{-1})	24±1	59±4**
Heart rate (beats min^{-1})	70±2	95±0.5**
Skin temperature (°C)	36.0±0.4	39.5±0.5**
Volume of O_2 consumed (ml kg^{-1} b wt)	3.18±1.08	3.90±1.06**
Pulmonary ventilation (lit. min^{-1})	39.39±2.65	53.72±3.96**
RQ	0.80±0.01	0.78±0.01**
Tidal volume (litre)	1.68±0.11	0.99±0.09**
Dead space ventilation (lit min^{-1})	0.62±0.05	0.43±0.05**
Alveolar ventilation (lit min^{-1})	26.05±2.20	30.11±2.09
Pulmonary water loss (g m^{-2} hr^{-1})	72.43±60187	115.96±7.01**
Sweating water loss (g m^{-2} hr^{-1})	492.64±57.90	760.23±57.79**

**P<0.01; *P<0.05.

Exposure of Hereford cattle change tidal volume and increased oxygen uptake (Hales and Findlay 1968). The panting in the exposed ox is the mechanism to maintain thermal balance with little extra energy expenditure. Increase in ventilation has been reported (Hales and Findlay 1968).

The correlation between sweating water loss and skin temperature was -0.3454 before exercise and -0.5696 after exercise. A negative correlation was found between rectal temperature and sweating water loss before and after exercise. Negative relationship in rectal temperature and sweating was also observed in cattle (Joshi *et al.* 1968). Taneja (1959) associated the lower skin temperature of zebu crosses in comparison to Shorthorn with their higher cutaneous evaporation.

Table 3. Components of the thermal balance (W/m^2) after 1 hr treadmill exercise

	H_s	E_t	M	S
Mean	2646.34	2129.14	1055.4	1572.60
±	±	±	±	±
SE	274.22	199.98	429.98	310.14

H_s, rate of environmental heat gain at the skin; E_t, rate of total evaporative heat loss; M, rate of metabolic heat production; S_t, rate of heat storage.

Table 4. Relationship between sweating water loss and body temperature and THI index and relative humidity in buffaloes

	Correlation between sweating water loss and			
	Skin temperature	Rectal temperature	THI index	Relative humidity
Before exercise	-0.3454*	-0.4678**	-0.0238	0.5380**
After exercise	-0.5695**	-0.4262**	0.0381	-0.0767

*P<0.05 **P<0.01.

Table 5. MSS values of sweating vs THI, relative humidity, skin temperature and rectal temperature before and after exercise

DF	Sweating vs skin temperature		Sweating vs THI		Sweating vs relative humidity		Sweating vs rectal temperature	
	Before exercise	After exercise	Before exercise	After exercise	Before exercise	After exercise	Before exercise	After exercise
1			207.32	838.84**	105542.61**	3324.25**	79792.97**	102705.20**
9			40498.93**	64089.18**	28795.01**	62468.30**	31656.08**	51425.98**
1	31949.28**	59188.83**						
6	39302.74**	20539.23**						

**P<0.01 * P<0.05.

Table 6. MSS of temperature vs VO_2 and pulmonary water loss, VO_2 vs alveolar ventilation, ΔVO_2 vs Δ dead space ventilation and Δ alveolar ventilation Vs Δ dead space ventilation

Source	DF	Air temperature vs VO_2		VO_2 vs alveolar ventilation		ΔVO_2 vs dead space ventilation		Δ Alveolar ventilation vs Δ Dead space ventilation		Air temperature vs pulmonary water loss	
		Before exercise	After exercise	Before exercise	After exercise	Before exercise	After exercise	Before exercise	After exercise	Before exercise	After exercise
Regression	1	0.14E-01	0.118E-01	2.77	3.41	43.74**	1.43				
Deviation	1	0.23E-01	0.62E-01	15.11*	3.68	33.78**	42.44**				
Regression	1									647.06**	315.40**
Deviation	13									654.37**	748.38**

**P<0.01 ; * P<0.05.

McLean (1963) reported a decline in skin and rectal temperature while sweating rates increased. Tennenbaum (1962) reported a linear correlation between evaporation from the skin surface of Friesian cattle and their surface temperature. These contradictions in above studies may be due to different experimental procedures, breeds and climatic conditions.

An increase in skin temperature consequent to rising ambient temperature evokes reflex sweating through the thermosensory impulses from receptors located in the skin (Joshi *et al.* 1968). The stronger the impulses the higher the rate of sweat secretion and thus a positive correlation result. But after some time a steady thermal gradient develops and moisture appears over the body surface which tends to lower its temperature due to high specific heat of water and its evaporation. This explains the negative correlations observed between sweating water loss and body temperature. A similar situation to that observed could have resulted in the experimental conditions during present investigation in buffaloes. In Braham × Shorthorn steers very significant reversals from positive associations in the early morning to negative associations at higher stresses have been observed (Schlegar and Turner 1965) which indicate that alterations occur in the state of thermal balance and patterns of sweating.

The negative rectal temperature and sweating water loss correlations observed in this study could be due to higher rectal temperature in relation to sweating water loss in hot humid conditions. This gives further indication that the skin of the buffalo must be adequently pre-conditioned by the previous environment for the sweat glands to respond to stimulation of higher temperature due to exposure or work (Joshi *et al.* 1968).

Temperature humidity index gives an indication of the environmental discomfort to the animals and values ranged between 79 and 90 during present experiment. These values were much above the comfort value of 70 (McDowell 1972), thus affirming the level of thermal stress to which the experimental buffaloes were exposed. The differences in climate chamber and open environment being ascribed to the stimulation of the sweat glands by solar radiation. Under present study relationship was observed between THI and the sweating rate of the buffaloes. However, results point out a negative correlation in the data recorded prior to exercise and positive but nonsignificant correlation after exercise.

The relationship between respiration rate and pulmonary water loss was 0.21 before exercise and -0.36 after exercise. The reversal from positive correlation before exercise to negative after exercise may be due to higher level of stress caused by continuous exercise at a speed of 5 km/hr on treadmill. The necessity to maintain thermal equilibrium in buffaloes might have caused the reversal effects as the body heat load changed.

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Performance of Awassi crossbred lambs in farmers' flocks of semi-arid region

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ABSTRACT

A field study was conducted on 55 Awassi crossbred lambs born out of Malpura ewes bred artificially using freshly diluted semen of Awassi rams. Their growth and survival rates from birth to 90 days of age were compared with Malpura lambs in the farmers' flocks of a cluster of villages around Avikanagar. Both crossbred and native lambs were kept on identical semi-intensive management throughout the period of this study. The birth weights of male and female crossbred lambs were significantly higher than birth weights of their counterpart Malpura lambs. The body weights of these crossbred lambs at the age of 90 days were still significantly ($P<0.05$) higher than the respective male and female lambs of native Malpura breed. Up to 90 days of average daily gain of crossbred lambs was consistently higher than their native counterparts, sex-wise. The survival rates of crossbred and native lambs from birth to 90 days of their age was exceptionally good (100%). It is apparent from this field study that improvement in growth and survival of Malpura sheep can be brought about by the use of Awassi rams of South-West Asia, which resemble the Malpura breed phenotypically.

Key words : Awassi, Crossbred lambs, Semi-arid region

The climatic features of place of inhabitation of this breed in Iraq, Lebanon, Jordon, Israel, Turkey and Cyprus (Epstein 1977, 1982, 1985) are similar to our arid and semi-arid regions. The productive attributes of this breed suit to our prevailing traditional system of husbandry of these 2 regions. Thus there seems to be a good scope of introducing productive and adaptive merits of Awassi breed into native Malpura sheep, which resemble each other phenotypically, for augmenting mutton, milk and wool production. To this effect, an attempt has been made to study the performance of crossbred lambs, born out of Malpura ewes which were artificially bred using freshly diluted semen of Awassi rams, in various farmer flocks.

MATERIALS AND METHODS

This study was conducted in a cluster of village of Malpura town in Rajasthan. This area is located in semi-arid region of country at longitude of 75 C - 28' E, at the latitude of 26 C - 26' N and at altitude of 320 M above MSL. The annual rainfall of this area is 400 -600 mm. The maximum and minimum ambient temperature ranged from 25 ° C to 46° C, 7° to 30° C, and the mean relative humidity ranged from 15 to 90%.

The ewes of Malpura breed in a 4 flocks of this village cluster were artificially bred, using freshly diluted semen of Awassi rams during July and August 1995, and lambed during December 1995 and January 1996. This study was conducted on 55 Awassi crossbred lambs born out of these artificially-bred ewes. The Malpura lambs born at similar time in flocks of farmers of this cluster, were used as control animals, for purpose of comparison.

