ICAR PUBLICATIONS

D. K. M. A.

636 (05) CODEN : IJASA4 **68** (11) : 1103-1206 (1998) II ISSN 0367-8318

636/639

The Indian Journal of Animal Sciences

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THE INDIAN JOURNAL OF
ANIMAL SCIENCES

Vol. 68, No. 11 November 1998

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Disease profile of sheep and goats in Tiruchirapalli District of Tamil Nadu

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Received : 20 October 1997; Accepted : 18 May 1998

ABSTRACT

The disease profile of sheep and goats in Tiruchirapalli district of Tamil Nadu is highlighted from sample survey data. The sample was drawn by stratified two-stage simple random sampling without replacement. Two parameters, viz. disease prevalence rate (DPR) and case fatality rate (CFR) were used as measures of the intensity of disease infliction among the ruminants. Further, relative frequencies of various diseases were obtained separately for each category of the animals. The proportions of cases treated, recovered, died and disposed were also worked out.

The DPR was 6% in sheep and 8% in goats; lambs and kids having the higher rates of 8% and 12% respectively. The CFR was over 60% in sheep and around 40% in goats. Prominent among the various ailments were enteritis, sheep/goat pox and pneumonia. Enteritis was conspicuous for the lion's share in the disease inflictions. While a good proportion of the animals had received treatment and recovered, very high proportionate mortality was noticed among the untreated cases.

Key words: Case fatality rate (CFR), Disease prevalence rate (DPR), Fractional exposures, Intensity of disease infliction, Risk population

The sheep and goat species are unique among the domestic animals in the sense that they can be maintained under diverse environments with a little expense on housing and feeding. At the same time, these animals provide us with wool, meat, milk, leather and manure. In view of the ever-increasing strain on our land resources, the small ruminants are indeed excellent alternatives to the traditional large animals to meet at least a part of the requirement of animal protein for the economically backward sections of our society. The utility of the animals could be enhanced by adopting suitable health protection measures: disease control being the one of maximum importance. An objective assessment of the relevant parameters, however, requires detailed information on disease prevalence among the animals. This paper is an attempt to highlight the disease profile of sheep and goats in a rural area.

MATERIALS AND METHODS

The data were obtained from a demographic survey of ovines in Tiruchirapalli district of Tamil Nadu (1984-85) conducted by the Indian Agricultural Statistics Research Institute (Arya *et al.* 1991). The climate of the area is generally hot and humid. The rainfall of the district during the year was 621.5 mm which is 26.2% below the normal rainfall (Government of Tamil Nadu 1988). The sample was obtained through stratified two-stage simple random sampling without replacement. Village and household having ovines were the sampling units at the 2 stages respectively. Data were collected

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Table 1. Category-wise incidence and intensity of disease and death in sheep and goats

Species	Category of animal	Population exposed	No. of animals inflicted by disease	No.of animals died	Disese prevalence rate (%)	Case fatality rate (%)
Sheep	Lambs	3033	247	151	8.14	61.13
	Adults	6363	341	213	5.36	62.46
	All sheep	9396	588	364	6.26	61.90
Goat	Kids	6227	745	292	11.96	39.19
	Adults	8381	429	209	5.12	48.72
	All goats	14608	1174	501	8.04	42.67

through periodical visits of trained investigators to the selected households of the 105 villages in the sample for 14 months. Detailed data on the incidence of mortality and disease in ovines, their treatment and recovery and related items of information were collected.

Two parameters were adopted as indicators of the intensity of disease-infliction. The first one, viz. disease prevalence rate is the ratio of the number of animals inflicted by disease to the risk population This gives the probability of an individual animal getting inflicted by disease The risk population was obtained by the fractional exposures method (Anderson and Dow 1948, Nadkarni *et al.* 1983). Thus the partial exposures of individual animals in any specified group were considered and total animal-years exposed during the survey period were computed The other measure used was the per cent case

Table 2. Disease-wise proportions (%) of incidence, treatment, recovery, death and disposal in various categories of sheep

Disease	Category of animal	Frequency of occurrence	Relative frequency of occurrence (%)	Proportion of cases		Treated cases			Untreated cases		
				treated	untreated	proportion recovered	proportion died	proportion disposed	proportion recovered	proportion died	proportion disposed
Enteritis	Lambs	148	59.92	60.81	39.19	77.71	22.29	-	6.90	89.66	3.44
	Adult sheep	145	42.52	58.62	41.38	62.35	37.65	-	1.72	90.00	8.28
	All sheep	293	49.83	59.73	40.27	70.28	30.28	1.7	4.23	89.83	5.94
Sheep pox	Lambs	24	9.72	20.83	79.17	-	100.00	-	-	100.00	-
	Adult sheep	53	15.54	33.96	64.04	-	100.00	-	-	100.00	-
	All sheep	77	13.09	29.85	70.12	-	100.00	-	-	100.00	-
Pneumonia	Lambs	3	1.21	100.00	-	100.00	-	-	-	-	-
	Adult sheep	18	5.28	55.55	44.45	10.00	90.00	-	12.50	62.50	25.00
	All sheep	21	3.57	61.90	38.10	30.77	69.23	-	12.50	62.50	25.00
Other diseases	Lambs	72	29.15	45.83	54.17	54.54	45.46	-	-	100.00	-
	Adult sheep	125	36.66	57.60	42.40	70.83	25.00	4.17	3.77	90.57	5.66
	All sheep	197	33.51	53.30	46.70	65.71	31.43	2.86	2.17	94.56	3.27
Over-all	Lambs	247	100.00	53.04	46.96	69.46	30.54	-	3.45	94.83	1.72
	Adult sheep	341	100.00	54.25	45.75	56.76	41.08	2.16	2.56	87.82	9.62
	All sheep	588	100.00	53.74	46.26	62.02	37.02	0.96	2.94	90.80	6.26

fatality rate (Schwabe *et al.* 1977). This is the number of deaths (due to disease) per 100 cases of disease-infliction.

Disease-wise details regarding the affected cases were utilized to obtain the relative frequencies of various diseases separately for each category of the ruminants. The proportions of cases treated, recovered, died and disposed of were also worked out.

RESULTS AND DISCUSSION

The incidence of disease tabulated separately for the categories : Lamb/kid and adult sheep/goats (Table 1) shows that the young animals in each species were more prone to be inflicted by disease. The disease prevalence rate (DPR), over all diseases, for lambs was 8% while that for kids was 12% . The adults of each species had almost equal probabilities of getting a disease infliction; the DPR being 5% . The overall values of DPR for sheep and goats were 6% and 8 % , respectively, thus indicating higher susceptibility of the goats as compared to the sheep.

The case fatality rate (CFR) in respect of sheep was over 60% in each category. In goats, the rate was 39% among kids and 49% among adults; the overall CFR for the species being 43% . It is thus seen that goats, though more susceptible to disease infliction, were less vulnerable to mortality. On the

Table 3. Disease-wise proportions (%) of incidence, treatment, recovery, death and disposal in various categories of goats

Disease	Category of animal	Frequency of occurrence	Relative frequency of occurrence (%)	Proportion of cases		Treated cases			Untreated cases		
				treated	untreated	proportion recovered	proportion died	proportion disposed	proportion recovered	proportion died	proportion disposed
Enteritis	Kids	454	60.94	74.67	25.33	77.29	21.83	0.88	16.52	80.87	2.61
	Adult goats	156	46.86	68.66	31.34	66.67	30.43	2.90	12.70	85.71	1.59
	All goats	610	55.79	72.82	27.18	74.21	24.32	1.47	15.17	82.58	2.25
Goat pox	Kids	8	1.07	37.50	62.50	-	-	100.00	-	100.00	-
	Adult goats	56	2.56	81.82	18.18	-	100.00	-	-	100.00	-
	All goats	64	1.62	63.16	36.84	-	75.00	25.00	-	100.00	-
Pneumonia	Kids	9	1.21	22.22	77.78	-	100.00	-	14.28	85.72	-
	Adult goats	19	4.43	94.74	5.26	-	100.00	-	-	100.00	-
	All goats	28	2.39	71.43	28.57	-	100.00	-	12.50	87.50	-
Other diseases	Kids	274	36.78	59.49	40.51	85.89	13.50	0.61	9.01	89.19	1.8
	Adult goats	198	46.15	68.69	31.39	81.62	18.38	-	4.84	95.16	-
	All goats	472	40.20	63.35	36.65	84.28	15.72	-	7.51	91.33	1.16
Overall	Kids	745	100.00	68.05	31.95	79.29	19.33	1.38	12.61	85.29	2.19
	Adult goats	429	100.00	70.16	29.84	67.79	31.23	0.98	8.59	90.62	0.79
	All goats	1174	100.00	68.82	31.18	75.00	23.76	1.24	11.20	87.16	1.64

other hand, sheep were greatly affected by the lethal effect of disease.

Disease-wise data on proportionate frequencies of incidence, treatment, recovery, death and disposal among the animals in the sample by category are given in Tables 2 and 3 for sheep and goats respectively. The diseases with relatively high frequencies of occurrence were enteritis, sheep/goat pox and pneumonia which together accounted for about 60 to 70% of disease inflictions; all other diseases together being responsible for the remaining cases. In fact, enteritis alone caused 50% and 56% of the inflictions in sheep and goats respectively. The percentage of affected cases in category of lamb/kid was 60. About 54% and 69% of the affected sheep and goats, respectively, received treatment for their ailments while 62% and 75% of them, respectively, recovered; most of the remaining cases succumbing to the respective disease. From among the untreated cases, 91% of the sheep and 87% of the goats died since very few of them had recovery from disease.

The duration of morbidity in the affected cases was usually a week or less. The cases lasting more than a week amounted to only 24% in sheep and 20% in goats. Over 50% of the disease inflictions were found to last up to 3 days only.

ACKNOWLEDGEMENTS

The authors express gratitude to the Director, IASRI, as well as Head, Division of Sample Survey Methodology and Analysis of Survey Data, for providing requisite facilities. Thanks are due to Ms Baby George for the excellent technical support in the preparation of this paper.

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Indian Journal of Animal Sciences **68** (11) : 1106-1108, November 1998

Immunomodulatory effect of a rodent bone marrow cytokine in improving antibody response in Newcastle disease vaccinated chicks

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Received : 17 November 1997; Accepted : 10 May 1998

ABSTRACT

Outbreak of Newcastle disease (ND) in spite of vaccination is not infrequent in India. To abrogate this vaccine failure condition a nonspecies specific 12.7 kD immunomodulatory cytokine (BIM) is used. One drop of BIM (conc. 0.3 μg / 25 μl) was applied via nasal or ocular route on 5th and 11th day post R_2B vaccination and antibody to ND virus was assayed after 2 and 6 weeks of vaccination. The mean antibody (Ab) titre at 2 weeks post-vaccination of control chicks was 1 in 517.7 while the vaccinated BIM treated group had a mean Ab titre of 1 in 1821 ($P<0.001$) indicating that the flock immunity of the BIM treated chicks with respect to R_2B vaccination is more at 2 weeks compared to only vaccinated group. At 6 weeks both the groups have comparable Ab titre (control 1 in 2132, BIM 1 in 1898). BIM is heat stable which dispense the use of cold chain. This immunomodulatory role of BIM opens up a new cytokine based immunopotentiation in chicks.

Key words : Bone marrow, Chicks, Cytokine, Immunomodulator, Vaccination

Outbreak of Newcastle disease (ND) in chicken is being observed in spite of vaccination with lentogenic and mesogenic strain of ND vaccine, which is causing alarming economic loss amongst farmers in India. This vaccine failure state on an average causes 40% mortality which may rise further if the birds suffer from prolonged immunosuppression (Ghoshal 1992-1994, Chakraborty 1997, Ghoshal *et al.* 1986, Ghoshal and Bhattacharyya 1985).

To abrogate this immunosuppressed situation the use of cytokines as adjuvants with the vaccines are now being considered (Taylor 1995, Brunt 1987, Holland *et al.* 1994, Mc Dyer *et al.* 1997). Ghoshal *et al.* (1987) and Ghoshal *et al.* (1990) isolated a heat stable low molecular weight nonspecies specific, immunomodulatory cytokine (BIM) from rodent bone marrow that improved both specific and nonspecific immune system, specially in immunocompromised hosts (Ghoshal *et al.* 1990, Ghoshal and Manna 1994, Ghoshal *et al.* 1997). Field trial report on BIM during 1986 to 1996, applied via nose or eye 4-times in broilers (life span 50-55 days prior to marketing) and once per month in layers reduced significantly incidence of a number of diseases including ND. The average mortality rate in the farms under trial dropped from 40% to less than 5% in vaccinated BIM-treated birds compared to vaccinated control though the mechanism was not known (Misra 1996).

In this paper the immunomodulatory effect of BIM in improving humoral immune response to ND in F_1 and R_2B strains vaccinated chicken was compared with the vaccinated control group.

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MATERIALS AND METHODS

Selection of chicks

At State Poultry Farm, Krishnanagar, Nadia, West Bengal, 160 chicks with mean body weight 40±5 g, age 1 day, layer, mixed breed Rhode Island Red (RIR) were randomly selected and maintained in 2 pens with 80 birds in each group, viz. (i) ND vaccinated birds (control group), and (ii) ND-vaccinated plus BIM treated.

Vaccination schedule

Vaccination with F-strain vaccine was performed on the 5th and 26th day-post-hatching. At 3 months of age, 160 birds were vaccinated with R_2B strain vaccine. The HA titre of the vaccine was 1 in 320.

Preparation of immunomodulator and schedule

BIM was prepared as per Ghoshal *et al.* (1990). In brief, femurs of rats were flushed with RPMI-1640 pH 7.3. The bone marrow cells (concentration 1×10^6 cells/ml) were cultured in serum free medium at 37°C for 18-24 hr. The cell-free supernatant

Table 1. Percentage of birds attaining different antibody titre in BIM group at different times post R_2B vaccination

Antibody titre	2 weeks post R_2B % of chicks in		6 weeks post R_2B % of chicks in	
	C	B	C	B
1 in 20	3.75	1	-	-
40	-	4	-	-
80	8	13	-	3
160	15	3	6	3
320	28	11	4.6	28
640	25	18	20	15
1280	15	13	29	14
2560	3	13	12	12.5
5120	-	7	-	-
> 5120	-	15	27	23
Mean Ab titre	1 in 517.7±527.3	1 in 1821±1937	1 in 2132±1883	1 in 1898±1931
Total protein mg%	13.38±4.1 n=80	30.6±5.2 n=80	30±4 n=65	38±3.6 n=64
Statistical significance	p < 0.001		NS	

C, Control; B, BIM treated; mean ± SD.

fluid was concentrated by lyophilization and subjected to sephadex gel filtration with tris-HCl buffer. Three fractions were obtained of which the first fraction (m.wt. ≃ 12.7 kD) shows the desired immunomodulatory activity.