The ewes after lambing were maintained on traditional type of management prevalent in this area. In addition, to daily grazing they were provided with groundnut and gram *churi*. The lambs were kept separate during grazing hours and during nights. The milk produced by these ewes was sufficient to rear their lambs, therefore no foster mother was needed. At the age of 30 days these lambs were provided with nibbling material in form of cut greens, in addition to suckling. Thus the managemental practices for crossbred Awassi and native Malpura lambs were identical throughout the period of study.

The birth weight of each lambs irrespective of the breed, was recorded with the help of a pan balance. The body weights of available lambs were recorded at the age of 90 days. The survival rates of these lambs were also recorded up to this age. The average daily gain was calculated from these data. Statistical analysis was done using standard methods of student 't' test.

RESULTS AND DISCUSSION

The average values of body weights at birth and at 90 days of age, in crossbred Awassi and native Malpura lambs are given in Table 1. The birth weights of crossbred lambs were significantly higher ($P<0.01$) than Malpura lambs irrespective

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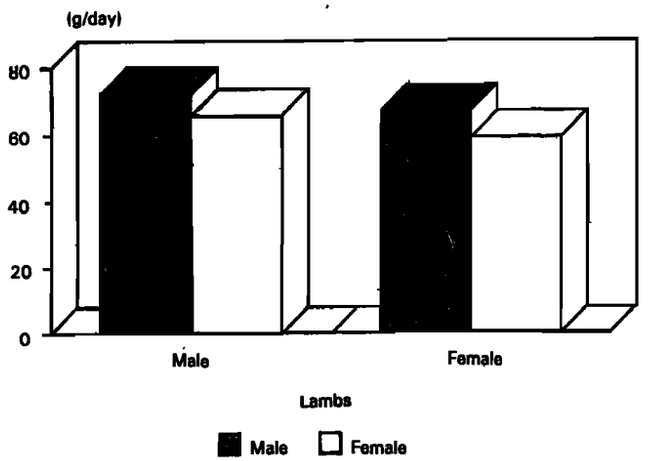


Fig. 1. Average daily gain in lambs (from day 0 to day 90).

of their sex. Similarly body weights of lambs of former breed were significantly higher ($P<0.05$) than latter breed at 90 days of their age. Sex-wise the birth weights of male and female crossbred lambs were also significantly higher ($P<0.01$) than their respective native counterparts. At 90 days the body weights of both male and female lambs of Awassi crosses, were again significantly higher ($P<0.05$) than the lambs of both the sexes of Malpura breed.

Table 1. Comparative growth rate of crossbred and native lambs

Breed and sex	Body weights	
	At birth	At 90 days
Awassi crosses		
Male	3.376±0.081 (21)	9.880±0.407 ((15)
Female	3.067±0.051 (34)	9.092±0.297 (25)
Pooled	3.185±0.051 (55)	9.474±0.256 (40)
Native Malpura		
Male	2.840±0.043 (15)	8.760±0.152 (14)
Female	2.682±0.038 (16)	8.000±0.145 (15)
Pooled	2.758±0.032 (31)	8.365±0.126 (29)

Table 2. Survival rates of lambs from birth to 90 days

Breed and sex	No. of lambs born	No. of lambs survived	Per cent survival rate
Awassi crosses			
Male	21	21	100.00
Female	34	34	100.00
Pooled	55	55	100.00
Native Malpura			
Male	15	15	100.00
Female	16	16	100.00
Pooled	31	21	100.00

The Awassi crossbred lambs grew at a faster rate than Malpura lambs under identical management systems of rural sector of semi-arid region of India. Almost similar results were obtained by Hill *et al.* (1993) when growth rate of Awassi lambs was compared with Coopworth lambs at New Zealand. According to Mavrogenis *et al.* (1980), the post-weaning lamb weight was the best criteria for selecting for growth because it was more heritable and less subjected to maternal influences than at other times. The adult body weight was genetically correlated with early growth (Mavrogenis 1988). It would be pertinent to mention here that by 90 days most of lambs are weaned in rural sector of semi-arid region of India and in this study also the weaning was complete by this time. Higher post-weaning weight of crossbred lambs than Malpura, recorded in present study may result in higher body weights of Awassi crossbred rams and ewes. The study to record adult body weights of these animals is in progress. The values of ADG were higher in crossbred than native lambs (Fig. 1). This superiority was visible in both the male and female crossbred lambs. Hybrid vigour in body weight gains was observed when Awassi crossed with Rambouillet and de France breeds at Turkey (Guney 1991 and Guney and Bicer 1986) and with Chios at Near East (Guirgis 1988) which was due to high feed efficiency of crossbred lambs.

Data pertaining to survival rates of crossbred and native lambs during first 90 days of their life have been provided in Table 2. Out of 55 crossbred Awassi lambs born all survived and in Malpura lambs all 31 lambs could survive. Thus the survival rate was 100% in crossbred and native lambs. In a study conducted at Nicosia, Cyprus, crossing of Chios, sheep with Awassi reduced the death rate and sustained the local offtake of meat and milk at the level of Chios (Constantinou 1991). In another study (Mavrogenis and Constantinou 1986) Awassi × Chios ewes had less abortions, sickness and mortality than purebred Chios ewes. Our results can very well be compared with these findings and can be interpreted on similar lines, in Indian conditions.

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Statistical model for estimating losses due to foot-and-mouth disease in India

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ABSTRACT

A stochastic model has been developed by incorporating a cost-benefit analysis system to study the merits and demerits of various strategies used to prevent, control or eradicate a disease in its operation area. This model will help in estimating the economic losses caused by morbidity and mortality and gain derived due to introduction of vaccination programme as a main strategy to control the disease. The stochastic model developed in the present study is .

$$R = \sum_{t=1}^{T} E(P_{t1}\ r_{t1} + P_{t2}\ r_{t2}) - v$$

Application of the model has been demonstrated by using data collected from FMD outbreak at the IVRI, Izatnagar cattle farm. The overall expected economic losses due to morbidity and mortality were Rs 15.44 and Rs 4.58 , respectively, which were 3.16 and 0.94%, respectively, of the average price value of animals. The overall total expected economic loss due to morbidity and mortality combined was Rs 20.02 amounting to 4.10% of the average price value of the animals in the farm. Though presently the total cost of vaccination/animal-dose is Rs 4.00 including Rs 3.50 for the price of a dose of vaccine, even then vaccination strategy for controlling the FMD' in this country is a profitable proposition in endemic areas.

Key words : FMD losses, Statistical model, Stochastic model

To evaluate the improvement in production and welfare, it is necessary to develop a stochastic model containing costs of resources required for implementing the programme and at least capable of estimating direct economic losses caused by FMD outbreak in a population.

It is difficult to exactly quantify the losses that will be caused by FMD at any given rate of incidence as they depend upon variable factors, viz. age, breed, condition, production state, immune status of host, pathogenicity of the virus strain and environmental factors such as the presence of organism causing secondary infections.

James and Ellis (1978) discussed in detail cost-benefit analysis of FMD control programme. They identified certain losses which were difficult to be quantified. They also established that the benefit exceeded the cost of a factor between 5:1 and 8:1 though not methodology has been mentioned to arrive at this conclusion.

Review of literature revealed that an attempt has been made to develop a stochastic model to assess the economic losses caused to develope a stochastic model to assess the economic losses caused by FMD. Keeping above situations in view, a stochastic model has been developed incorporating all possible factors responsible for economic losses due to FMD. This model will enable the investigator to simulate all possible situations going to arise in a FMD outbreak so that merits of various strategies could be ascertained.

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STOCHASTIC MODEL

Assumptions and notations

Various assumptions and factors are considered to develop the aforesaid model. These are :

(i) The animal will follow one of the 3 stages, viz. the animal will get infected by FMD and will die (ID), the animal will get infected by FMD and will survive (ID), it will not be infected by FMD (I).

(ii) Individual infected by a virus type in a particular time interval will acquire immunity after recovery against that for a definite period.

(iii) The animal will die or survive provided that has been infected in the same time interval or in any time interval.

(iv) The incubation period is constant.

Each epidemic should be divided into intervals of the length of incubation period of the disease in such a way that, in so far as possible, there is a clustering of cases in the middle of each interval.

Although the disease operates in continuous time, for the development of the model we shall assume that risk of attack of disease occurs at discrete interval. This discrete approximation to continuity is usually reasonably good because the time interval under study is short as compared to average life span of an economically productive animal.

Following are some necessary notations which will be used for the development of this model.

p_i = probability of getting infected during ith interval of epidemic.

$q_i = (1-p_i)$ = probability of not getting infected during ith interval of epidemic.

a_i = probability of dying during ith interval given that animal got infected before or during ith interval of epidemic.

$\bar{a}_i = 1-a_i$ = probability of not dying during ith interval given that animal got infected before or during ith interval of epidemic

p_{ij} = probability associated with jth event (got infected and survived, got infected and died, not-infected) during ith interval of epidemic.

T= The complete epidemic period in intervals after outbreak of epidemic

t= Variable time index taking interger value from 1 to T

A_t = sale price of animal died during interval t.

B_t = sale price of byproducts of animal died during interval t.

B_{it} = cost of infrastructure attributab'e to the care of a given animal died during t-th interval.

L_{it} = cost of labour attributable to the care of a even animal died during t-th interval

M_{it} = cost of medical care attributable to the care of a given animal died during t-th interval

C_{it} = total cost attributable to the care of a given animal died during t-th interval, mathematically,

$$C_{it} = B_{it}+L_{it} + M_{it}$$

where A_t, B_t, B_{it}, L_{it} and M_{it} will be calculated on per animal basis for different groups of animals such as calves, young calves, heifers, animals in milk, dry and working animals, and appropriate weightage will be given to each group of animals depending on its proportion in the population.

B_{2t} = cost of infrastructure attributable to the care of a given animal got infected during t-th interval.

L_{2t} = cost of labour attributable to the care of a given animal got infected during t-th interval

M_{2t} = cost of medical care attributable to the care of a given animal got infected during interval t.

E_t = loss attributable to a given animal got infected during t-th interval.

M_{2t}, B_{2t} and L_{2t} will be calculated on per animal basis for different groups of animals such as calves, young calves, heifers, animals in milk, dry animals, and working animals and appropriate weightage will be given to each group of animals depending on its proportion in the population. For each group, loss will be calculated on per animal basis separately; then E_t will be calculated using these losses and their respective weights.

It is difficult to quantify losses like deleterious effect on food conversion efficiency, secondary mastitis infection, reduction in agricultural output attributable to lameness of draught animals, restriction on world trade, and deviation from the optimal production system due to risk and uncertainty caused by FMD. If it will be possible to estimate or quantify these losses, it should also be included in the calculation of E_t.

V = cost of vaccination attributable to a given animal

C_{2t} = total cost attributable to a given animal got infected in t-th interval, mathematically

$$C_{2t} = B_{2t}+ L_{2t} + M_{2t}$$

r_{ij} = monetary loss associated with jth event during ith interval of epidemic

$R_{1\bar{D}}$ = expected monetary loss if animal got infected and survived

R_{1D} = expected monetary loss if animals got infected and died

R_1 = expected monetary loss if animal did not get infected (zero in this case)

Associated probabilities and development of the model

Let us consider that the first interval begins before the day when first case of FMD takes place. The 3 possible events which can take place in this interval are :

(i) The animal enters into state ID, (gets infected and survives)

or

(ii) The animal enters into state ID (the animal gets infected and dies)

or

(iii) The animal enters into state I (the animal does not get infected)

These events are exhaustive and mutually exclusive.

The probabilities associated with these events in t-th interval would be, respectively, as follows:

$$P_{t1} = P_1 \prod_{i=1}^{t} \bar{\alpha}_i + q_1 p_2 \prod_{i=2}^{t} \bar{\alpha}_i + \ldots + p_t \bar{\alpha}_t \prod_{i=1}^{t-1} q_i \quad \text{(i)}$$

$$P_{t2} = p_1 a_1 \prod_{i=1}^{t-1} \bar{a}_i + q_1 p_2 a_1 \prod_{i=2}^{t-1} \bar{a}_i + \ldots + p_t a_t \prod_{i=1}^{t-1} q_i \quad \text{(ii)}$$

$$p_{t3} = \prod_{i=1}^{t} q_i \quad \text{(iii)}$$

The probability of getting infected and probability of death due to FMD will be estimated by Natarajan and Singh (1977). The monetary losses associated with events in t-th interval are given, respectively, as follows :

$$r_{t1} = E_t + C_{2t} \quad \text{(iv)}$$

$$r_{t2} = A_t + C_{it}-B_t \quad \text{(v)}$$

$$r_{t3} = 0, \text{ for all t.} \quad \text{(vi)}$$

The expected monetary loss over a period of T intervals associated with first, second and third events, are given by

$$R_{1D} = \sum_{t=1}^{T} P_{t1}\, r_{t1} \qquad \text{(iv)}$$

$$R_{1D} = \sum_{t=1}^{T} P_{t2}\, r_{t2} \qquad \text{(v)}$$

$$R_{1} = \sum_{t=1}^{T} p_{t3}\, r_{t3} = 0 \qquad \text{(vii)}$$

Thus, the total expected monetary loss due to FMD over a period of T intervals associated with these three events is given by:

$$R = R_{1\bar{D}} + R_{1D} + R_{1}$$

$$= \sum_{t=1}^{T} (p_{t1}r_{t1} + p_{t2}r_{t2} + p_{t3}r_{t3}) - V \qquad \text{(viii)}$$

Since $r_{t3} = 0$ for all t, then expression (viii) reduces to

$$R = \sum_{t=1}^{T} (P_{t1}\, r_{t1} + p_{t2}\, r_{t2}) - V. \qquad \text{(ix)}$$

The expression (IX) is our stochastic model to determine the economic loss due to FMD.

Application of the model

To demonstrate the application of the model, only the following direct economic losses were considered as they could be estimated precisely.

(a) Value of milk loss due to reduction in milk yield,

(b) Value of meat loss due to reduction in weight or growth rate,

(c) Cost of medicines,

(d) Cost of increased human labour hours for additional care.

(e) Any other associated costs, viz. arrangement of extra infrastructure.

FMD outbreak started in the farm with 1 Brown-Swiss animal identified as a case and vanished on day 24 from the day of outbreak. In total, 121 out of 670 animals of different breeds, grades, age-groups and sexes were got infected by the disease. It is only 4 days incubation period attack rate curve which shows 2 peaks and the first peak is higher than the second peak (Fig.1). Since this curve strictly coincides with the theoretical FMD epidemic-curve, 4 days incubation period was considered a suitable class-interval for grouping the total FMD period into classes or periods.

In this study, the total FMD period of 24 days was grouped into 6 periods considering 4 days as class interval. The cases were distributed into these 6 periods and p_i and a_i were calculated using Natarajan and Singh (1977):

p_i or $a_i = D_i/\{(N_i - D_i) + a_i D_i\}$

where

D_i = Number of animals became cases or died in ith period (interval) of epidemic.

N_i = Number of susceptibles or number of animals just before the ith period.

a_i = fraction of time for which each dead animal susceptible before becoming the case.in i-th period lived in this period

Using the formula given in (X), p_i, q_i and $\bar{a}_e$ were estimated utilizing the actual data (Table 1)

Table 1. Estimates of p_i, q_i, a_i and $\bar{a}$

Period of infection	$\hat{p}_i$	$\hat{q}_i$	$\hat{a}_i$	$\hat{\bar{a}}_i$
1	0.006	0.9940	0.0	1.0
2	0.0359	0.9641	0.0	1.0
3	0.0389	0.9611	0.08	0.92
4	0.0494	0.9506	0.0	1.0
5	0.0330	0.9670	0.0279	0.9721
6	0.0135	0.9865	0.0	1.0

Table 2. Expected direct economic losses due to morbidity and mortality

Period of infection	Average economic losses due to morbidity	p_{t1} morbidity	Expected economic losses due to mortality	Average p_{t2} economic losses due to mortality		Expected economic losses due to morbidity	Total expected losses due to morbidity and mortality
I	105.06	0.0060	0.63	0	0	0	0.63
II	83.06	0.0417	346	0	0	0	3.46
III	58.63	0.0739	4.33	453.36	0.0063	2.85	7.18
IV	33.70	0.1181	3.98	0	0	0	3.98
V	13.86	0.1429	3.40	423.05	0.0041	1.73	4.13
VI	4.13	0.1544	0.64	0	0	0	0.64
Overall			15.44 (0.94)			4.58 (0.94)	20.02 (4.10)

Note : Values in parentheses indicate the per cent economic losses to the average value of animals.

The average economic losses due to morbidity varied from Rs 4.13 to 105.06 establishing declining trend showing maximum and minimum losses during the first and last periods of the epidemic. But the same trend was not repeated by expected economic losses due to morbidity because P_{tl} followed just the reverse trend of average economic losses. The expected economic losses registered an increasing trend from first to third period of epidemic and then decreasing trend was depicted up to the end of the epidemic. The highest average economic loss observed in the beginning of the outbreak is a natural psychological human phenomena as the maximum efforts are put in the beginning of any epidemic to control it as early as possible.

The expected economic losses due to mortality and total expected economic losses due to morbidity and mortality did not follow any trend because mortality is a rare event in this disease.