The BIM group received 1 drop of BIM, (cytokine concentration 0.3 μg/25 μl), once a day, via eye or nose on the 5th and 11th day post R_2B vaccination. The control group received only the scheduled vaccination.

Blood collection and antibody assay

Blood was collected from the wing vein after 2 and 6 weeks post-R_2B strain vaccination. R_2B immune status of ND was determined by standard H I test (Rai 1985).

Statistical analysis was performed by students 't' test and Wilcoxan Composite rank sum test.

RESULTS

After 2 weeks post R_2B strain vaccination the BIM treated group (Table 1) showed a significant increase in antibody titre to ND virus compared to control (Table 1).

At 2 weeks post R_2B strain vaccination 3.75% of the control birds compared to 5% of vaccinated and BIM-treated ones showed an antibody titre of <1 in 80 in spite of 2 F strain and 1 R_2B strain vaccinations against ND (Table 1). Analysis of flock immunity status of the 2 groups showed that 50% of the flock in vaccinated control had an antibody titre range between 1 in 320 and 1 in 2 560. In contrast, 50% of the BIM group had the antibody titre range between 1 in 1 280 and > 1 in 5 120.

Thus, the humoral immune response of BIM-treated birds at 2 weeks post R_2B strain vaccination was significantly higher (P<0.001) than that of control (Table 1). At 6 weeks both the groups showed 100% protective titre 1 in 80 or above (Table 1).

The total protein concentration in serum of the control bird at 2 weeks post R_2B vaccination was significantly lower than that of BIM-treated, viz. 13% vs 30 mg%, P<0.001, (Table 1). At 6 weeks the protein concentration increased significantly in the control group from 13 to 30 mg% (P < 0.001), slight increment is also noticed in the BIM group (30% as against 38 mg%) at 6 weeks or between control and BIM group at 6 weeks 30 mg% vs 38 mg% (Table 1).

DISCUSSION

It is conventional that layer chicks are twice vaccinated with F strain vaccine within the first and fourth week post hatching for protection against ND. R_2B strain vaccination is generally performed after 75 days in layers.

Following this schedule here we observed that at 2 weeks post R_2B strain vaccination, 3.75% of the control layer chicks had a very insignificant protective antibody titre of 1 in 20 (Table 1). These layer birds, therefore, in spite of vaccination suffer from a state of immunosuppression and might be potential targets for ND virus attack.

To overcome this immunosuppressive condition a modulation of the microenvironment of the immune organs seems to be essential (Bloom 1986, Khwaja and Linch 1994, Kelley 1996).

Homeostasis in the immune organs are dependent upon the production and secretion of a cascade of cytokines with a wide variety of biological functions including haemopoietic and immunomodulatory (Taylor 1995, Holland *et al.* 1994, Mc Dyer *et al.* 1997). Current report suggest that a disruption of the microenvironment by altering normal production level of cytokines seems to be a prerequisite in the establishment of pathological condition (Taylor 1995, Mc Dyer *et al.* 1997, Ghoshal and Manna 1994, Ghoshal *et al.* 1997). Can external application of proper cytokines in optimum doses reestablish the disrupted microenvironment, triggering specific immune function that fights disease?

Bone marrow is the chief organ from where lineage restricted immunocompetent cells originate from pluripotent stem cells depending upon specific signal input from the periphery that modulate production and function of specific haemopoietic cytokines that in turn regulate haemopoiesis. Studies on immunocompromised rodents showed that a low molecular weight protein secretion from bone marrow gradually diminishes as disease progressed, be it microbial or tumor (Ghoshal and Manna 1994, Ghoshal *et al.* 1997). Administration of this cytokine (BIM) seems to restore immunocompetence in these immunocompromised rodents.

BIM is secreted from bone marrow mononuclear cells (Ghoshal *et al.* 1990). This nonspecies specific cytokine improves both the specific and nonspecific immune systems and has autocrine (on bone marrow) and paracrine (on thymus, brain, kidney etc.) effect (Ghoshal *et al.* 1987, Ghoshal *et al.* 1990, Ghoshal and Manna 1994, Ghoshal *et al.* 1997).

Application of BIM improved both T and B cell functions in immunosuppressed rodents. It also modulated macrophage and neutrophil phagocytic activities (Ghoshal *et al.* 1997). Optimum BIM secretion *in vivo* was under the feedback control of vestibular nuclei of brain (Ghoshal *et al.* 1995). Stress of any form seems to disrupt the secretion of BIM and the animal became immunoincompetent (Ghoshal *et al.* 1995). Because of its nonspecific nature BIM is used on chicks to correct immunoincompetence as observed in most of the farms even after R_2B vaccination against ND. Two drops of BIM, applied on the 5th and 11th day post R_2B strain vaccination seems to improve the mean flock antibody response of the birds (Table 1) and 5% of the birds showed antibody titre less than 1 in 80. Gross immunosuppresive state (Ab titre 1 in 20) exists in only 1% compared to 3.75% in control (Table 1) and the remaining 4% had a titre of 1 in 40. Approximately 50% of the BIM-treated birds have an antibody titre of 1 in 1 280 and above compared to 18% of control i.e. only vaccinated ones (Table 1) after 2 weeks of R_2B strain vaccination. Thus, the flock resistance to the ND virus is much more in the BIM group than that of the control (vaccinated ones) within 2 weeks of R_2B strain vaccination.

At 6 weeks both the groups have 100% protection. The reason for the transient immunoincompetence of some control birds and overall depressed antibody response compared to BIM-treated was for at least 2 weeks even after R_2B vaccination needs detailed investigation.

The heat stable character of BIM (stable up to 40°-42°C) dispense the use of cold chain. Field trial has been conducted for more than 10 years in approximately 0.5 million birds with success. This opens up a new cytokine-based immunopotentiation in chicks, as was reported previously in rodents.

ACKNOWLEDGEMENTS

We thankfully acknowledge the help and encouragement received from the Superintendent and staff of State Poultry Farm, Krishnanagar; Dr Ashim Bhattacharyya, ex Director, Directorate of Animal Husbandry, Government of West Bengal; Dr Bijoyesh Choudhury, ex RO, IAH and VB; Professor P Bhattacharyya, Bose Institute. The project was supported partly by a CSIR adhoc RA project of Dr D Ghoshal.

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Indian Journal of Animal Sciences 68 (11) : 1109-1113, November 1998

Cell-mediated immune response to bovine herpes virus (BHV-1) infection in experimental animals

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Received : 4 September 1997; Accepted : 15 June 1998

ABSTRACT

Leucocyte migration inhibition (LMI) test was used to study the cell-mediated immune response in rabbits after experimental infection with BHV-1. The BHV-1 was propagated in MDBK cell line cultures with titre of $10^{8.5}$ $TCID_{50}$/ml and used for antigen preparation. The optimum concentration of antigen used was 100 µg/ml for LMIT. Rabbits (10) divided into 2 groups (A and B) of 5 each, including control were used. The peak LMI response was observed 30th day post infection (PI) with 62.90% migration inhibition in group A and 25th day PI in group B with 60.59% migration inhibition. The overall LMI response showed by the rabbits of group A was not significantly different (P>0.05) from the rabbits of group B. However, there was significant positive correlation (P<0.005) between 2 groups.

Key words : BHV-1, Cell-mediated immune respoonse , IBR, Leucocyte migration inhibition

Bovine herpes virus-1 is responsible for infectious bovine rhinotracheitis or infectious pustular vulvovaginitis (IBR/IPV) in cattle. The infection is also associated with the other clinical conditions including keratoconjunctivitis and encephalomyelitis. There has been a growing awareness that with many viruses. Cell-mediated immunity (CMI) may be the major means of defence mechanisms. The factors that allow activation and those responsible for persistence of herpes viruses are poorly understood, but it is thought that CMI may be involved in the overall process. In this study leucocyte migration inhibition test was used to study CMI against BHV-1 in rabbits as experimental model.

MATERIALS AND METHODS

Cell and virus

MDBK cell cultures were procured (National Facility for Animal Tissue and Cell culture, Pune and IVRI, Bangalore) for virus propagation.

Table 1. Febrile response in rabbits of group A and group B due to IBR virus infection

Days postinfection	Group A^{x}	Group B*
0	38.2	38.0
1	38.2	38.0
2	39.2	38.7
3	39.8	39.0
4	40.6	39.8
5	41.4	40.1
6	40.1	40.3
7	40.8	41.0
8	40.5	39.6
9	39.5	39.6
10	39.1	39.2
11	38.7	38.6
12	38.4	38.2

x Each group constitutes four rabbits.
*Each value represents average of four observations.

Virus

A field IBR isolate from an aborted cow, (Suri Babu and Mallick 1983) maintained in the lab was used for antigen preparation.

Preparation of virus

Stock virus was prepared in MDBK cell lines. MDBK cells were grown to confluency in roller bottles. Eagles minimum essential medium (EMEM) for growth and maintenance of cell cultures was supplemented with 10% calf serum and antibiot-

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Table 2. Leucocyte migration inhibition (LMI) responses of peripheral blood leucocytes in rabbits infected with infectious bovine rhinotracheitis virus-group A

Days post-infection	Unsensitized rabbits area of migration in square inches			*Sensitized rabbits area of migration in square inches			Mean % area of inhibition
(5 days interval)	without antigen	with antigen	Migration	without antigen	with antigen	Mean % area of migration	
0	13.21	13.56	1102.64	12.16	12.53	103.04	3.04
5	13.69	14.20	103.72	14.16	9.83	69.42	30.58^{S}
10	17.01	18.52	108.87	23.65	15.67	66.25	33.75^{S}
15	14.90	15.64	104.96	16.98	9.99	58.83	41.17^{SS}
20	14.32	14.56	101.67	19.15	9.85	51.43	48.57^{SS}
25	15.30	16.03	104.77	14.88	6.48	43.54	56.46^{SS}
30	14.52	15.38	105.92	13.83	5.13	37.10	62.90^{SS}
35	14.25	14.91	104.63	16.96	9.54	56.20	43.80^{SS}
40	16.35	16.55	101.22	17.18	11.15	64.90	35.10^{S}
45	15.69	16.17	102.86	13.13	9.50	72.39	27.61^{S}

*Average of 4 readings using 4 rabbits.
S = Significantly different from unsensitized (control) rabbits ($P<0.05$).
SS = Highly significantly different from unsensitized (control) rabbits ($P<0.01$).
% LMI = 100 minus mean per cent area of migration in infected rabbits.
Tests were considered positive when area of migration inhibition was more than 21%.

Table 3. Leucocyte migration inhibition (LMI) responses of peripheral blood leucocytes in rabbits infected with infectious bovine rhinotracheitis virus-group B

Days post-infection	Unsensitized rabbits area of migration in square inches			*Sensitized rabbits area of migration in square inches			Mean % area of inhibition
(5 days interval)	without antigen	with antigen	Migration	without antigen	with antigen	Mean % area of migration	
0	14.93	15.36	102.88	13.92	14.16	101.72	-1.72
5	16.98	17.33	102.06	16.46	11.48	69.91^{S}	30.09^{S}
10	15.28	15.99	104.64	20.24	12.64	62.45^{SS}	37.45^{SS}
15	17.05	18.28	107.21	16.16	9.02	55.81^{SS}	44.19^{SS}
20	16.08	16.53	102.79	16.52	7.85	47.52^{SS}	52.48^{SS}
25	18.51	19.48	105.24	12.66	4.99	39.41^{SS}	60.59^{SS}
30	17.28	17.46	101.24	13.71	5.80	42.30^{SS}	57.70^{SS}
35	16.28	16.81	103.25	14.20	8.98	63.23^{SS}	36.77^{SS}
40	17.58	18.01	102.44	15.64	10.51	67.19^{SS}	32.81^{SS}
45	16.25	16.67	102.58	14.24	10.67	74.92^{SS}	25.08^{SS}

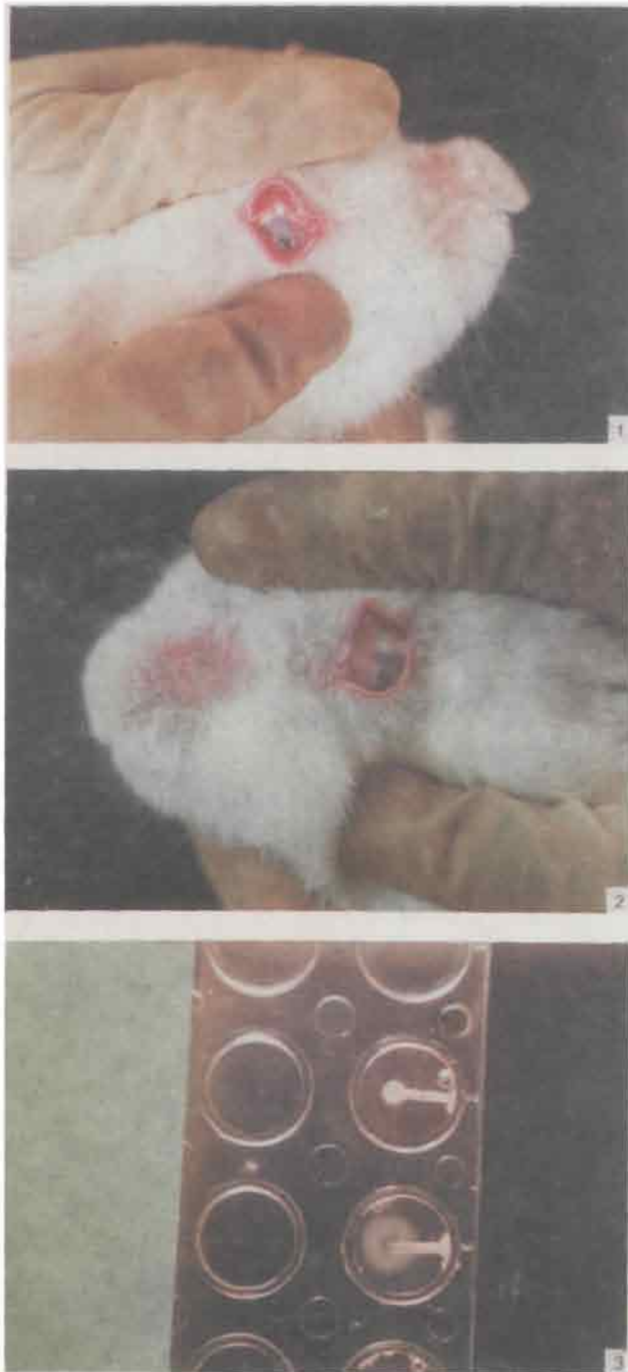
*Average of 4 readings using 4 rabbits.
S = Significantly different from unsensitized (control) rabbits ($P<0.05$).
SS = Highly significantly different from unsensitized (control) rabbits ($P<0.01$).
% LMI = 100 minus mean per cent area of migration in infected rabbits.
Tests were considered positive when area of migration inhibition was more than 21%.

ics and adjusted to pH 7.2. The cells were infected with IBR virus; virus was allowed to absorb at 37°C for 60 min. The infected monolayers were incubated at 37°C until the cells exhibited cytopathic effect was greater than 60%. Infected cultures were frozen and thawed thrice to release the virus. Cell debris was removed by centrifugation at 3 000 rpm for 15 min and stored at -70° C. The harvested infected tissue culture fluid was titrated and $TCID_{50}$ was calculated (Reed and Muench 1938). The virus titre was $10^{8.5} TCID_{50}$/ml.