The overall expected losses due to morbidity and mortality were Rs 15.44 and Rs 4.58, respectively, which were 3.16% and 0.94% of the average price value of animals. The overall total expected economic loss due to morbidity and mortality was Rs 20.02 amounting to 4.10% of the average value of animals in the farm.

An animal is vaccinated with 3 doses during first year and 2 doses during every subsequent year against the FMD. The price of 1dose of IVRI vaccine is Rs 3.50. Assuming the cost of storage, inoculation, etc. of the vaccine to be on a bit higher side, let it be Re 0.50. Then the total cost of vaccination per dose per animal will be Rs 4.00. Even at this cost of vaccination, the vaccination strategy for controlling the FMD in the country is a profitable proposition. Even if there is no mortality in the herd, which is a common phenomena in indigenous breeds, the vaccination strategy is still a profitable proposition in Indian context.

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Different levels of adoption of buffalo husbandry practices among farmers of different levels of social participation

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ABSTRACT

Social participation has highly strong association with the level of adoption of housing (24.83**), feeding (24.36**), breeding (20.54**), disease prevention (45.67**) and sale-purchase (27.53**) practices. The KI increased with increased level of social participation. The Buffalo Management Index (BMI) also increased with increased level of social participation. The BMI in different categories were 67.51, 69.80, 72.01 and 77.78 in no, low, medium and high level of social participation. The age at first calving (AFC) significantly decreased ($P < 0.05$) with increased level of participation. The AFC and calving interval (CI) were lowest in buffaloes of respondents of high social participation.

Keywords : Buffalo husbandry practices, Husbandry practice adoption, Social participation

The effect of social participation of buffalo keepers on adoption various buffalo husbandry practices has been studied in this paper.

MATERIALS AND METHODS

The present study was undertaken in Haryana state, i.e. the home tract of Murrah, in 6 districts, viz. Rohtak, Sonepat, Jind, Bhiwani, Hisar and Gurgoan. Two villages having maximum population of Murrah buffaloes were selected purposefully from each district. The farmers who kept one or more buffaloes in the area were considered and a sample size of 25 respondents was selected from 5 categories, viz. agricultural landless labourers, marginal, small, medium and large farmers from each village by proportional allocation. Thus, 300 repondents were selected having 50 respondents from each district and 25 respondents from each village. A list of various recommended practices from 8 components of buffalo husbandry were collected from 20 experts. A set of 10 most important recommended practices in each aspects were selected based on highest score points in order of merit. These 8 components of buffalo husbandry practices, viz. housing, feeding, disease prevention, breeding, calf rearing, milking, sale-purchase and care management practices. Each selected practices was given '2', '1' or '0' score to perfectly adopted, partially adopted and not adopted respectively. The level of adoption of various practices among adapters were classified into 3 categories as under :

(1)	Less than mean	- 1 SD	Low adapters
(2)	Between mean	±1 SD	Medium adopters
(3)	More than mean	+ 1 SD	High adopters.

The frequencies of the respondents falling in low, medium and high level of adoption have been worked out with the help of chi-square test and determined the association between different level of social participation the level of adoption of various practices. The social participation, viz. low, medium and high, of buffalo keepers were calculated on the basis of participation contact with extension in various village organizations such as Panchayats, Samities, Co-operative and Dairy milk co-op societies.

(i)	No participation	No membership of any organization
(ii)	Low	Membership of one organization
(iii)	Medium	Membership of more than one organization
(iv)	High	Office bearer of any organization

The adoption index which is more reliable method to measure the status of adoption of various practices among the respondents. It is determined by the method developed by Mahipal and Kherde (1991) and Rao *et al.* (1992) for the study as given below :

$$\text{Adoption index} = \frac{\text{Number of scores obtained}}{\text{Expected maximum scores}} \times 100$$

To estimates the status of adoption of buffalo husbandry practices in the study area of Murrah tract, the BMI was calculated as given on next page.

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Table 1. Adoption level of buffalo husbandry practices among respondents of differential level of social participation

Category	Number	Housing practices			Feeding practices			Breeding practices			Milking practices		
	(%)	Low	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High
No participation	81	70	10	1	33	41	7	17	48	16	18	57	6
	(27.0)	(34.1)	(12.8)	(5.9)	(40.7)	(24.8)	(13.0)	(28.8)	(32.0)	(17.6)	(23.7)	(32.2)	(12.8)
Low participation	126	86	32	8	35	72	19	29	64	33	35	70	21
	(42.0)	(42.0)	(41.0)	(47.1)	(43.2)	(43.6)	(35.2)	(49.2)	(42.7)	(36.3)	(46.1)	(39.5)	(44.7)
Medium	82	45	30	7	12	45	25	13	35	34	22	43	17
	(27.3)	(22.0)	(38.5)	(41.2)	(14.8)	(27.3)	(46.3)	(22.0)	(23.3)	(37.4)	(28.9)	(24.3)	(36.2)
High participation	11	4	6	1	1	7	3	-	3	8	1	7	3
	(3.7)	(2.0)	(7.7)	(5.9)	(1.2)	(4.2)	(5.6)	-	(2.0)	(8.8)	(1.3)	(4.0)	(6.4)
Column total number (%)	300 (100.0)	205 (68.3)	78 (26.0)	17 (5.7)	81 (27.0)	165 (55.0)	54 (18.0)	59 (19.7)	150 (50.0)	91 (30.3)	76 (25.3)	177 (59.0)	47 (15.7)
x^2 =		24.83**			24.36**			20.54**			10.38^{NS}		

Figures in parentheses indicate the percentage of respondents

Category	Number	Calf rearing practices			Disease prevention practices			Sale-purchase practices			Care management practices		
	(%)	Low	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High
No social participation	81	14	48	19	25	45	11	-	57	24	2	38	41
	(27.0)	(26.4)	(30.4)	(21.3)	(52.0)	(29.0)	(11.3)	-	(35.6)	(19.0)	(11.8)	(29.0)	(27.0)
Low social participation	126	25	65	36	18	72	36	9	67	50	5	55	66
	(42.0)	(47.2)	(41.1)	(40.4)	(37.5)	(46.5)	(37.1)	(64.3)	(41.3)	(39.7)	(29.4)	(42.0)	(43.4)
Medium social participation	82	14	40	28	5	35	42	5	35	42	10	34	38
	(27.3)	(26.4)	(25.3)	(31.5)	(10.4)	(22.6)	(43.3)	(35.7)	(21.9)	(33.3)	(58.8)	(26.0)	(25.0)
High social participation	11	-	5	6	-	3	8	-	1	10	-	4	7
	(3.7)	-	(3.2)	(6.7)	-	(1.9)	(8.2)	-	(0.6)	(7.9)	-	(3.1)	(4.6)
Column total number (%)	300 (100.0)	53 (17.6)	158 (52.7)	89 (25.7)	48 (51.7)	155 (32.3)	97 (4.7)	144 (53.3)	160 (42.0)	126 (5.6)	17 (43.7)	131 (50.7)	152
x^2 = Table value at 5% = 12.59 at 1% = 16.81 df = 6			7.29^{NS}			45.67**			27.53**			10.05^{NS}	

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

Table 2. Mean knowledge scores of various practices among respondents of different level of social participation

Practices (Number)	No participation (81)	Low participation (126)	Medium participation (82)	High participation (11)	Overall mean (300)
Housing practices	2.87^{a}±0.09	3.16^{b}±0.07	3.56^{c}±0.09	3.81c±0.29	3.20±0.05
Feeding practices	3.67^{a}±0.10	4.04^{b}±0.09	4.26^{b}±0.12	4.27^{b}±0.44	4.01±0.06
Breeding practices	4.32^{a}±0.12	4.61^{a}±0.10	4.95^{b}±0.11	5.27^{b}±0.30	4.65±0.06
Milking practices	4.09^{a}±0.11	4.18^{a}±0.08	4.25^{a}±0.12	5.18^{b}±0.22	4.27±0.06
Calf rearing practices	3.49^{a}±0.10	3.53^{a}±0.09	3.79^{ab}±0.12	4.36^{b}±0.33	3.62±0.06
Disease	3.72^{a}±0.11	3.80^{ab}±0.08	4.09^{b}±0.13	4.90^{c}±0.41	3.91±0.06
Average	22.18^{a}±0.43	23.31^{a}±0.36	24.92^{b}±0.54	27.81^{c}±1.62	23.61±0.26

$$BMI = \frac{\text{Total number of scores obtained in all the practices}}{\text{Expected maximum number of scores in all the practices}} \times 100$$

To determine the knowledge index (KI) only the 'high cost' and 'low cost' practice were taken. The knowledge index was calculated as described by Kumar and Pushkaran (1990).

$$KI = \frac{\text{Knowledge score obtained}}{\text{Expected maximum scores}} \times 100$$

RESULTS AND DISCUSSION

Adoption level of various practices among the respondents of different social participation

The detailed distribution of the respondents on the basis of level of social participation in various buffalo husbandry practices is given in Table 1. Out of 300 respondents , 82 (27.3%) respondents were having medium level of participation and only 11 (3.7%) were having high social participation in various village organizations, viz. Dairy Milk Co-operative Societies, Village Panchayats and Panchayat Samities. A considerable number of (42.0 %) buffalo keepers fall in low participation group. It is interesting to note that out of 300 respondents, 81 (27%) respondents were not having any social participation. This seeks support from the earlier finding of Sinha *et al.* (1974) who indicated that farmers associated with dairy business had no social participation. However, the results of the present investigation are in line with these of Saini *et al.* (1977) who also observed that dairymen were having either nil, low, medium or high level of social participation.