Rabbits (10) apparently healthy and maintained under proper care, were divided into 2 groups of 5 each including control.

Infection of rabbits

In each group 4 rabbits were inoculated with IBR virus @250µl containing 50 µg/rabbit by different routes. Rabbits of group A were inoculated intramuscularly (I/M) and rabbits of group B were inoculated by intraconjunctival and intradermal routes. Control rabbits were inoculated with MEM medium. Prior to virus inoculation all rabbits were treated with



Figs 1-3. 1. IBR virus induced keratoconjunctivitis in rabbit 5 days after inoculation. 2. Control rabbit no conjunctivits. 3. Leucocyte migration inhibition test (LMIT). A. LMIT control without antigen showing migration of leucocytes from the rabbits infected with IBR virus. B. LMIT with antigen (IBR) showing migration inhibition of leucocytes from rabbit infected with IBR virus.

dexamethasone @ 4mg/day i/m for 7 days and rectal temperature was recorded daily. Blood was collected from both control and infected rabbits of 2 groups at 5 days interval, for 45 days to study the CMI by using LMIT.

LMI procedure

Antigen

IBR virus was used as antigen for LMIT and the estimated protein concentration in the virus was 40µg/10µl of virus (as per to Lowry Method).

Separation of leucocytes

Leucocytes were separated as per Kalaimathi *et al.* (1992).

Counting of viable and dead cells

One part of 0.5 % Trypan Blue solution was added to 1 part of the cell suspension and the mixture was kept for 10 min at 4°C. Viable and dead cells counts were made by counting unstained and stained cells respectively using a haemocytometer and total count of cells was calculated. Cell concentration in the growth was adjusted to 4×10^7/ml..

Preparation of capillaries and test proper

Capillaries were prepared (Rosenberg and David 1971). After uniformly suspending the cells in RPMI-1640 growth medium the capillaries were filled and one of the capillaries was sealed with semisoft paraffin wax. The sealed capillaries were placed in a test tube and centrifuged at $150 \times g$ for 1-2 min. Capillary tubes were removed and cut at cell liquid interphase. The cut pieces containing packed leucocytes were fixed in migration chambers with help of semisoft paraffin wax. Chambers were slowly filled with RPMI-1640 growth medium and covered with cover-slips avoiding formation of air bubbles. Four chambers were kept with medium containing specific antigen and other 4 chambers were kept as control without addition of antigen. The chambers were placed in a moist container and incubated at 37°C for 18-24 hr. Migration zone was recorded using camera-Lucida and then the area of migration zone measured with planimeter. Per cent area of migration

Table 4. Comparison of leucocyte migration inhibition (LMI)* responses of pheripheral blood leucocytes from rabbits of group A and group B to IBR virus infection

Days post-infection	Mean per cent area of leucocyte migration		Mean per cent area of leucocytes migration inhibition*	
	Group A	Group B	Group A	Group B
0	103.04	101.72	-3.04	-1.72
5	69.42	69.91	30.58	30.09
10	66.25	62.45	33.75	37.55
15	58.83	55.81	41.17	44.19
20	51.43	47.52	48.57	52.48
25	43.54	39.41	56.46	60.59
30	37.10	42.23	62.90	57.70
35	56.20	63.23	43.80	36.77
40	64.90	67.19	35.10	32.81
45	72.39	74.92	27.61	25.08

ˣEach value represents mean of 4 readings.

* % inhibition = 100-per cent area of migration in infected rabbits.

was calculated according of David and David (1971) using the following formula.

$$\text{Per cent migration} = \frac{\text{Average area of migration of cells with antigen}}{\text{Average area of migration of cells without antigen}} \times 100$$

RESULTS

Propagation of virus

The cell culture fluids were harvested after there was maximum CPE. The CPE showed mainly focal rounding and shirnkage of cells with increased granularity and finally clumping of cells giving the appearance of grape like clusters which is characteristic of herpes virus.

Clinical observations

Rabbits appeared depressed 2-3 days after inoculating. Rabbits developed conjunctivitis within 24 hr after inoculation of IBR virus into the conjunctival sac. Occular lesions were characterized by hyperaemia and edema of conjunctiva (Fig. 1). Rabbits exhibited photophobia and conjunctivitis persisted for 10 days. The eyes of control rabbits remained normal (Fig. 2). Bilateral nasal discharges were also noted from 4th day after infection. Temperature of the infected rabbits was also increased from 2 DPI persisted up to 8 DPI. Thereafter, it gradually decreased (Table 1).

Leucocyte migration inhibition test (LMIT)

Results of this test are expressed as mean per cent area of migration, compared to the antigen free leucocytes controls. "Student 't' test" was used to compare the mean per cent area of migration between control rabbits (unsensitized) and infected rabbits (sensitized). Tests were considered negative when the area of migration inhibition was less then 21%.

The results of LMIT of IBR infected rabbits of group A and group B are given in Tables 2, 3. Photographic illustration of LMIT are presented in Fig. 3.

The mean per cent area of leucocyte migration inhibition (LMI) in rabbits of group A increased in the presence of IBR antigen from the fifth day of infection onwards, reaching a peak mean of LMI responses of 62.90 on 30th day of infection and gradually decreased to 27.61 by 45th day of infection. The LMI response of the rabbit ranged between 30.58 and 62.90. A highly significant increase in LMI response ($P<0.01$) with a mean per cent area of leucocyte migration of 41.17 was observed from 15th day of infection onwards and maintained till 30th day of infection with a mean per cent migration of 62.90 and slowly receded as days advanced.

No significant difference was observed between the per cent areas of leucocyte migration of control rabbits and rabbits those were infected 5 days after infection the per cent area of migration observed in infected rabbits was 59.42 whereas in control rabbits it was 103.72.

The LMI response in the group B ranged between 30.09 and 60.59 on 5th day and 25th day, PI respectively. The rabbit showed a highly significant increase ($P<0.01$) in LMI response from 10th day PI with mean per cent area of migration of 37.55 and maintained the increased trend till 25th day PI with a mean per cent area of migration of 60.59 as that of group A rabbit.

Comparison of leucocyte migration inhibition (LMI) response of groups A and B

With the mean per cent area of LMI between the periods of the 2 groups compared, there was a significant increase in LMI response shown by the group A from 10th day to 30th day post infection (Table 4). The same trend continued in group B rabbits till 25th day post-infection. The difference between the 2 groups slowly narrowed towards the end of experiment and a significant decrease in LMI response was recorded from 30th day in group B rabbits while it started a little late by 35th day in group A.

The overall LMI responses shown by the rabbits of groupA were not significantly different when compared to that of group B rabbits with overall mean of 42.21 and 41.91, respectively, indicating the equal responses shown by both the groups of rabbits.

DISCUSSION

BHV-1 was probably introduced into this subcontinent by over ambitious and intensive development programmes by introducing new germplasm through importing exotic cattle and frozen semen. Then indigenous cattle were inseminated with it to cause great economic losses to livestock industry (Wiseman *et al.* 1979). To overcome this situation it is quite essential to study the immune responses of host against BHV-1 infection to control by means of preventive measures.

After inoculation with specified antigen into rabbits depression, conjunctivitis and lacrimation were noted within 24 hr and remained for 10 days (Figs 1, 2). These results were in agreement with Luption *et al.* (1980). Suri Babu *et al.* (1985) also reported similar findings in experiment infection of rabbits.

Leucocytes were separated for LMIT by using density gradient method with Ficoll hipaque and lytic technique by using ammonium chloride solution. The yield of leucocytes was 98% in both methods, but viability was more in ficoll hipaque method. Srinivasulu (1983), Kalaimathi and Rajendran (1994) also reported similar findings in their studies.

In this study positive CMI response by LMIT was observed on to 5 DPL in rabbits of groups A, B with 30.58 and 30.09% of migration inhibition, respectively (Tables 2, 3) which was considered positive. The LMI response continued to rise, reaching the peak at 30th day and 25th day in groups A and B respectively. These findings were in agreement with Field and Smith (1984) who reported the same to bovine respiratory syncytial virus in cattle, whereas Wisniewski *et al.* (1993) noted LMI response on 7 DPI in calves infected with IBR virus.

The results of LMI response of groups A and group B are compared and there was significant increase (P<0.05) in both groups from 10 day to 30th day and decreased towards end of experiment (Table 4). These findings are in agreement with results of Wisniewski *et al.* (1993) in calves infected with IBR virus. The overall LMI responses showed by groups A and B were not significantly (P<0.05) different and it may probably indicate the equal response of immune system to different routes used in this study for inoculation.

It can be concluded that there was LMI responses in rabbits to BHV-1 virus infections and further it was confirmed by LMIT. It is suggested that the rabbit may be taken as laboratory model for the study of immune responses to IBR virus infection nevertheless studies are warranted to critically assess the usefulness of these tests to envisage immune status of an animal with respect to reinfections to assess the recurrances of infection due to BHV-1.

ACKNOWLEDGEMENT

We thank the Principal, College of Veterinary Science, Tirupati, for providing required facilities.

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Indian Journal of Animal Sciences **68** (11) : 1114-1118, November 1998

Chicken natural killer cells – Target cell susceptibility and modulation by interferon inducers*

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Received : 22 April 1997; Accepted : 14 May 1998

ABSTRACT

Cytotoxicity assays were carried out using splenic and peripheral blood non-adherent mononuclear cells as effectors on xenogeneic (vero and BHK-21 cells) and allogeneic (CEF) targets by MTT colorimetric assay and chromium release assay (CRA). CRA was unreliable due to high spontaneous release. MTT cytotoxicity assays clearly showed the susceptibility of xenogeneic targets to chicken natural cell-mediated cytotoxicity. Healthy, secondary CEF cultures were also susceptible to lysis. Non-adherent, T and B depleted effectors were more efficient than non-adherent non-depleted cells at any given E:T ratio suggesting NK cell-mediated lysis. Peripheral blood exhibited more NK activity than their splenic counter parts. Lymphokine activation of killer cells resulted in increased cytotoxic activity at higher E:T ratios. Peripheral blood NK cell activity on vero cells was significantly increased after 48 hr of vaccination with R_2B strain of Ranikhet disease vaccine than that of at 24 hr postvaccination. A role for interferons is suggested. Synthetic RNA poly (I:C) had no augmentative effect on splenic NK cell activity after 3 hr of intra-peritoneal injection.

Key words: Chicken, Interferon inducers, Modulation, Natural killer cells, Target cells

Natural killer cells are one of the important mediators of natural cell-mediated cytotoxicity (NCMC). Unlike the T and B cells, no antigen specific receptors have been demonstrated on NK cells. Apart from their structural and morphological uniqueness, NK cells are also functionally distinct in the sense that, they can mediate nonspecific cytotoxicity on various microbes, infected cells and normal/transformed cells of syngeneic, allogeneic or even xenogeneic origin (Herberman 1986). Although mammalian NK cells have been studied in great details, little is known about their avian counterparts in spite of the fact that a lot has been learnt about the ontogeny of immune system as a whole from the avian species. Cells-mediating NK activity were detected in peripheral blood, spleen and in gut-associated lymphoid tissue in chicken (Lillehoj and Chai 1988). They have been variously shown to be non-phagocytic, non- adherent, non-B, non-T lymphomononuclear cells (Fahey and York 1987) with small lymphocyte (Sieminski and Mashaly 1992), lymphoblastoid, LGL (Schat *et al.* 1986) or granulocyte morphology (Mandi *et al.* 1990). The present investigation was carried out to study the functional characteristics of chicken NK cells.

* Part of M.V. Sc. Thesis of the first author approved by the Deemed University IVRI, Izatnagar, Uttar Pradesh.

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MATERIALS AND METHODS

Apparently healthy outbred broiler chicks, either sex, obtained from the Institute's poultry farm, were maintained under proper hygienic conditions without any vaccination and antibiotic feed additives.

Culture media

Peripheral blood mononuclear cells (PBMC) were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated, colostrum deprived, new- born calf serum (NBCS), 2.0 mM L-glutamine, benzyl penicillin (100 I U/ml), streptomycin sulphate (100 µG/ml) and N-2 hydroxyl ethyl piperazine-N'-2 - ethane sulphonic acid (HEPES) (25 mM). This is designated as RPMI growth medium (RPMI-GM). Baby hamster kidney (BHK-21) cells and vero cells were propagated in Eagle's minimum essential medium (MEM) supplemented with NBCS (10%), tryptose phosphate broth (0.3%) and L-glutamine (0.03%) with penicillin (1 00IU/ml) and streptomycin (100 µG/ml). Cultures were maintained in MEM (Eagle's) with 2% NBCS. Cells were subcultured once in 3 days. Chicken embryo fibroblasts (CEF) were obtained from 9-11-day-old embryos as per the standard protocol and were cultured in Medium-199 (M-199) supplemented with

Table 1. MTT cytotoxicity assay

Targets	Per cent cytotoxicity at different E:T ratios			
	100:1	50:1	25:1	10:1
vero*,[1]	26.25± 4.48	18.95± 1.60	10.92± 3.00	11.74± 4.22
BHK-21*,[2]	8.4± 2.88	7.63± 2.56	10.69± 1.74	12.09± 3.69
CEF*,[1]	N.D	27.19± 4.48	19.61± 7.80	25.02± 7.30
vero**,[1]	37.53± 3.75	30.28± 3.45	21.28± 2.63	17.64± 3.94

* Effectors-splenic non-adherent cells; ** Effectors - peripheral blood non-adherent cells; [1]. Mean ± SE of 6 experiments; [2] Mean ± SE of 4 experiments; ND Not done.

10% NBCS, tryptose phosphate broth (0.3%) and L-glutamine (0.03%) and antibiotics.

Preparation of MTT solution

MTT stock solution was prepared in phosphate buffered saline (PBS) @ 5mg/ml. Filtered stocks were aliquoted and stored frozen in dark. MTT working solution was made just before use by diluting the stock solution with PBS to give a final concentration of 1mg/ml and used within 2 weeks.

Raising of anti thymocyte (ATS) and antibursacyte serum (ABS) in ducks

Anti T and anti B sera were raised in ducks (Mishra and Jaiswal 1984). The cytotoxic titres of ATS and ABS were determined by tryptan blue microcytotoxicity assay with thymocytes and bursacytes respectively, using guineapig serum as a source of complement (1/5 dilution). The dilution of ATS/ABS showing more than 90% cytotoxicity was taken as the working dilution in subsequent experiments for depleting T and B cells.

Isolation of effector cells

Splenic and peripheral blood mononuclear cells were obtained (Ding and Lam 1986, Haddad and Mashaly 1992).

Table 2. Comparison of cytotoxicity mediated by NK cells and LAK cells

Targets : BHK-21
Effectors : Splenic non-adherent cells
Assay : MTT cytotoxicity assay

E:T rations	Per cent cytotoxicity *	
	NK cells	LAK cells
50:1	7.63±2.56	13.34±3.22
25:1	10.69±1.74	16.06±4.28
12.5:1	12.09±3.69	12.64±4.29

*Mean ± SE of 4 separate experiments.

Glass/plastic adherent cells were removed by incubating the mononuclear cells thrice in petri-dishes (100mm dia. 1 hr/ incubation) @ 2 × 108 cells/plate. in RPMI-GM. The resulting non-adherent cells were collected and kept at 4 ° C till further processing.

Removal of T and B cells using ATS and ABS

Plastic/glass non-adherent cells were depleted of T and B cells by successive treatments with ATS and ABS followed by lysis with guineapig complement. The dead cells were removed by passing the cells rapidly through autoclaved nylon wool columns twice.

Cytotoxicity assays

In vitro cytotoxicity assays were carried out to test the lytic potentials of chicken NK cells on various target cells like BHK-21, vero and CEF.

Standard 18 hr MTT cytotoxicity assay and chromium release assays were carried out as per Espevik and Meyer (1986) and Meyers and Schat (1990), respectively.

Lymphokine activated killer (LAK) cells

Preparation of conditioned medium (CM) : Conditioned medium was prepared (Keller 1992). Briefly, the splenic mononuclear cells were incubated with 10 μg/ml of con A, @ 2×10^6/ml. After 24 hr, the medium was harvested, centrifuged and the filtered supernatants were stored frozen in aliquots. For determining any enhancement in the cytolytic potential of lymphokine activated killer (LAK) cells, BHK-21 was used as target because this cell line was less susceptible than vero and CEF cells. Conditioned medium as a source of cytokines, was added in equal volumes to all the wells and the test was performed as mentioned earlier using MTT with proper NK cell controls. The cytotoxic potential of LAK cells was compared with that of NK cells.

Influence of R_2B strain of Ranikhet disease vaccine on natural cell-mediated cytotoxicity

Healthy birds (4) of 6 weeks of age were used in this experiment. Each bird received 1 dose of R_2B vaccine (10^5 EID50) intramuscularly. Cytotoxic activity of peripheral blood non-adherent mononuclear cells collected from these vaccinated birds on vero cells was determined at 0, 24 and 48 hr postvaccination by MTT colorimetric assay.

Modulation of NCMC by polyinosinic-polycytidylic acid [Poly(I:C)]

Apparently healthy (10), 6-8 weeks-old, broiler chicks of either sex were used in this study (6 as control and 4 as experimental). Each bird of the experimental group received 1 ml of poly (I:C), (1mg/ml in PBS) intra-peritoneally and the control group received 1 ml of sterile PBS alone. Birds of both groups were sacrificed 3 hr after injection and spleens collected. The

Table 3. Influence of Ranikhet disease vaccine (R_2B) on natural cell-mediated cytotoxicity

Target : Vero
Effector : Peripheral blood lymphocytes
Assay : MTT cytotoxicity assay

Hours post-vaccination	Per cent cytotoxicity at different E:T ratios#		
	50:1	25:1	12.5:1
0	20.39±1.38	13.75±0.26	14.05±2.01
24	12.81±3.01	14.88 ± 2.05	16.01 ± 2.11
48	44.64±7.86**	31.60±3.64*	29.05±2.51*

Mean ± SE of 4 experiments; * $P< 0.03$; ** $P< 0.06$.

cytotoxic activity of splenic non-adherent mononuclear cells on vero cells was determined by MTT assay (Espevik and Meyer 1986).

Statistical analysis

The results were tested for their statistical significance by students 't' test.

RESULTS

MTT cytotoxicity assays

With vero, the per cent cytotoxicity generally increased as the E:T ratios increased and with BHK-21 the reverse was seen (Table 1). When CEF were used as targets, the cytotoxicity mediated by the effectors depleted of T and B cells were as good as T and B undepleted effectors (data not shown). But the per cent lysis without T and B cells were slightly higher because removal of T and B cells causes a relative increase in the NK cell numbers among the effector cells since the latter are added in the same numbers as the former corresponding to a particular E:T ratio. Hence, the subsequent experiments (Table 1) with CEF were done only using non-adherent mononuclear cells as effectors without further depleting T and B cells.

Cytotoxicity assays clearly showed that cells mediating natural cell-mediated cytotoxicity are also present among the peripheral blood non-adherent cells. Vero cells were the best xenogenic target with both splenic and peripheral blood non-adherent cells. Chromium release assays were carried out to compare with MTT colorimetric assay. The spontaneous release of the isotope from the labeled cells was too high (35 to 60%) and the results obtained were highly inconsistent and insensible (data not shown).

LAK cells exhibited an increased cytotoxic activity when compared with their uninactivated counterparts (Table 2). A 6% increase was found at the E:T ratios of 50:1 and 25:1. At 12.5:1 the difference was negligible.

Ranikhet disease vaccination had a marked stimulatory effect on peripheral blood NK cell activity. The effect was more pronounced at 48 hr postvaccination rather than that of at 24 hr (Table 3). At 48 hr postvaccination, the increase was statistically significant ($P<0.05$) at the E:T ratios of 25:1 and 12.5:1. Immunomodulatory role of poly(I:C) on chicken natural cell- mediated cytotoxic activity was assessed by MTT assay. Poly (I:C) though slightly increased the splenic NK cell activity, in the treated group, it had no significant immunomodulating activity (Table 4).

Table 4. Modulation of natural cell-mediated cytotoxicity by Poly (I:C)

Target : Vero
Effector : Splenic non-adherent cells
Assay : MTT cytotoxicity assay

Group	Per cent cytotoxicity at different E:T ratios			
	100:1	50:1	25:1	10:1
Control*	26.25± 4.48	18.95± 1.60	10.92± 3.00	11.74± 4.22
Treated#	29.66± 3.88	19.02± 4.35	12.86± 1.16	13.23± 2.03

Mean ± SE of 6 experiments; # mean± SE of 4 experiments.

DISCUSSION

MTT assay is widely being used to study the *in vitro* effects of lymphokines, chemosensitivity of tumor cell lines, bactericidal assays and in cell-mediated cytotoxicity assays because MTT basically detects the live cells (Espevik and Meyer 1986 and Heo *et al.* 1990).No literature is available at present on the use of MTT in assessing cell-mediated cytotoxicity in chickens and farm animals.

The effectors of avian spontaneous cell-mediated cytotoxicity are plastic/glass/nylon-wool non-adherent, non-T, non-B lymphocytes as their mammalian counter parts (Lam and Linna 1980, Sharma and Okažaki 1981). Cells-mediating NK activity is present in spleen, peripheral blood and in gut-associated lymphoid tissue in chicken (Fahey and York 1987).

Many allogeneic and xenogenic target cells were screened for their susceptibility to MHC-unrestricted, spontaneous, NK-cell-mediated lysis in many animal species (Susan and Krakowka 1985) and chicken (Leibold *et al.* 1980, Sharma and Okazaki 1981, Haddad and Mashaly 1991). No report is available at present on the susceptibility of vero and BHK-21 cells to chicken natural cell- mediated cytotoxicity. The results of MTT cytotoxicity assays of the present investigation clearly showed that these cells are efficiently lysed by chicken NK cells. Vero cells were better targets compared to BHK-21 cells. NK cells were demonstrated in the peripheral blood of chickens (Haddad and Mashaly 1991, 1992). Experiments conducted in the present study support these findings. Since (i) plastic/glass adherence removes majority of the monocyte/macrophages, (ii) cytotoxic T lymphocytes can not lyse these targets due to MHC incompatibility (if at all they express any) and, (iii) lack of priming to these target cells, it can be concluded that the cytotoxicity is mediated by NK cells.

Chicken NK cells can lyse various transformed (Sharma

and Okazaki 1981) and virus-infected allogeneic target cells (Meyers and Schat 1991). On the otherhand, allogeneic, healthy secondary CEF and cultured-chicken kidney cells were resistant to chicken NK cell-mediated lysis obtained from specific inbred lines of chickens (Sharma and Coulson 1979, Meyers and Schat 1990, respectively). Interestingly, in this study healthy, allogeneic CEF was susceptible to NK cell-mediated cytotoxicity. NK cell activity was greatly influenced by the genetic makeup and age of the donor birds (Sharma and Coulson 1979, Sharma 1981, Sharma and Okazaki 1981, Chai and Lillehoj 1988). Moreover, murine and human NK cells express killer inhibitory receptors (KIRs) for specific MHC class I allotypes . Expression of corresponding MHC class I molecules on the target cells protect them from NK cell-mediated lysis (Janeway and Travers 1996). In view of these reports and the data obtained in this investigation, it can be concluded that NK cells from the outbred, broiler strain of chickens are cytotoxic to healthy, allogeneic CEF.

Natural killer cell activity was modulated by cytokines. Interleukin-2 (IL-2), a T-cell derived cytokine is a potent enhancer of NK cell activity *iv vivo* and *in vitro* . Conditioned medium containing the crude mixture of various cytokines is being used as a source of IL-2 to activate chicken NK cells *in vitro* (Keller 1992, Keller *et al.* 1992). Even in this study splenic NK cell activity increased, though not significantly, upon incubation with conditioned medium. This moderate increase in NK activity could be due to shorter incubation with CM. Moreover, in this assay the CM was added to the E:T mixture rather than using pre-ctivated NK cells.

Interferons (IFN) augment the cytolytic activity of NK cells (Herberman 1986). Human adenovirus (Mandi *et al.* 1982, 1984), Marek's disease virus (Sharma 1981) or M.D vaccine Herpes virus of Turkeys vaccine (Sharma 1981, Heller and Schat 1987) enhanced NK cell activity in chickens by inducing interferons.

RDV, a potent inducer of IFN *in vivo* and *in vitro* (Bankowski and Kaleta 1972), can augment splenic NK cell activity in chicken (Ding and Lam 1986). Mishra *et al.* (1984), reported that R_2B vaccine can give significant protection by 50 hr post-vaccination, to challenge with virulent Mukteshwar strain of RDV through interferon. No report is available at present on the augmentation of NK activity by Mukteshwar vaccine strain of Ranikhet disease virus. So, in the present investigation the immunomodulating role of this vaccine virus on peripheral blood NK activity was studied. Cytotoxicity was significantly higher at 48-hr postvaccination ($P<0.05$) than at 0 or 24 hr postvaccination at all E:T ratios. The delay in the onset of augmentation may be due to the low dose of the virus used and the type of virus (mesogenic strain) used for vaccination. Synthetic polynucleotides like Poly (I:C), are very potent inducers of interferons and thus can augment NK cell activity (Quinn 1990). Our results showed that Poly (I:C) does not have any significant augmentative activity on chicken splenic NK cells. This is similar to previous observations (Heller and Schat 1987, Ding and Lam 1986). In poly (I:C), the ineffectiveness could be due to rapid hydrolysis by nucleases (Hartmann *et al.* 1987) in chickens *in vivo* because, poly (I:C) efficiently induced IFN in CEF cultures *in vitro* (Ding and Lam 1986).

To show the superiority of MTT-based cytotoxicity assay over chromium release assay, long term (18 hr) cytotoxicity assays were performed with 51Cr labeled-vero, BHK-21 and CEF as targets. Our results also clearly showed that for long-term cytotoxicity assays 51Cr-based assays are not suitable because of high spontaneous release (Heo *et al.* 1990, Keller *et al.* 1992) and in such situations MTT-based assays are more reliable and reproducible.

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Indian Journal of Animal Sciences **68** (11) : 1119-1120, November 1998

Resistogram typing of *Salmonella* serotypes of avian origin

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Received : 22 April 1997 ; Accepted : 14 May 1998

ABSTRACT

The resistogram typing of the 65 *Salmonella* isolates of poultry and its environment has shown 36 different patterns. Among the various dyes and chemicals used organisms evinced a high rate of resistance to the chemicals such as 0.7% copper sulphate (81.54%) and 0.003% malachite green (78.46%) followed by 0.09% acriflavine (69.23%) and 0.0006% phenyl mercuric nitrate (67.69%). The copper sulphate and malachite green have the least discriminating ability in the differentiation of strains in resistogram typing. Among the dyes and chemicals used only 3 out of 65 isolates have shown resistance to 0.7% 4-chloresorcinol.