Table 3. Mean knowledge indices of various practices among respondents of differential level of social participation

Practices	Number	Low	Medium	High	Overall index
	(81)	(126)	(82)	(11)	(300)
Housing practices	47.83	52.16	59.33	63.50	53.33
Feeding practices	61.16	67.33	71.00	71.16	66.83
Breeding practices	72.00	76.83	82.50	87.83	77.50
Milking practices	68.16	69.66	70.83	86.33	70.16
Calf rearing practices	58.16	58.83	63.16	72.66	60.33
Disease prevention practices	62.00	63.33	68.16	81.66	65.0
Overall average knowledge Index	61.61	64.75	69.22	77.25	65.58

The results reveals that social participation had highly strong association with the level of adoption of housing, feeding, breeding, disease prevention and sale-purchase which is quite evident from their high Chi-square values (Table 1). Social participation were not found to have any significant association with the adoption of milking, calf rearing and care management practices. Sheron and Kumar (1988) also observed that social participation was not found to have any significant relationship with adoption of different dairy practices. A critical analysis showed that a majority of the respondents having high participation were found either in high adopters or medium adopters group.

Table 4. Mean adoption scores of various practices among respondents of different levels of social participation

Practices	No participation (81)	Low participation (126)	Medium participation (82)	High participation (11)	Overall mean (300)
Housing practices	$9.92^{a}\pm0.242$	$11.50^{b}\pm0.208$	$12.45^{c}\pm0.255$	$13.63^{c}\pm0.509$	11.41 ± 0.143
Feeding practices	$12.98^{a}\pm0.197$	$13.59^{b}\pm0.152$	$14.13^{c}\pm0.190$	$14.45^{c}\pm0.593$	13.61 ± 0.103
Breeding practices	$13.83^{a}\pm0.208$	$13.96^{a}\pm0.192$	$14.69^{b}\pm0.229$	$16.09^{c}\pm0.368$	14.21 ± 0.100
Milking practices	$13.18^{a}\pm0.183$	$13.64^{ab}\pm0.167$	$13.71^{ab}\pm0.227$	$14.54^{c}\pm0.692$	13.57 ± 0.109
Calf rearing practices	$14.32^{a}\pm0.179$	$14.18^{a}\pm0.150$	$14.62^{a}\pm0.215$	$15.90^{b}\pm0.414$	14.40 ± 0.101
Diseases prevention practices	$13.37^{a}\pm0.189$	$14.51^{b}\pm0.130$	15.20 ± 0.202	$16.45^{d}\pm0.340$	14.46 ± 0.104
Sale purchase practices	$14.97^{a}\pm0.119$	$14.96^{a}\pm0.132$	$15.30^{a}\pm0.159$	$16.90^{b}\pm0.392$	15.13 ± 0.081
Care management practices	$15.27^{a}\pm0.166$	$15.34^{a}\pm0.120$	$15.00^{a}\pm0.208$	$16.45^{b}\pm0.577$	15.27 ± 0.091
Average management practices	$108.03^{a}\pm0.918$	$111.68^{b}\pm0.668$	$115.23^{c}\pm0.965$	$124.45^{d}\pm1.983$	112.13 ± 0.506

The means bearing different superscripts differ significantly ($P < 0.05$) within the practices.

Table 5. Adoption index in various practices among respondents of different levels of social participation

Social participation level (Number of respondents)	No participation (81)	Low participation (126)	Medium participation (82)	High participation (11)	Overall index (300)
Practices :					
Housing practices	49.60	57.50	62.25	68.15	57.05
Feeding practices	64.90	67.95	70.65	72.25	68.05
Breeding practices	69.15	69.80	73.45	80.45	71.05
Milking practices	65.90	68.20	68.55	72.70	67.85
Calf rearing practices	71.60	70.90	73.10	79.50	72.00
Disease prevention practices	66.85	72.55	76.00	82.25	72.30
Sale-purchase practices	74.85	74.80	76.50	84.50	75.65
Care management	76.35	76.70	75.00	82.25	76.35
Buffalo management index	67.51	69.80	72.01	77.78	70.08

Mean knowledge of various practices among respondents of different levels of social participation

The level of social participation influenced significantly ($P < 0.05$) the mean knowledge scores of various practices (Table 2). The mean knowledge scores had increased with increased level of social participation. The KI had also increased with increased level of participation (Table 3). This implied that higher the level of social participation higher would be K I. The overall knowledge indices were 61.61, 64.75, 69.22 and 77.25 for the respondents having no, low, medium and high level of social participation respectively. The respondents having high level of social participation obtained maximum values of KI in all the practices when compared to other categories. The present findings tally with the past findings of Malik (1978) who also found that social participation had significant influence on the knowledge about dairy innovations.

Adoption of various practices among respondents of different levels of social participation

The average adoption scores in various practices significantly ($P < 0.05$) differed with the level of social participation (Table 4). The overall total scores increased significantly ($P < 0.05$) with increased level of social participation. The respondents having high social participation obtained higher average adoption indices (Table 5) in all the components of buffalo husbandry practices to those respondents having either no participation or low participation. The BMI in different categories were 67.51, 69.80, 72.01 and 77.78 in no, low, medium and high-level of participation respectively. This implied that BMI increased with increased level of social participation in all the practices. This may be due to the fact that the respondents who have high social participation especially in Dairy Milk Co-operative Societies and Panchyats generally develop leadership qualities and favourable outlook towards new scientific practices. It acts as an antidote for dogmas and superstition and as an inducing factor for adoption of improved practices. The results are in agreement with the past findings of Coman (1951), Mikkilineni (1976) and Sohi and Kherde (1980), but contrary to the contentions of Malik and Sohal (1980) and Sheoran and Kumar (1980) who found that social participation had no significant ($P < 0.05$) effect on adoption of dairy practices.

Age at first calving and calving interval in buffaloes influenced by level of social participation of respondents

The AFC significantly decreased ($P < 0.05$) with increased level of social participation (Table 6). The AFC were 47.77, 45.66, 45.39 and 44.27 months, respectively, for the respondents having no, low, medium or high social participation. The CI was lowest (14.09 months) among the buffaloes of the respondents having high social participation when compared to others. However, no significant difference was observed in CI for different levels of social participation.

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Table 6. Age at first calving and calving interval in buffaloes influenced by level of social participation of respondent

Variables	No participation (81)	Low participation (126)	Medium participation (82)	High participation (11)	Overall mean (300)
Age at first calving	47.77a±0.513	45.46b±0.340	45.39b±0.401	44.27b±0.973	46.02±0.237
Calving interval	15.21±0.161	15.23±0.291	14.95±0.187	14.09±0.322	15.11±0.140

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Short Communication

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Performance of weaned crossbred calves on feeding of grain-replaced diets and green berseem in early life

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Key words : Crossbred calves, Weaned calves, Feeding of calves, Grain, Berseem

Calves can be raised on good quality, all-roughage diets after 6 months of age with optimum growth (Maheshwari and Ranjhan 1969) while all-roughage diet is unsatisfactory before 6 months of age (Srivastava *et al.* 1979). At present it is also difficult to spare grain for the feeding of livestock considering the prime need of ever growing human population. Hence, a study was conducted to raise the crossbred calves reared on a milk-containing diet up to 8 weeks of age, on the feeding of grainless/low grain concentrate mixture along with leguminous fodder during the early life of 2 to 5 months.