Key words : Resistogram typing , *Salmonella* serotypes

In general, typing within a bacterial species is restricted essentially to the use of serology or phage or to colicine susceptibility. There are a few other unexplored methods of typing such as resistogram which is not commonly applied in contrast to serological or phage typing (Hinton 1985). Many antibacterial substances when tested in critical concentration overlooked in the past because antiseptics are commonly tested only in crude steps and often against a single arbitrarily choosen "standard" organism of the species. On the other hand, both phage and colicine typing are based on sophisticated form of selective toxicity. The more immediate method of using the selective toxicity of chemical substances has hitherto not been fully explored.

The present study is aimed at screening poultry and its environment, viz. cloacal swabs of culled, sick and reproductive birds and also from those caecal swabs collected from dressed broiler birds, fish-meal meant for incorporating into poultry feed and poultry feed as a whole for *Salmonella* organisms. These organisms were subjected to resistotyping with sodium arsenate, phenyl mercuric nitrate, boric acid, acriflavine, 4-chloresorcinol, copper sulphate and malachite green to assess the usefulness of these chemicals in resistotyping.

MATERIALS AND METHODS

Strains

The different strains of *Salmonella* were isolated from 3 450 materials of avian sources and environment such as cloacal swabs, poultry feeds, poultry litter, fish-meal and caecal swabs. The isolates were serotyped at the Central Research Institute, Kasauli, Himachal Pradesh.

Dyes and chemicals

The resistogram typing (Elek and Higney 1970) with per cent concentration (w/v) mentioned was used for the study, sodium arsenate (A) (0.75), phenyl mercuric nitrate (B) (0.0006), boric acid (C) (3.0), acriflavine (D) (0.09), 4-chloresorcinol (E) (0.7), copper sulphate (F) (0.7) and malachite green (G) (0.003). The concentrations were optimal for the *Salmonella* isolates of this study as well.

Resistotyping

Of 8 nutrient agar base plates 1 was used as control and to the remaining 7 was added either the dye or the chemical of the stipulated concentration in 3 ml amount when added to 27 ml of the agar base. The strains were grown overnight in 1 ml of nutrient broth in 10 ml of screw capped bottles and diluted to 1:10 with the addition of 9 ml of sterile distilled water. Each petri-dish plate was dried for 20 min at 37°C before inoculation at a particular point for bacterial growth by means of Takatsky microtitre loop and a reference point was encircled with glass marking pencil for subsequent reading. The spots of inocula were allowed to disappear before the plates were incubated at 37°C for 18 hr. The growth pattern was recorded as either full confluent growth or with confluent growth of very small colonies or non-confluent growth or more than 10 colonies or absence of growth.

RESULTS AND DISCUSSION

The strains differing in their description by 1 bracket

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Table 1. Resistotype of *Salmonella* strains belonging to different serotypes

Serotypes	Resistotype patterns*
S. typhimurium	BDFG(2) ; (B) (C) DFG (1) ; BCD (F) G-1 ; (C) (D) FG (1) ; (C) (D) (F) G - (1) ; ABDF (1) ; (A) B (C) DFG (1) ; DFG (1) ; (B) - (1) ; D - (1) ; G - (1)
S. virchow	ABCDFG - (2) ; BCDFG - (3) ; CDFG - (2) ; (A) BCDFG - (1) ; (A) CDFG - (1)
S.kentucky	DFG - (2) ; BCDFG - (3) ; CDFG - (2) ; (A) BCDFG - (1) ; (A) CDFG - (1)
S. sandiego	BFG-(2) ; ABDFG- (1) ; BD(F)G-1; (A) FG - (1)
S. senftenberg	(A)B(C)(F)-(1) ; A(C)F-(1) ; ABDFG- (1) ; B - (1)
S. butantan	AB (C)D(F)G-(1);(A)BD(F)G-(1);A(B)G-(1); BDFG-(1)
S.chester	ABDFG-(1) ; ABDEFG - (1) ; (A) B (C) D (F) G - (1)
S. nchanga	(A) B (C) EF - (1) ; (A) DFG - (1)
S. havana	BD(F) G-(2); ABD(F) G - (1) ; D(F) G - (1) CDFG - (1)
S. wentworth	(B) (C) - (2)
S. bredeney	BDG - (2)
S. cerro	AB (C) DEFG - (1)
S. albany	AB (C) -(1)
S. litchfield	DFG - (1)
S. newport	ABC(F) - (1)
S. bareilly	BDFG - (1)
S. telhashomer	(A)BDFG - (1)
S. gallinarum	AD(F)G-(1)
S. agona	B(C)-(1)
S. california	BFG-(1)
S. braenderup	B-(1)

(intermediate) have been regarded as identical and those differing by a letter or by 2 or more brackets regarded as belonging to a different class. The resistogram pattern of individual serotypes is grouped under Table 1 for 65 *Salmonella* isolates.

Each capital letter denotes that the strain evinces full growth on a medium containing the test concentration of the dye/chemical. A letter within brackets denotes the strain exhibiting partially inhibited growth in the presence of the antibacterial dye or chemical.

Among the dyes and chemicals used greater resistance was evinced by the majority of the isolates to copper sulphate, and among the isolates resistance was seen in descending order to malachite green, acriflavine, phenyl mercuric nitrate, boric acid, sodium arsenate and 4-chloresorcinol.

Somerville *et al.* (1983) differentiated *S.typhi* based on this technique. This technique is economical and easy to perform. The resisto gram typing of all the 65 *Salmonella* isolates comprising 21 serotypes showed that 36 different patterns of resistogram could be obtained distinguishing the serotypes also. Among the various dyes and chemicals used the organisms evinced a high rate of resistance to the chemicals such as copper sulphate (81.54%) and malachite green (78.46%) followed by acriflavine (69.23%) and phenyl mercuric nitrate (67.69%). This observation indicated that the *Salmonella* are more commonly encountered in an atmosphere containing these chemicals probably copper sulphate which is used as a feed additive in the feed (Hinton 1985) and other chemicals because of industrial pollution. The copper sulphate and malachite green have the least discriminating ability in the differentiation of strains in resistogram typing. The value of these chemicals in differentiating the strains is much less compared to other chemicals.

Among the dyes and chemicals used, only 3 isolates among 65 isolates have shown resistance to 4-chloresorcinol, indicating the rarity of this chemical in resistogram typing. Hence this chemical will be a useful adjunct with acriflavine (69.23%), phenyl mercuric nitrate (67.69%), boric acid (43.08%) and sodium arsenate (41.54%), to which *Salmonella* exhibited a reasonable variation in the resistogram pattern.

In situations wherein serotyping is not possible, the resistogram types can be used to establish the strain identify. Thus under such circumstances the use of resistotyping with dyes and toxic chemicals can be very useful in the strain identification in an epidemiological situation because of its simplicity.

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Indian Journal of Animal Sciences 68 (11) : 1121-1125, November 1998

Evaluation of fixation devices for resistance of bending and rotations in supracondylar femoral fracture fixation in dogs

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Received : 20 October 1997; Accepted : 15 May 1998

ABSTRACT

Transverse supracondylar femoral fracture was done in 20 dogs, divided equally in groups, A, B, C and D. Fixation was done using cross intramedullary pins (group A), single pin passed through the intercondyloid fossa (group B), standard stainless steel plate (group C) and properly contoured horn plate (group D). Intramedullary pins (groups A and B) could resist bending forces more effectively compared to plates (groups C and D) and maximum bending was recorded in stainless steel plated animals. Rotation of the distal fragment was more with single pin fixation (group B) than any other group. Horn plating effectively counteracted both rotational and bending forces and thus provided rigid fracture fixation.

Key words : Bending, Dogs, Fixation devices, Rotation, Supracondylar femoral fracture

Fractures of the distal femur, close to or involving the condyles, are frequently reported in dogs (Alcantara and Stead 1975, Kolata *et al.* 1974). Small and curved distal fragment and proximity of the fracture to the joint make effective reduction of such fractures difficult. Several modified techniques of pin and plate fixation have, therefore, been used to treat such cases with variable results (Campbell 1976, Parker and Bloomberg 1984, Whitney and Schrader 1987, Lewis *et al.* 1993). Some of the basic forces acting at the fracture site include rotational, bending and shearing forces. Any fixation technique used to immobilize the fracture should be able to neutralize these inherent forces to prevent motion at the fracture site, which inturn would enhance vascular ingrowth and fracture healing (De Young and Probst 1985). The ability of a particular technique to resist these forces *in vivo* can only be measured by recording the angular deformities (such as bending and rotation) at the fracture site. Hence, in the present study, an attempt has been made to evaluate different techniques of fixation used for supracondylar femoral fracture by recording the angles of cranial/caudal bending, medial/lateral bending and rotation at the fracture site.

MATERIALS AND METHODS

Clinically healthy adult mongrel dogs (20) of either sex, divided randomly into groups, A, B, C and D were kept off feed and water for 24 hr and 12 hr, respectively. Left lateral femoral region was prepared for aseptic surgery. The animals were premedicated with atropine sulphate (0.65 mg) and triflupromazine hydrochloride (1 mg/kg), given intramuscularly, 15 min before the induction of anaesthesia with 5% solution of thiopental sodium. The animals were secured in right lateral recumbency and the femoral condyles were approached through lateral parapateller arthrotomy (Piermattei and Greeley 1979). The condyles were exposed and a transverse fracture was created 'just above' the condyles using an orthopaedic saw.

In animals of group A, cross intramedullary pins were used to immobilize the fracture fragments, in the manner of Rush pins (Howard 1991) whereas, single Steinmann pin was used by normograde technique through the intercondyloid fossa, just cranial to the cruciate ligaments in group B. In animals of group C, a 4-hole stainless steel bone plate (4 cm long, 12 mm wide and 1.5 mm thick) and screws were fixed, whereas in group D properly contoured-horn plates with 5 holes (6.5 cm long, 14 mm wide and 3 mm thick) were fixed along the carniolateral aspect of the bone. After the fixation of fracture with a particular technique, the arthrotomy incision was closed using 3-0 catgut in simple continuous pattern. The muscles, fascia and skin were sutured in a routine manner.

Postoperatively, all the animals were administered (intramuscularly) with streptopenicillin (0.5 g bid) for 6 days and phenylbutazone (300 mg) for 3 days. Regular cleaning and antiseptic dressing of the surgical wound was undertaken till the sutures were removed after 8-10 days. All the animals were maintained up to 90 days, then sacrificed and both the femurs were collected. Craniocaudal and mediolateral radiographs of both the femurs were taken to measure the carniocaudal and mediolateral angulation/bending. The ex-

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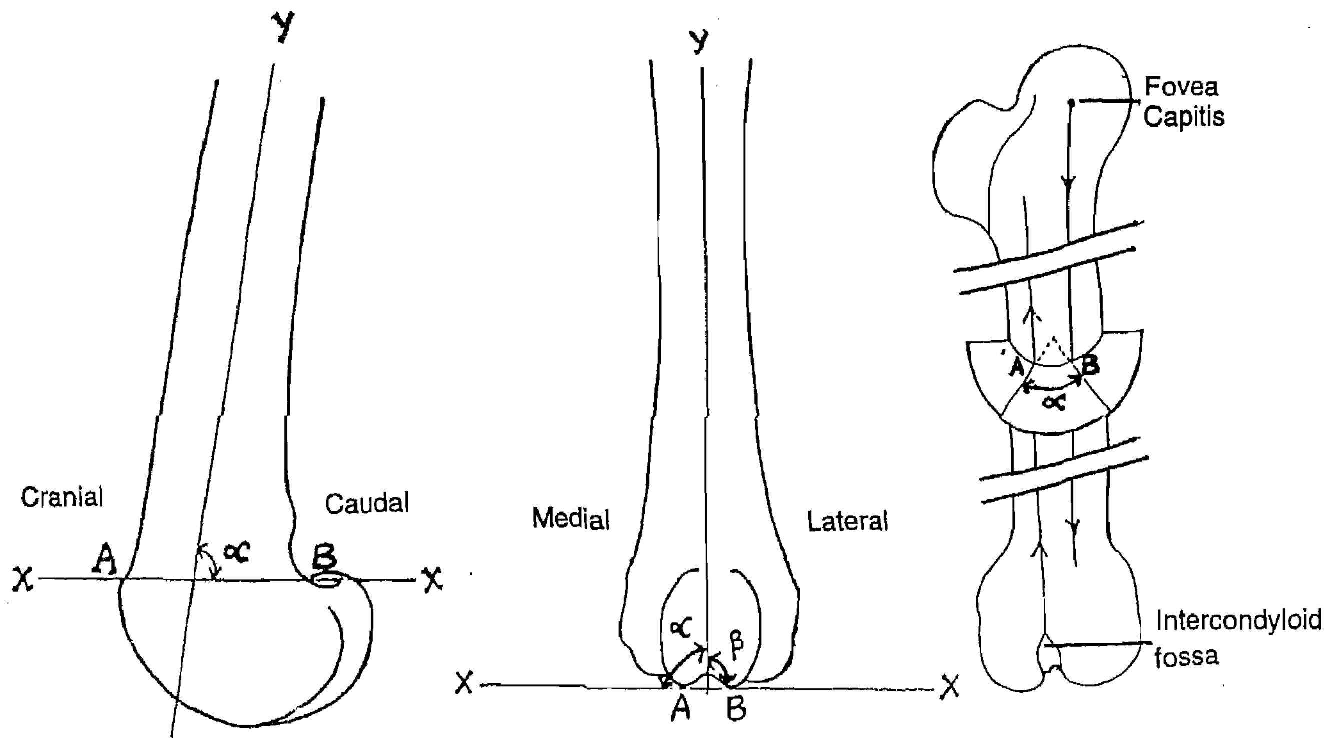


Figs 1-3. 1. Measurement of cranicocaudal angulation at the fracture site. 2. Measurement of mediolateral angulation at the fracture site. 3. Measurement of rotation at the fracture site.

tent of rotation at the fracture site was measured using the test bone itself.