Table 1. Composition of calf-starters

Ingredients	Group 1	Group 2	Group 3	Berseem
Crushed maize	50	25	0	-
Wheat bran	20	48	76	-
Groundnut-cake	27	24	21	-
Mineral mixture	2	2	2	-
Common salt	1	1	1	-
Chemical composition				
OM	91.68	91.80	91.01	87.33
CP	21.34	22.20	22.22	23.52
EE	3.03	2.33	1.97	2.60
TCHO	67.31	67.27	66.82	61.21
CF	7.87	8.57	10.08	20.20
NFE	59.44	58.70	56.74	41.01

Crossbred calves (18) reared up to 8 weeks of age on milk feeding with grain-replaced calf-starter and berseem fodder (Mondal *et al.* 1996) were shifted to experimental feeding comprising 6 calves in each of the 3 groups on the basis of body weight. Each group was allotted to one of the 3 nutritional treatments, viz. conventional feeding of concentrate mixture (control, group 1), concentrate mixture containing 50% grain in comparison with group 1 in group 2, and concentrate mixture without any grain in group 3 (Table 1). Green berseem was fed *ad lib.* in all the groups. Concentrate mixture was fed *ad lib.* up to 13 weeks of age then the amount was fixed to 1 kg/calf a day to have excess to berseem fodder in all the calves up to 20 weeks of age. All the calves were weighed in the morning before feeding and watering at weekly intervals to know the pattern of gain in body weight. Calves were housed in a well ventilated, clean and dry pucca shed. Health coverage and necessary preventive measures were taken throughout the experiment.

Table 2. Intake and digestibility of nutrients, and plane of nutrition during digestion trials

Items	Group 1	Group 2	Group 3	Mean ±SE
Body weight (kg)	57.17	61.75	61.08	61.00±8.72
$W^{0.75/kg}$	20.78	22.02	21.78	21.53±2.38
Intake/day (kg)				
Concentrate	1.10	0.87	0.81	
Berseem	1.04	1.42	1.36	
Total	2.14	2.29	2.17	2.20±0.37
per 100 kg BW (kg)	3.72	3.71	3.56	3.66±0.30
Per kg $W^{0.75}$ (g)	102.47	103.95	99.12	101.85±9.44
Digestibility coefficient (%)				
DM	71.20	73.20	74.76	73.05±1.51
OM	73.47	76.14	77.51	75.71±1.66
CP	65.85	71.92	74.14	70.64±3.41
EF	53.52	47.76	51.31	50.86±4.36
TCHO	76.86	78.69	79.80	78.45±1.34
Nutritive value				
TDN (%)	68.23	69.74	71.04	69.67±1.43
DCP (%)	14.29	15.95	16.61	15.62±0.77
TDN intake/kg $W^{0.75}$ (g)	69.76	72.49	70.38	70.88±5.52
DCP intake/kg $W^{0.75}$ (g)	14.56	16.57	16.46	15.87±1.26

Feed intake in all the groups was recorded daily for the individual calf. A digestion trial was conducted at 13 weeks of age taking 3 calves from each group. Samples of feeds, residue and faeces on 24 hr basis were collected following the standard procedures and preserved after aliquoting for fur-

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ther analysis. Proximate analysis was done as per AOAC (1990) and statistical analysis as per Snedecor and Cochran (1967).

The chemical composition of 3 concentrates and green berseem revealed more or less isonitrogenous status on dry - matter basis (Table 1). The CP content in the concentrate mixures was kept a little higher than the NRC (1989) requirement to take care for the decrease in CP content of berseem due to maturity at later stages during the feeding period.

Mean values of DM intake during digestion trial varied nonsignificantly between the groups (Table 2) and replacement of grain with wheat bran reduced the intake of concentrate, but it was compensated by higher intake of berseem. The DM intake in this study was higher than the values reported on the feeding of all-roughage diet of berseem hay (Srivastava *et al.*, 1979). The difference might be either due to nature of roughage or due to shorter milk feeding period in this study which provided an opportunity for increase in stomach capacity (Goel and Soni 1973).

Digestibility of all the nutrients except EE showed an increasing trend (Table 2) due to replacement of maize grain with wheat bran although difference between the groups remained nonsignificant. Higher proportion of berseem in DM intake in groups 2 and 3 favoured better digestibility of nutrients. The effect on digestibility was reflected in the nutritive value of the diets of 3 groups. Mean plane of nutritive determined digestion trial was similar between the groups.

Table 3. Growth performance of crossbred calves

Items	Group 1	Group 2	Group 3	Mean±SE
Initial body wt (kg)	40.00	41.67	41.00	40.89±5.12
Final body wt (kg)	89.17	94.33	90.83	91.44±10.12
ADG (8 / day)	585.33	627.00	593.17	601.83±67.09
Total gain (kg)	49.17	52.67	49.83	50.56±5.64
Total DMI (kg)	181.69	191.80	176.71	183.40±20.91
Feed : gain ratio	3.67	3.66	3.59	3.64±0.21

Average daily gain (ADG) in the body weight of calves was significantly not influenced by the composition of concentrate mixture (Table 3). There was also nonsignificant effect of diet on feed : gain ratio between the groups and performance of the calves was comparable with the observation of Panda *et al.* (1995) on the feeding of grain-based diets. Katiyar *et al.* (1972) could not observe additional benefit of feeding high grain concentrate in the diet of growing crossbred calves. From the overall performance of calves it may be concluded that the cereal grain may be conveniently dispersed from the concentrate mixture with wheat bran when adequate amount of berseem fodder is available for feeding the calves.

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Statistical modelling for describing fish production in India

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ABSTRACT

Various statistical modelling approaches, viz. polynomial function fitting approach, non-linear mechanistic growth modelling approach, and ARIMA time-series analysis approaches are discussed for describing marine, inland as well as total fish production of the country during 1950-51 to 1994-95. The underlying assumptions are examined by statistically sound procedures. In view of the presence of the autocorrelations among observations in the 3 data sets, it is found that the ARIMA time-series analysis approach is most appropriate. The identified models are then used to forecast the future fish production.

Key words : Fish Production, Forecasting, Statistical modelling, Time-series analysis

Today's world is witnessing a resurgence in the consumption of fish due to the new awareness about its low cholesterol fat content. Although the inland fish production in the country has increased almost 10 folds during the last 4 decades, yet there is hardly any modelling effort in this area. In comparison with this, the marine fish production in the country has not increased at the same pace during this period. However, some isolated attempts have been made in the past to explore the trends in marine fish production. The techniques generally used are: (i) Graphical approach (CMFRI, 1989; James 1992), (ii) Polynomial function fitting approach (Alagaraja and Srinath 1980 Yogamoorthi and Sivashankar 1994), and (iii) time -series analysis approach (Noble and Sathianandan 1991). In view of the economic importance of both inland and marine fisheries, it is highly desirable to study their growth pattern in a comprehensive manner. Accordingly, in this paper various statistical modelling approaches, viz. Polynomial function fitting approach, Non-linear mechanistic growth modelling approach, and ARIMA time-series analysis approach are applied for studying marine, inland as well as total fish production data of the country.

MATERIALS AND METHODS

Data on all-India marine, inland and total fish production during the perod 1950-51 to 1994-95 available in the report of Fisheries Division, Ministry of Agriculture (Government of India) were used for the analysis. As far as the first approach is concerned, we have fitted polynomial functions of first and second degree to the 3 data-sets using methods of least squares. Before taking a final decision about the suitability of the model, the main assumptions of randomness and normality of residuals are examined by applying the Run test (Siegel and Castellan 1988) and Shapiro - Wilk test (Shapiro and Wilk 1965) respectively.

It may be mentioned here that the above approach involves fitting of models in which all the parameters appear in a linear manner. However, for a more realistic modelling, it is desirable to apply non-linear models and to this end, an attempt is made regarding the second approach to fit mechanistic growth models. The 5 nonlinear models considered are:

(i) *Logistic model :*

$$Z_t = c/ [1 + a \exp(-b (t-1))] + \varepsilon \quad (1)$$

(ii) Gompertz model :

$$Z_t = c \exp [-b \exp (-a(t-1))] + \varepsilon \quad (2)$$

(iii) Monomolecular model :

$$Z_t = c - (c-a) \exp [-b (t-1)] + \varepsilon \quad (3)$$

(iv) Richards model:

$$Z_t = c/ [1 + \exp (a(t-1))]^{1/d} + \varepsilon \quad (4)$$

(v) Morgan - Mercer- Flodin (MMF) model :

$$Z_t = (bc + at^d) / (c+t^d) + \varepsilon \quad (5)$$

In the above equations Z_t represents the fish production at time t; a,b,c,d are constants, and ε denotes the error term. All these models are fitted to the data by employing Levenberg - Marquardt nonlinear iterative procedure (Prajneshu 1991; Ratkowsky 1990).

The main assumption in the above discussed approaches is that the successive observations are statistically independent. To handle a situation in which this assumption is vio-

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lated, we can use the approach of time - series analysis methodology (Brockwell and Davis 1991). ARIMA time - series models, traditionally expressed as ARIMA (p,d,q) combine as many as 3 types of processes, viz. autoregression (AR) of order p; differencing d times to make a series stationary and moving average (MA) of order q. It may be pointed out that this methodology applies only to stationary data, a characteristic of which is that the mean and variance are constant over time. Three stages for carrying out the analysis are : (i) Identification ; (ii) Estimation and (iii) Diagnostic checking.