Craniocaudal angulation was measured using the mediolateral radiographs of both the femurs. Using a tracing paper, a line 'X' was drawn along the junction of epiphysis and metaphysis, passing through the points A and B (Fig. 1). Another line 'Y' was drawn parrallel to the longitudinal axis of femoral diaphysis. The angle (alpha) formed at the intersection of X and Y was measured for both 'test' and 'normal' femur bones. The extent of reduction in the angle 'alpha' in test bone as compared to that of normal bone was considered as degree of caudal bending.

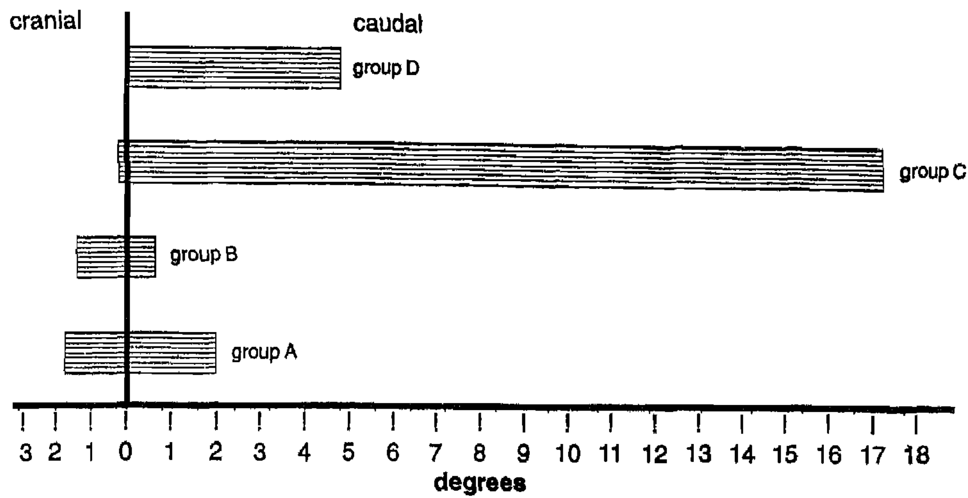
Mediolateral angulation was determined using the carniocaudal radiographs of both femurs. Using a tracing paper, a line 'X' was drawn connecting the lowest points of medial and lateral femoral condyles (points A and B). The line 'Y' was drawn parallel to the longitudinal axis of the femoral diaphysis (Fig 2). The angles obtained at the transaction of 'X' and 'Y' lines were designated as α and β (medial and lateral, respectively). The angles α and β of the test bone were compared with that of contralateral normal bone. If there was any decrease in α, it was considered as medial bending, if there was decrease in β, it was considered as lateral bending.

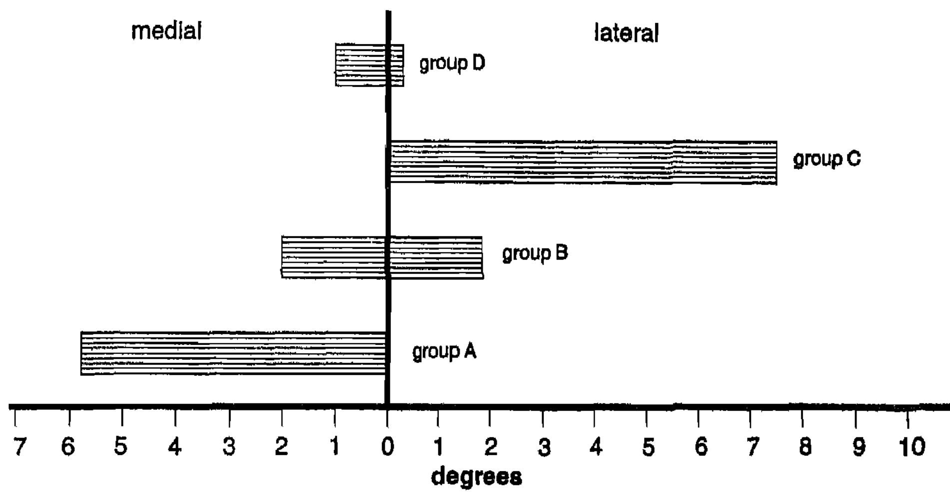
An attempt was made to determine the extent of rotation at the fracture site using the bone specimens. After the bones were collected, the moisture from bone surface was removed using a piece of tissue paper. The landmarks were located-fovea capitis at the proximal end and intercondyloid fossa at the distal end. Then using a thin thread soaked in ink, a line was drawn from fovea capitis along the long axis of femoral diaphysis. Another parallel line was drawn from the centre point of intercondyloid fossa along the femoral diaphysis. The angle of the arch formed between the 2 parallel lines was measured at the mid-diaphysis (between A and B) using a scale (Fig 3). This angle was compared with that of contralatral bone. If there was any reduction in this angle, it was considered as medial rotation of distal fragment and if there was an increase in the angle, it was considered as lateral rotation.

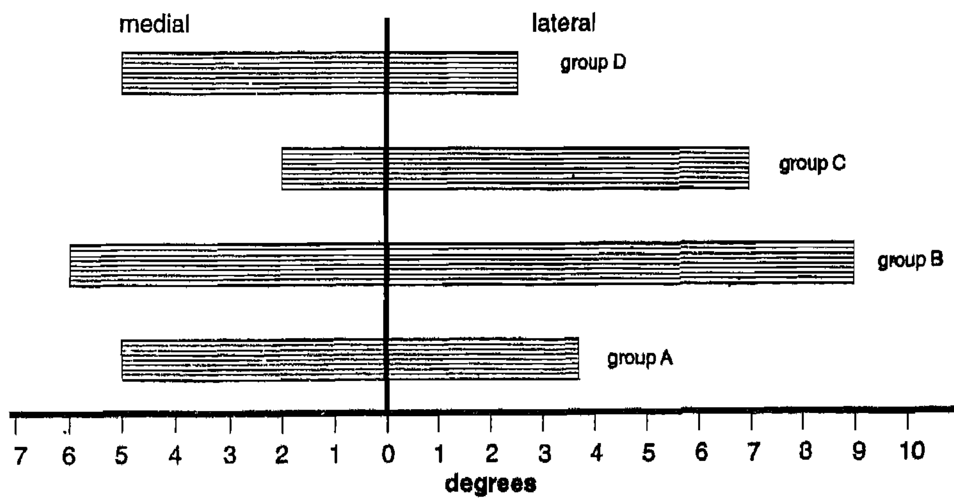
The length of the test bones was also measured using radiographs taken on day 90 and were compared with that of contralateral bone. Craniocaudal views of the radiographs taken after collecting the bones were utilized for the purpose.

RESULTS AND DISCUSSION

In animals of groups A and B, craniocaudal bending was lesser than the animals of groups C and D (Fig. 4) indicating that IM pins resisted bending forces more effectively due to the position of IM pins within the medullary cavity near the neutral axis of the bone (Smith 1985, Schwarz 1991). One animal in group A and 3 animals in group B showed slight cranial bending of bone suggesting little over reduction of the distal







Figs 4-6. **4.** Mean cranial/caudal bending at the fractures site (in degrees) in animals of different groups. **5.** Mean medial/lateral bending at the fractures site (in degrees) in animals of different groups. **6.** Mean medial/lateral rotation at the fractures (in degrees) in animals of different groups.

fragment at the time of fixation. Caudal bending of bone was maximum in animals of group C suggesting that stainless steel plates failed to neutralize bending force effectively. This may be attributed to relatively small length and less strength of stainless steel plates in relation to the bone. Stress can be better distributed over a greater length of loaded bone, if length

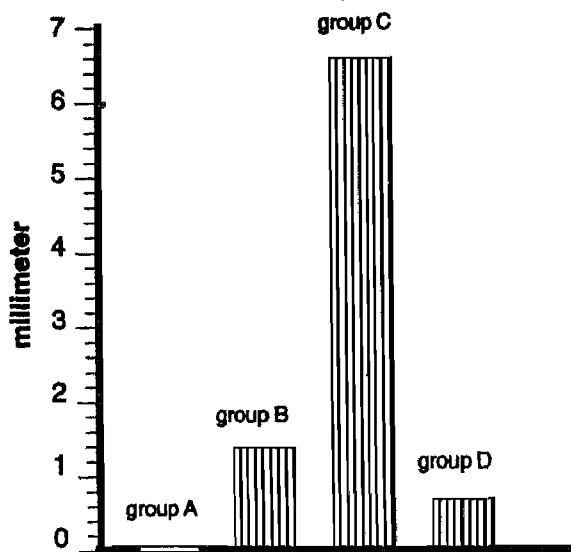


Fig. 7. Mean shortening of test bones (in milimeters) when compared with contralateral normal bones in animals of different groups.

of plate is increased (Perren 1989). In this study, however, as the fracture was very close to the epiphysis and also due to the caudal bend of the distal femur, it was not possible to fix longer stainless steel plates. It was interesting to note that, when the length of bone increased, the degree of bending also increased, indicating that the bending stress acting at the fracture site was proportional to the bone length. In animals of group D bending was less than that of animals of group C, this was attributed to the fixation of properly contoured (to the lateral surface of distal femur) horn plate along the long axis of the bone. Secondly, relatively longer plates were fixed with more number of screws in animals of this group. Further, horn plate being more elastic than stainless steel plate, can bounce back to its original position after some degree of bending and thus maintain axial alignment (Singh 1986).

Medial/lateral bending was also less with IM pinning groups as compared with plating groups. All the animals of group A showed bending (Fig 5); this may be attributed to slight malalignment at the time of fixation rather than subsequent bending as suggested by Newton and Nunamaker (1985) with Rush pins. Either medial or lateral bending was seen in animals of group B, suggesting that with single IM pin bending can occur at any direction. In animals of group C maximum lateral bending might be due to the heavy muscle mass on the lateral side. In animals of group D, medial/lateral bending was also less than that of group C, indicating fracture fixation with horn plates.

Rotational force is present in most fractures and is specially a problem in transverse/slightly oblique long bone fractures that do not interdigitate (De Young and Probst 1985). In this study, animals of all groups showed either medial or lateral rotation of the distal fragment (Fig. 6), suggesting that rotation of distal fragment can occur at any direction after supracondylar fracture fixation in femur. A relatively less rotation was seen in animals of groups A and D, indicating rigid fracture fixation. Maximum rotation was seen in animals of group B followed by group C.

Maximum rotation seen in animals where single IM pin fixation was done (group B) confirms the observations of De Young and Probst (1985) and Smith (1985). One animal of this group, which developed osteomyelitis, recorded maximum rotation (25°-laterally). It may be attributed to unstability at the fracture site because of delay in healing and necrosis at the fracture ends. A slight rotation was seen in animals of group A where cross IM pins were used. However, De Young Probst (1985) observed that 2 IM pins aid in counteracting rotational forces. Among groups C and D, rotation was more in stainless steel plate animals (group C). Rotational forces are least efficiently stabilized by plate fixation, unless frictional forces from fragment compression also are used (Perren 1979).

In this study, some degree of shortening was seen in animals of all groups (Fig. 7), however, maximum shortening was seen in animals of group C. It could be attributed to bending at the fracture site. The extent of shortening was directly proportional to the degree of bending at the fracture site, confirming that shortening of bone was attributed mainly to bending. Shortening was least in group A (nil), followed by groups D and B.

Our study suggests that intramedullary pin(s) can resist bending stress more effectively than plates. Rotation of distal fragment was maximum with single pin fixation. Properly contoured horn plates can effectively counteract bending and rotational stresses and thus provide rigid fixation of supracondylar femoral fractures.

ACKNOWLEDGEMENTS

We thank Mr H C Setia, for the help in taking radiographs.

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Indian Journal of Animal Sciences **68** (11) : 1126-1129, November 1998

Paralytic effect of fasciolicidal agents on rhythmic motility of *Fasciola gigantica*

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Received: 17 November 1997; Accepted : 8 May 1998

ABSTRACT

Effect of some clinically used fasciolicides was assessed on the rhythmic motility of *Fasciola gigantica* to evaluate their possible action on the neuromuscular system of the fluke. The mature flukes collected from the local abattoir were either mounted isometrically or incubated in Hedon-Fleig solution. Hexachlorophene (10^{-6} M), niclofolan (10^{-5} M) and oxyclozanide (10^{-6} M) induced rapid, persistent and tonic paralysis of the flukes. However, rafoxanide (3×10^{-4} M) caused slow, partial and flaccid paralysis of the fluke. Albendazole and triclabendazole did not show any paralytic effect on fluke rhythmicity. The gross visual motility was inhibited completely within half an hour of incubation and the flukes were paralyzed in tonic state by hexachlorophene (10^{-5} M), niclofolan (10^{-5} M) and oxyclozanide (10^{-5} M), while the same was inhibited slowly by rafoxanide, albendazole and triclabendazole. It is concluded from the results that hexachlorophene, niclofolan and oxyclozanide produced their fasciolicidal effect by affecting neuromuscular system of the fluke whereas rafoxanide, albendazole and triclabendazole may have effect on energy metabolism and/ or may be affecting the tegument of the fluke. The results may facilitate developing combination therapy against *F. gigantica*.

Key words: *Fasciola gigantica*, Fasciolicides, Paralysis

In the absence of effective vaccines against helminth parasites, chemotherapy remains to be the most effective means to combat the parasitic infection. There are some preliminary reports on the effects of fasciolicidal agents on rhythmic motility of *Fasciola hepatica* (Fairweather *et al.* 1984) and acetylcholinesterase of *F. hepatica* and *F. gigantica* (Durrani 1980). Carbon tetrachloride induced tonic paralysis of *F. gigantica in vitro* (Kumar *et al.* 1995a). However, such studies are completely lacking with respect to commonly used fasciolicides on the rhythmic motility of *F. gigantica*, an economically important liverfluke in the Indian subcontinent. Thus, keeping in view the importance of neuromuscular physiology of trematodes in general (Geary *et al.* 1992) and that of *F. gigantica* in particular, an effort has been made in this study to evaluate the acute effect of different concentrations of the available fasciolicidal agents as potential targets for the development of selective fasciolicidal agents., on the motility of *F. gigantica in vitro*.

MATERIALS AND METHODS

Collection, mounting and isometric recording of spontaneous motility of *F. gigantica* was done as per Kumar

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Table 1. Effect of different concentrations of various fasciolicides on gross visual motility of *Fasciola gigantica* after 15 min, 30 min and 4 hr of incubation

Fasciolicidal agent	Concentration (M)	Gross visual motility at different intervals			
		0 hr	15 min	30 min	4 hr
Hexachlorophene	10^{-7}	+++	+++	+++	±
	10^{-6}	+++	++	++	0
	10^{-5}	+++	±	0	0
Oxyclozanide	10^{-7}	+++	+++	+++	±
	10^{-6}	+++	++	++	0
	10^{-5}	+++	±	0	0
Niclofolan	10^{-7}	+++	+++	+++	±
	10^{-6}	+++	++	++	0
	10^{-5}	+++	±	0	0
Rafoxanide	10^{-7}	+++	+++	+++	±
	10^{-6}	+++	+++	+++	0
	10^{-5}	+++	+++	++	0
Albendazole	10^{-7}	+++	+++	+++	+
	10^{-6}	+++	+++	+++	+
	10^{-5}	+++	+++	+++	+
Triclabendazole	10^{-7}	+++	+++	+++	+
	10^{-6}	+++	+++	+++	±
	10^{-5}	+++	++	++	±
Control		+++	+++	+++	+++
Vehicle		+++	+++	+++	+++

0, Nil; ±, feeble; +, poor; ++, moderate; +++, good; n, 8 to 12.

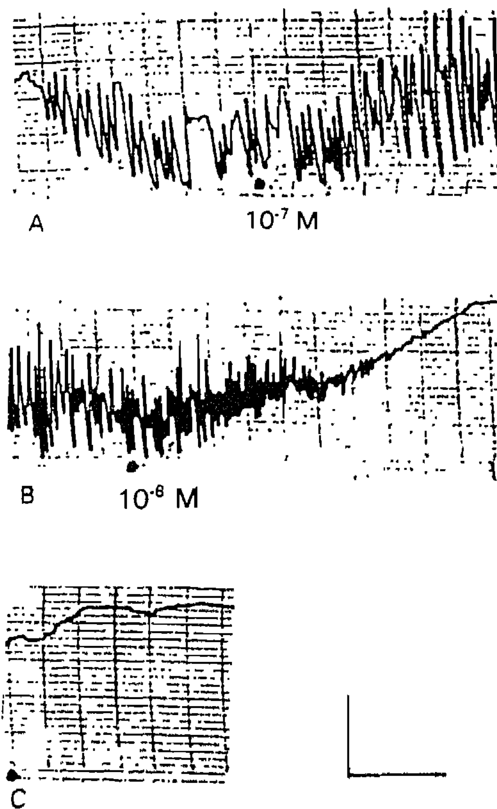

Fig. 1. Effect of hexachlorophene (10^{-7} and 10^{-6} M) on the rhythmic motility of isometrically mounted *F. gigantica*. A. hexachlorophene 10^{-7} M; B. hexachlorophene 10^{-6} M; C. wash. Calibration: vertical-500 mg; horizontal-2 min.

et al. (1995b). After recording the normal rhythmicity of the fluke for 1/2 hr, the lowest concentration (from 10^{-7} to 3×10^{-4} M, based on the concentrations used by Fairweather *et al.* 1984) of particular fasciolicidal drug was administered to the organ bath, and then the rhythmicity was further recorded for 5 min. The bath solution was then replaced with normal H-F solution and after 5 min next higher concentration was administered. A fresh fluke was always mounted to record a new set of concentration-responses.

Flukes (8-12) were also incubated in different concentrations (10^{-7} to 10^{-5} M) of various fasciolicidal agents at 38°±1° C for 4 hr and gross visual motility was recorded at 0, 1/4, 1/2, 1, 2, 3 and 4 hr intervals after incubation. The motility was graded 0, ±, +, ++ and +++ representing nil, feeble, poor, moderate and good motility, respectively. Hexachlorophene, rafoxanide, oxyclozanide and triclabendazole were first suspended in dimethyl sulfoxide (DMSO- 0.1%) and final dilutions were made in H-F solution. Albendazole and niclofolan were directly diluted in H-F solution. All the fasciolicidal agents were applied in different molar concentrations. Six recordings were made for each drug and representative tracings are given wherever required. Comparative studies with vehicle were also undertaken.

RESULTS AND DISCUSSION

Normal functioning of neuromuscular physiology is very important in helminth parasites to maintain their feeding site and other vital coordinating functions. Although, understanding of neuromuscular physiology of trematodes and mode of action of available fasciolicides are still in preliminary stage, results of some studies on *F. hepatica* (Fairweather *et al.* 1984, Holmes and Fairweather 1984) and *F. gigantica* (Kumar *et al.* 1995b, Kumar and Tripathi 1996, Tripathi and Kumar 1997) revealed that neuromuscular physiology could be the important target for fasciolicidal agents.

Hexachlorophene (HCP, 10^{-7} M) caused marked increase in the tone marked by rise in base line and amplitude of rhythmic

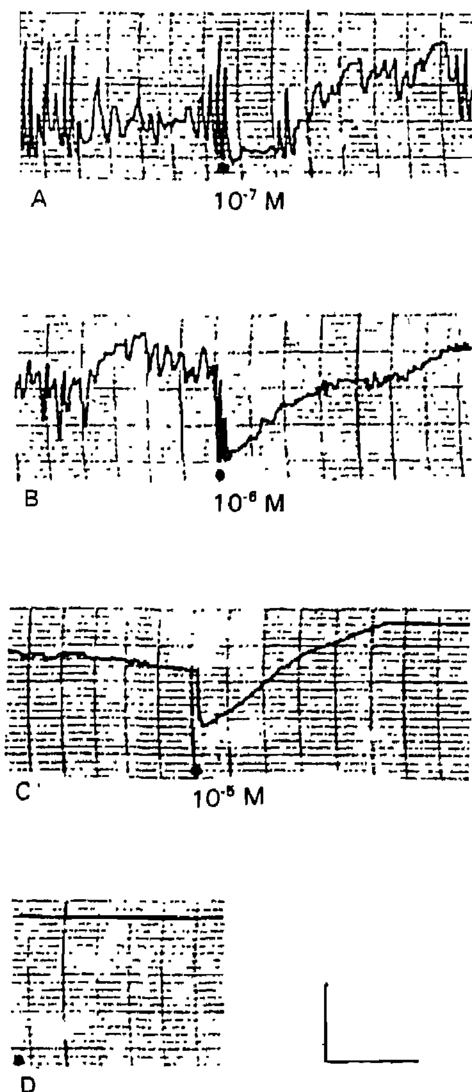

Fig. 2. Effect of niclofolan (10^{-7}, 10^{-6} and 10^{-5} M) on the rhythmic motility of isometrically mounted *F. gigantica*. A. niclofolan 10^{-7}M; B. niclofolan 10^{-6} M; C. niclifolan 10^{-5} M; D. wash. Calibration: vertical-500 mg; horizontal-2 min.

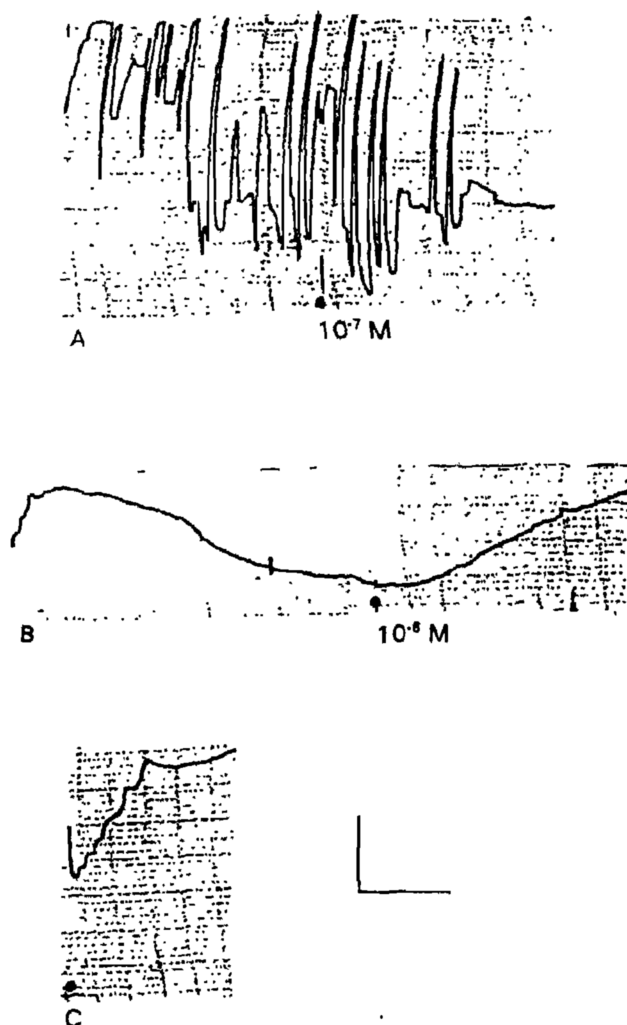

Fig. 3. Effect of oxyclozanide (10^{-7} and 10^{-6} M) on the rhythmic motility of isometrically mounted *F. gigantica*. A. oxyclozanide 10^{-7} M; B. oxyclozanide 10^{-6} M; C. wash. Calibration: vertical-500 mg; horizontal-2 min.

contractions within 2-3 min of its application. The effect disappeared slowly on washing. At (10^{-6} M) concentration of HCP, there was a gradual rise in base line with concomitant reduction in the amplitude of rhythmic contractions of the fluke. The onset of effect was almost immediate and the fluke exhibited persistent spastic paralysis. The effect was irreversible during the period of recording for about 1/2 hr (Figs 1A-C). Almost similar effect was produced by niclofolan (10^{-7} M). However, it caused initial fall in base-line tension followed by gradual increase in the tone at all the concentrations (10^{-7}, 10^{-6} and 10^{-5} M). The inhibition of rhythmicity and rise in base-line tone was concentration-dependent. Complete spastic paralysis was set in at 10^{-5} M (Figs 2A-D). Oxyclozanide (10^{-7} and 10^{-6} M) also exhibited dose-depeandent increase in the base-line tone following its administration and led to irreversible spastic paralysis (Figs. 3A-C). None of the drugs (HCP, niclofolan and oxyclozanide) induced phasic

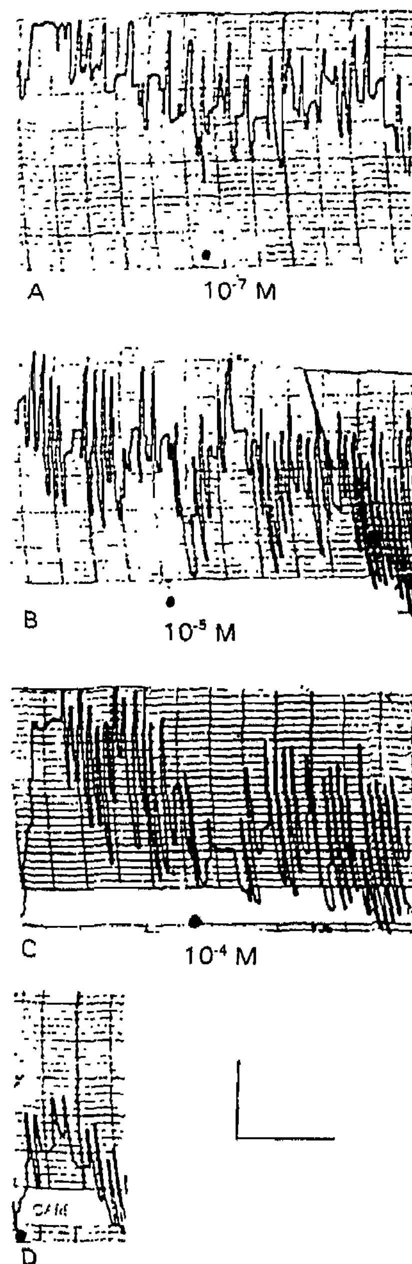

Fig. 4. Effect of rafoxanide (10^{-7}, 10^{-5} and 10^{-4} M) on the rhythmic motility of isometrically mounted *F. gigantica*. A. rafoxanide 10^{-7} M; B. rafoxanide 10^{-5} M; C. rafoxanide 10^{-4} M; D. wash. Calibration: vertical-500 mg; horizontal-2 min.

contractions of the fluke. Thus, the results indicate their direct impact on the neuromuscular system of *F. gigantica*. The effect is supplemented by the observations on the gross visual motility wherein, the motility of *F. gigantica* was inhibited completely within 1/2 hour of incubation in 10^{-5}M concentration of HCP, niclofolan and oxyclozanide. The affected fluke did not respond to a prick (Table1). Kaila (1982) has observed that HCP, niclofolan and oxyclozanide have many effects on the neuromuscular system. Apparently similar observations were made by Fairweather *et al.* (1984) on *F. hepatica* collected from the experimentally infected rats with

certain differences such as induction of initial phasic conduction, which was not observed by us. Many workers concluded that HCP, niclofolan and oxyclozanide inhibit energy metabolism of *F. hepatica* (Cornish and Bryant 1976, Edward *et al.* 1981). However, rapid and spastic paralytic effect induced by these fasciolicidal agents does not appear to involve energy metabolism of the fluke which could lead to slow flaccid paralysis and death due to starvation. In the base line tone gradual and steep rise could be due to slow penetration of the drug through the thick tegument of *F. gigantica*.

Rafoxanide did not induce paralysis at 10^{-7} M to 10^{-4} M concentrations but at these concentrations, it increased the amplitude of rhythmic contractions with concomitant decrease in base-line tension of the fluke. At 3 $\times 10^{-4}$ M, rafoxanide caused partial flaccid paralysis (Figs 4A-D). Rafoxanide induced spastic paralysis of *F. hepatica* (Fairweather *et al.* 1984) but the same is not true for *F. gigantica* which exhibited slow partial flaccid paralysis. However, our results are in agreement with the results of Ogunbinade and Guwaze (1981) on *F. gigantica* carried out by kymographic recording. Furthermore, rafoxanide (10^{-5} M) reduced the gross visual motility of *F. gigantica* within 1/2 hr of incubation (Table1) and it was reported that rafoxanide inhibited the energy metabolism of *F. hepatica in vitro* (Cornish and Bryant 1976). Thus, slow partial flaccid paralytic effect of rafoxanide on *F. gigantica* may be because of its inhibitory role on the energy metabolism of the flukes.