At the identification stage, two graphical devices, viz. estimated autocorrelation function (acf) r_k and estimated partial autocorrelation function (pacf) ϕ_{kk} are used with a view to tentatively select one or more candidate ARIMA models. Denoting the number of observations by n, and the number of computable lags by $k_{(l.c.)}$,

$$r_k = \sum_{t=1}^{n-k} (Z_t-\bar{Z})\ (Z_{t+k}-\bar{Z}) / \sum_{t=1}^{n-k} (Z_t-\bar{Z})^2 \qquad (6)$$

and

$$\phi_{kk} = [r_k - \sum_{j=1}^{k-1} \hat{\phi}_{k-1,j}\ r_{k-j}\ ] / [1- \sum_{j=1}^{k-1} \phi_{k-1,j}\ r_j]; \ k=2,3... \qquad (7)$$

where

$$\hat{\phi}_{11} = r_1, \hat{\phi}_{kj} = \hat{\phi}_{k-1,j} - \hat{\phi}_{kk}\ \hat{\phi}_{k-1,\ k-j},\ k=3,4...,j=1,2,...$$

If the r_k values tail off to zero rapidly, it indicates that the original series is stationary. Otherwise successive differences, are computed and the above procedure is continued till stationary it is achieved. Patterns based on spikes in ϕ_{kk} and r_k values are used to select the appropriate values of p and q which are, respectively, the orders of AR and MA. At the estimation stage, precise estimates of AR and MA parameters are obtained using Melard's algorithm (Melard 1984). Finally, at the diagnostic checking stage, an appropriate model is selected based on the following goodness of fit statistics (Koreisha and Yoshimoto 1991; Jurado *et al.* 1995; Pukkila *et al.* 1990);

(i) Akaike's Information criterion (AIC):

$$AIC = \ln V^*(p,q) + (2/n)\ (p+q),$$

where V^* is an estimate of white noise variance obtained by fitting the corresponding ARIMA model.

(ii) Bayesian Information Criterion (BIC):

$$BIC = \ln V^*\ (p,q) + (p+q)\ [\ln (n)/n]$$

(iii) Root mean squared error (RMSE);

$$RMSE = [\ \sum_{t=1}^{n} (Z_t - \hat{Z}_t)^2 / n]^{1/2}$$

(iv) *Mean absolute error (MAE):*

$$MAE = \sum_{t=1}^{n} |\ Z_t - \hat{Z}_t\ | /n.$$

The lower the values of these statistics, the better is the model. A statistically adequate model is one whose random shocks are independent. To test this assumption, we use the following statistic :

Box - Lung statistic (Q) :

$$Q = (n - d) \sum_{j=1}^{k} r_j^2\ (\hat{a}) \sim \chi^2_{k-m},$$

where $r^2_j(\hat{a})$ denotes the squared of corresponding to residuals of j th lag and m denotes the number of parameters estimated in the ARIMA model. Moreover, as an additional check, the significance of computed residual autocorrelations of the selected model is tested by applying the following approximate t-test :

$$t = r_k\ (a) / [\ 1 + 2 \sum_{j=1}^{k-1} r_j\ (a)^2\ ]\ n^{-1/2} \sim t_{n-1}. \qquad (8)$$

RESULTS

(i) Polynomial function fitting approach

The polynomial function of first degree fitted to the data on fish production in various years (t) of marine (Z_M), inland (Z_I) and total (Z_T) are represented by eqns. (9), (10) and (11), respectively, and the corresponding summary statistics are presented in Table 1:

$$Z_m = 2.509 + 0.456\ t \qquad (9)$$

$$Z_I = -0.611 + 0.375\ t \qquad (10)$$

$$Z_T = 1.920 + 0.830\ t \qquad (11)$$

The corresponding quadratic functions fitted to the data are given by eqns. (12), (13) and (14), respectively, and the corresponding summary statistics are presented in Table 1:

$$Z_M = 6.268 - 0.032\ t + 0.109\ t^2 \qquad (12)$$

$$Z_I = 2.652 - 0.051\ t + 0.004\ t^2 \qquad (13)$$

$$Z_T = 8.933 - 0.084\ t + 0.020\ t^2 \qquad (14)$$

(ii) Nonlinear mechanistic growth modelling approach

Attempts have been made to fit nonlinear growth models to each of the 3 data sets, respectively, using NLR option on the SPSS-PC Version 4.0 available at IASRI, New Delhi. We tried several sets of initial values for each of the data set. In respect of the 3 models, viz. Monomolecular, Gompertz, and Morgan-Mercer-Flodin models, there was no convergence (i.e. successive residual sum of squares, to be arbitrarily small, say 10^{-8}) even after the maximum number of model evaluations (i.e. 300) which was permissible under the software package. In case of the remaining two models, viz. logistic, and Richards models convergence did take place after about 70 model evaluations but it did not ensure global convergence as shown in Table 2. However, in contrast to the logistic model, the parameter estimates for Richards model are quite realistic.

(iii) ARIMA time- series analysis approach

For each of the 3 data sets the values of r_k and ϕ_{kk} for k=1,2,..,16 lags computed using eqns. (6) and (7) given in Table 3. As the r_k values of the original series do not drop to zero rapidly, none of the data sets reflect stationarity. However, it for all the data sets, the same is achieved for first differencing, i.e. d=1. Furthermore, there is no significant spikes at initial

Table 1. Summary statistics of polynomial function fitting of first (second) degree

Statistics/Data	Marine	Inland	Total
R^2	0.892(0.958)	0.903(0.977)	0.904(0.974)
RMSE	3.475(3.062)	1.771(1.450)	3.778(3.366)
Run test (|Z|)	4.571(2.593)	5.854(5.021)	5.198(4.419)
Shapiro Wilk test (W)	0.912(0.966)	0.868(0.968)	0.865(0.976)

lags in both the r_k and Φ_{kk} values in respect of transformed marine data which shows the absence of autoregression (AR) as well as moving average (MA) processes. Thus the following ARIMA (0,1,0) model is suggested for describing the marine fish production data :

$Z_{M,t} = Z_{M,t-1} + a_{t3}$

where a_t represents the random shock term assumed to be a white noise process (Brockwell and Davis 1991).

The patterns of r_k and Φ_{kk} for inland data suggests the following set of value for p and q as reasonable values :

$p_1 = 1, q_1 = 1$ and $p_2 = 1, q_2 = 0$.

The AIC, BIC, RMSE and MAE values for both the candidate models, viz. ARIMA (1,1,1) and ARIMA (1,1,0) are computed and given in Table 4. Between these two candidate models, the former is preferred as it entails lower values for all the 4 criteria, i.e.

$$Z_{I,t} - Z_{T,t-1} = \mu (1-\phi_1) + \phi_1 (Z_{I,t-1} - Z_{T,t-2}) - \theta_1 a_{t-1} + a_t, \quad (16)$$

where μ is a constant and ϕ_1 and θ_1 denote, respectively, the AR and MA parameters.

In the case of total fish production, both r_k and θ_{kk} values of the differenced series tail off after lag 1. Hence the following ARIMA (1,1,1) model is suggested for describing the data set :

$$Z_{T,t} - Z_{T,t-1} = \mu (1-\theta_1) + \phi_1 (Z_{T,t-1} - Z_{T,t-2}) - \phi_1 a_{t-1} + a_t. \quad (17)$$

Eqns. (15), (16) and (17) are fitted to the respective data by using Melard's algorithm of SPSS-X Trends package V 4.0 available at IASRI, New Delhi and the parameter estimates are given in Table 5.

Adequacy of the models

To test the adequacy of the fitted models of the values of Box-Ljung statistic along with tabulated values at 5% level are presented in Table 5. For all the three data sets, it is found that the assumption of independence of random shock is not violated. As an additional check, the approximate t-test given in eqn (8) is used to test the significance of computer residual autocorrelations of the fitted models. These values, given in Table 6 for each data separately, show that the computer residuals are uncorrelated as all the calculated values are less than the tabulated t-value, viz. 2.020 at 5% level. Hence the identified models given by (15), (16), and (17) adequately describe the fish production data of the country.

DISCUSSION

We bring out the salient features of the results obtained by employing various statistical modelling procedures to the marine, inland and total fish production data of the country during the period 1950-51 to 1994-95. Under the polynomial function fitting procedure, Table 1 shows satisfactory results regarding the goodness of fit statistics, viz. R^2 and RMSE. However, the assumption of normality of residuals is satisfied only for polynomial function of second degree as the tabulated value for Shapiro-Wilk test for 44 degrees of freedom at 5% level is 0.944. Furthermore, the assumption of

Table 2. Fitting of growth models by non-linear regression to all-India marine fish production data

Statistic	Model	Logistic			Richards		
		I	II	III	I	II	III
Initial Values	a_0	8.363	13.045	17.727	0.179	0.202	0.215
	b_0	0.016	0.015	0.015	4.610	5.014	6.551
	c_0	50	75	100	40	50	90
	d_0	-	-	-	5.340	5.340	5.340
Parameter estimates	a	96164760	116955602	163141967	0.372	0.465	0.355
	b	0.039	0.037	0.039	31.112	37.877	34.307
	c	47443933	577018815	80488648	124.895	114.888	206.895
	d	-	-	-	9.637	12.032	9.182
R^2		0.956	0.956	0.956	0.956	0.956	0.956
RMSE		1.228	1.228	1.228	1.508	1.508	1.508
Run test (|Z|)		2.139	2.139	2.139	2.139	2.139	2.139
Sharpio Wilk test (W)		0.975	0.975	0.975	0.975	0.975	0.975

Table 3. Values of r_k and θ_{kk} for fish production data

Lag/data	Marine			Inland			Total		
	Original series	Stationary series (d=1)		Original series	Stationary series (d=1)		Original series	Stationary series (d=1)	
	r_k	r_k	θ_{kk}	r_k	r_k	θ_{kk}	r_k	r_k	$\theta\theta_k\theta$
1	.877	-.118	-.118	.902	.291	.291	.894	.070	.070
2	.767	-.119	-.135	.804	.060	-.027	.791	.006	.001
3	.672	-.042	-.076	.723	.293	.310	.710	.028	.027
4	.575	.112	.082	.651	.156	-.021	.615	.127	.123
5	.519	.141	.160	.583	.130	.126	.554	-.219	.206
6	.476	.003	.072	.512	.206	.197	.499	.100	.081
7	.430	.089	-.035	.444	.069	.121	.440	-.139	.162
8	.376	.153	.024	.377	-.093	-.127	.385	-.077	-.100
9	.319	.119	.039	.311	.013	-.089	.322	.100	.111
10	.296	.089	.068	.259	-.090	-.156	.279	.058	048
11	.243	.023	-.295	.211	.020	.135	.233	.011	.012
12	.191	-.352	.060	.151	.107	.039	.178	-.288	-.288
13	.138	.103	.023	.104	-.178	-.154	.124	.045	.122
14	.098	.890	.017	.057	-.129	.015	.076	.044	.011
15	.042	.025	.012	.016	.080	.092	.029	.039	.008
16	.004	.096	-.180	-.022	-.091	-.054	.100	.047	.113

randomness of residuals is rejected for all the data sets as the tabulated value at 5% level is 1.96. Thus the models represented viz. eqns. (9) through (14), are not adequate to describe the given fish production data.

Regarding the second approach of statistical modelling, 5 mechanistic growth models were considered for the 3 data sets. It was found that only Richards model provides realistic parameter estimates. A perusal of R^2 and RMSE values given in Table 2 in respect of this model indicates that this model provided a good fit to the data. Futhermore, the assumption of normality of residuals is not violated at 5% level. However, the assumption of randomness of residuals is rejected at 5% level for all the three data sets. Thus, none of the five growth models considered is adequate to describe the data sets.

Table 4. ARIMA summary statistics for inland fish production data

Statistic/Model	ARIMA (1,1,1)	ARIMA (1,1,0)
AIC	51.952	57.018
BIC	51.090	54.445
RMSE	0.428	0.440
MAE	0.311	0.322

The above studies indicate that successive observations in the three data sets are not independent. Accordingly, the possibility of using the third approach of statistical modelling viz. ARIMA time-series modelling, is explored. Applying this approach, it is found that the marine fish production data are adequately represented by a white noise model, i.e. ARIMA (0,1,0) given by eq. (15). Since each value in white noise series is a (random) step away from the previous value, it implies

Table 5. Fitting of ARIMA models for fish production data

Statistic/Data	Marine	Inland	Total
Constant*	0.498	0.380	0.088
ϕ_1	--	0.283	0.906
ϕ_1	--	0.619	0.820
Box-Ljung Statistic (Q)	11.227	12.962	10.860
Tabulated $\chi 2$ values	25.000	22.400	22.400

*The random shock (a_t) is the constant term for ARIMA model fitted to marine data.

Table 6. Absolute t-values of residual acf for fish production data

Lag/Data	Marine	Inland	Total
1	0.781	0.106	0.231
2	0.798	0.503	0.530
3	0.287	1.470	0.299
4	0.772	0.300	0.744
5	0.986	1.752	1.566
6	0.027	0.208	0.652
7	0.640	0.757	1.258
8	1.100	0.623	0.766
9	0.881	0.434	0.643
10	0.609	0.804	0.314
11	0.176	0.840	0.871
12	1.950	0.733	1.137
13	0.817	1.253	0.451
14	0.725	0.991	0.646
15	0.206	1.074	0.377
16	0.806	1.042	0.811

Table 7. Forecast values for fish production (in lakh tonnes) using ARIMA time-series models

Data/ Period	Marine		Inland		Total	
	Observed	Predicted	Observed	Predicted	Observed	Predicted
1993-94	26.49	26.258	19.95	18.218	46.44	45.001
1994-95	27.47	26.988	20.49	20.929	47.26	47.958
1995-96	--	27.466*	--	21.001*	--	49.423*
1996-97	--	28.466*	--	21.350*	--	50.839*
1997-98	--	28.964*	--	21.993*	--	52.210*

*Forecast values.

that, apart from random fluctuations, the marine fish production during any year essentially depends on its value during the preceding year. Furthermore, a mixed autoregressive and moving average model identified for the total and inland fish production indicated that the fish production during any year is a weighted function involving fish production during two preceding years as well as of the most recent disturbance.

From the above, it is evident that ARIMA time - series modelling approach is the best one for the data sets under consideration. Accordingly, this approach is used to forecast the fish production. However, before attempting this, the models are first fitted to the data ignoring the last two observations and the two forecast values on the basis of these models are compared with the actual observations. A perusal of Table 7 shows that the observed and forecast values for each data set are quite close to each other. This further strengthens our view about the appropriateness of the identified models. Finally, the fish production is forecast for three more years and the values are given in Table 7.

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2.2 SHORT RESEARCH NOTES not more than 1 500 words (about 4-5 typed pages), which deal with (a) research results which are complete but do not warrant comprehensive treatment, and (b) descriptions of new material or improved techniques or equipment, with supporting data. Such notes require no headed sections.

2.3 CRITICAL RESEARCH REVIEWS pointing out lacunae in research and suggesting possible lines of future work.

2.4 Contributors are requested to ensure that the research papers or notes submitted for publications have a direct bearing on animal production or open up new grounds for productive research. Basic types of papers and notes which relate to investigations in a narrow specilalized branch of a discipline, may not form an appropriate material for this journal.

3.1 THE TITLE should be short, specific and informative. It should be phrased to identify the content of the article and include the nature of the study and the technical approach, which is essential for key-word indexing and information retrieval.

3.2 In addition, a SHORT TITLE not exceeding 50 latters should be provided separately for running head lines.

3.3 The BYLINE should contain, in addition to the names and initials of the authors, the place where research was conducted. Change of address should be given as a footnote and correspondence address separately.

4. The ABSTRACT, written in complete sentences, should not have more than 150 words. It should contain a very brief account of the materials, methods, results, discussion and conclusions, so that the reader need not refer to the article except for details. It should not have references to literature, illustrations and tables.

5.1 The INTRODUCTORY part should be brief and limited to the statement of the problem or the aim of the experiment. The review of literature should be pertinent to problem.

5.2 Relevant details should be given of the animal, MATERIALS AND METHODS, including experimental design and the techniques employed. Where the methods are well known, the citation of a standard work is sufficient. Mean results with the relevant standard errors should be presented rather than detailed data. The statistical methods used should be clearly stated.

5.3 The RESULTS AND DISCUSSION should preferably be combined to avoid repetition.

5.4 The result should be supported by brief but adequate tables, or graphic or pictorial material, wherever necessary. Self-explanatory tables should be typed on separate sheets and carry appropriate titles. The tabular matter should not exceed 20% of the text.

5.5 The data should be so arranged that the tables would fit in the normal lay-out of the page. All weights and measurements should be in metric units.

5.6 The DISCUSSION should relate to the limitations or advantage of the author's experiments in comparison with the work of others.

5.7 Line-drawings should be clearly drawn in black waterproof ink on smooth, tough paper. Photographs should be large, unmounted, glossy prints of good quality. They should be clear and relevant to the subject. Line-drawings and photographs should have legends, which should also be supplied on a separate sheet. Tables and illustrations should not reproduce the same data.

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7 All articles are sent to referees for SCRUTINY and authors should meet criticism by improving the article.

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