Benzimidazoles, albendazole (10^{-7}-10^{-4} M) and triclabendazole (10^{-7}-10^{-4} M) did not induce any alteration in the rhythmic motility of *F. gigantica*. At these concentrations, the fasciolicidal agents reduced the vigorous motility of the flukes to feeble, after 4 hr of incubation (Table1). Thus, both the agents seem to exert slow effect on the fluke which could be because of an inhibition of energy metabolism and inhibition of secretion of enzymes and sloughing away of the tegument by albendazole (Vertinskaya *et al.* 1988) and triclabendazole (Bennett and Kohler 1987, Stitt and Fairweather 1993). Similar slow effect of albendazole and triclabendazole was observed in *F. hepatica* (Fairweather *et al.* 1984). Benzimidazoles also acted slowly and thus indicating their influence on the biochemical processes of the parasite (McCracken and Stillwell 1991).

In conclusion, the present results suggest that HCP, niclofolan and oxyclozanide have marked effect on the neuromuscular system of *F. gigantica*. The findings could be beneficial to develop combinations of available fasciolicidal agents. Further studies on the neuromuscular physiology of trematodes are recommended which would help in development of newer and selective antitrematodal agents.

ACKNOWLEDGEMENT

We thank the Director of the Institute for the facilities.

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Indian Journal of Animal Sciences **68** (11) : 1130-1131, November 1998

Coccidiosis amongst calves. 1. A relation between oocysts per gram faeces and faecal consistency

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Received : 18 October 1997; Accepted : 4 June 1998

ABSTRACT

Clinical cases of eimeriosis exhibiting diarrhoeic syndrome were diagnosed in calves. In general, animals shedding over 2 000 oocysts were showing only the symptoms of diarrhoea. Reexamination of faecal samples has been recommended after 9 days from the onset of diarrhoea in unusual cases. Age-wise variation of the oocyst count from 10 diarrhoeic cases revealed presence of 3 species, viz. *Eimeria bovis* (23.95 %), *E. zuernii* (46.12 %) and *E. subspherica* (29.93%).

Key words : Calves, Clinical coccidiosis, Faecal consistency, Oocysts per gram faeces (OPG)

In India bovine coccidiosis is seen throughout the year (Ruprah 1985). The order of prevalence of different species of the parasite is *E. auburensis* , *E. bovis*, *E. ellipsoidalis*, *E. zuernii*, *E. subspherica*, *E. cylindrica*, *E. canadensis* and *E. alabamensis*. During search for cases of neonatal calf diarrhoea, calves were seen to shed eimerian oocysts and hence, an attempt was made to find out a relationship between oocysts per gram (OPG) of faeces and cases of diarrhoea.

MATERIALS AND METHODS

The materials were collected from calves between the age group of 15 days and 5 months separated from dams and kept in one shed. The calves were offered colostrum within 1 hr of birth @ 6% of their body weight. Colostrum were fed to the calves up to 5 days of age, followed by whole milk @10% of their body weight twice a day and from fifteenth day onward besides milk crushed grains, bran and fodder were also provided up to 2 months of age.

Rectal faecal samples (63) collected from individual animals were brought to the laboratory in icebox. Oocysts were counted to workout OPG (Georgi 1974). Speciation of oocysts was carried out on the basis of their shape-index, structure of oocysts and sporulation time.

RESULTS AND DISCUSSION

Out of 63 samples, 22 were collected from the cases of diarrhoea wherein OPG ranged from 0 to 22 000 (5626.92 ± 7853.41). In general, OPG of animals passing formed stool

Table 1. Relationship between OPG and faecal consistency

Range of oocyst output	Frequency	
	Diarrhoea	Solid faeces
0 - 500	7	36
501 - 1000	2	3
1001 - 2000	3	2
2001 - 4000	6	0
4001 - 8000	0	0
8001 - 16000	3	0
> 16000	1	0
	22	41

ranged from 0 to 1650 (272.27 ± 412.13). Calves which passed more than 2 000 oocysts were having only the symptom of diarrhoea, were between 1 month and 1-2 months of age (Table 1). No significance difference of OPG between the animals passing loose stool and formed faeces was observed, which may be attributed to heterogenicity of variance in the 2 populations. Out of 63 samples, OPG count of less than 2000 was observed in 53 samples, 12 samples were collected from the animals suffering from diarrhoea. When 0-1600 range of oocyst output was further classified at 200 class-interval, out of 12 diarrhoeic samples 5 samples were having oocyst count of 0-200 and 4 samples were negative to coccidian oocyst. Lower oocyst count but presence of diarrhoea may be attributed to examination of faecal sample earlier to formation of oocysts (Ruprah 1985).

This was further supported by our present findings. Because, during this study 3 diarrhoeic animals had an initial OPG of 50,600 and 400. When sampling was done from the same animal after 9 days, there was increased OPG count (22 000; 1 000 and 1050 respectively). This observation tends

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Table 2. Differential oocyst count from 10 individual animals suffered from diarrhoea

Name of the species	Animals (% oocyst count)						(n = 1240)			
	I	II	III	IV	V	VI	VII	VIII	IX	X	Overall
E.zuernii	63.00	72.72	40.15	87.50	39.25	52.53	63.95	8.69	30.90	53.60	46.12
E. bovis	12.00	9.09	33.85	3.57	29.90	13.29	13.95	37.68	29.69	34.02	23.95
E. subspherica	25.00	18.19	26.00	8.93	30.85	34.18	22.10	53.63	39.41	12.38	29.93

to conclude that OPG count may be a good diagnostic aid provided repeated sampling from the herd is done.

Boughton (1945) reported that 5000 to 10000 oocysts/g of faeces may occur in clinical cases. Munyua and Ngotho (1990) also observed high number of oocyst/g from the cases of clinical coccidiosis. In this investigation as many as 22 000 oocysts were observed in 1 animal. High oocyst count (> 2000 OPG) and persistence of diarrhoea are the features of this study which invariably is the reflection of clinical cases of coccidiosis. Because high oocyst output of *E. zuernii* and *E. bovis* besides manifestation of severe dysentery was recorded by Levine (1985). In this study, 2 species as described by Levine (1985) besides presence of *E. subspherica* were recorded which is suggestive of mixed infection of these three species. Besides this, when faecal samples of 10 diarrhoeic animals were counted, differential oocysts count revealed 3 species (Table 2).

Besides relationship between high oocyst count (> 2000 OPG) and diarrhoeic syndrome age-wise variation in oocysts count was also observed during coprological examination. It was noticed that oocyst output was inversely proportional with the age i.e OPG up to 1 month (2094.44 ± 5189.01), 1-2 month (1795.45 ± 4279.39) and more than 2 months (316.66 ± 471.96) which indicates that younger animal serve as a potential source of infection. Mage and Reynal (1993) reported that suckling calves suffer most followed by calves while housed and least percentage when put to graze. Krotenov *et al.* (1990) found also clinical symptoms of pneumonia and enteritis at 6-24 weeks of age whereas, Rudetskii (1989) reported the incidence of clinical coccidiosis at 6-16 weeks of age. Hasbullah *et al.* (1990) could find less oocyst output in the higher age group of animals. This finding corroborates with their findings on correlation of oocyst output and age group. But in this investigation most of the cases were less than 1 month to 2 month - old.

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Indian Journal of Animal Sciences **68** (11) : 1132-1134, November 1998

Histogenesis of the metanephric duct system in goat

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Received: 14 January 1998; Accepted : 7 April 1998

ABSTRACT

Histogenesis of the duct system of metanephros was studied in goat embryos of different age groups ranging from 6 to 350 mm crown rump length (CRL). The primordium of duct system originated in the form of ureteric bud from caudal part of the mesonephric duct in embryos of 8 mm CRL (17 days). It divided into the cranial and caudal primary branches at ampullated end by 18 mm CRL (30 days). Each one of these redivided dichotomously in to secondary branches in embryos of 22 mm CRL (34 days). Subsequent branches (3rd, 4th and 5th orders) became evident by 35 mm CRL (46 days). These formed network of channels throughout the gland by 45 mm CRL (50 days) and arranged as medullary rays in embryos of 70 mm (56 days) to 120 mm CRL (74 days). The straight collecting ducts, coursed upward into the cortex and terminated finally as arched collecting ducts by 160 mm CRL (89 days). The ducts of all orders showed epithelial reaction for PAS-positive and Sudanophilic material in embryos of 180 mm CRL (96 days) and onwards. Their orientation and structure did not change further in embryos of subsequent ages except a gradual increase in staining intensity for PAS-positive and sudanophilic materials.

Key words : Goat, Histogenesis, Intra- renal ducts, Metanephros, Nephrogenic blastema, Ureteric bud

The development of metanephros in animals follows an accelerated time course when compared with the human (Bremer 1916, Canfield 1980). In ungulates it becomes functional at an early embryonic age due to low permeability of placenta (Davies 1952). Literature on the development of metanephros in general and its duct system in particular is sparse in goat (Chandra 1964). This paper describes the primordial origin and differentiation of intra-renal ducts in goat embryos of early to late (near term) gestation.

MATERIALS AND METHODS

This study was conducted on 65 goat embryos ranging from 6 mm to 350 mm in crown-rump length (CRL). These, as per calculated age, were divided into 5 age groups (Gopinath *et al.* 1997) and fixed in 10% neutral buffered formalin. The smaller embryos (6 mm to 35 mm CRL) were processed as a whole for paraffin embedding. From the rest (45 mm to 350 mm CRL), the metanephroi were dissected out and then processed for paraffin sectioning. Serial sections of 5-7 μm thickness from both the groups were stained with routine Harris haematoxylin and eosin, Gomori's method for reticulum, periodic acid Schiff's (PAS) stain for polysaccharides and Sudan Black-B stain for fat (Luna 1968).

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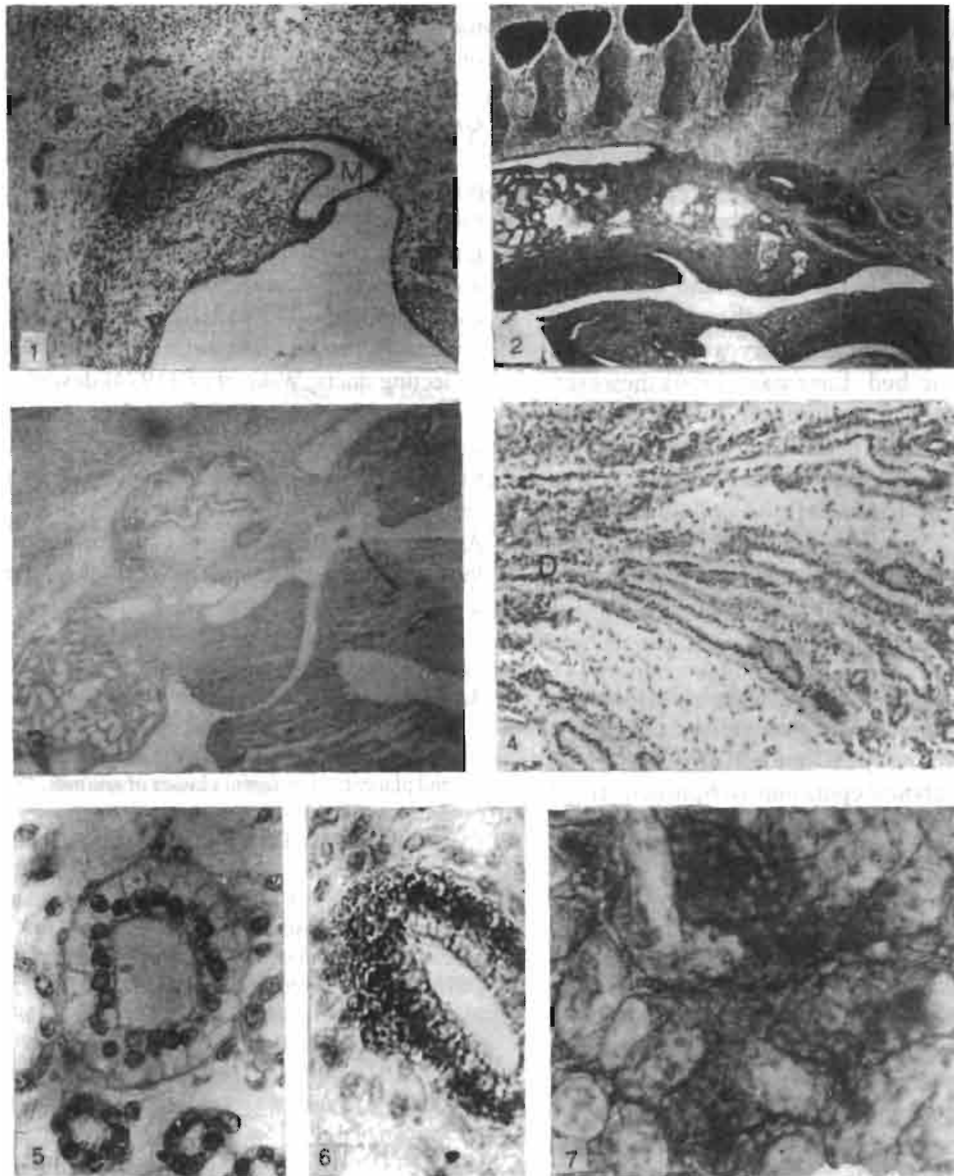
RESULTS AND DISCUSSION

Group I (0-30 days)

The duct system of metanephros in goat, like that of the other animals, developed from the ureteric bud. The latter evaginated from the caudal part of the mesonephric duct in embryos of 8 mm CRL (17 days) and grew craniad over the mesonephros by 12 mm CRL (21 days). It's distal end was ampullated and capped by nephrogenic blastema by this stage of development (Fig.1). The epithelial cells lining this primordial duct for whole of the length stained lightly with eosin. Their nuclei were highly basophilic and stacked in 2-3 layers. The nucleoli occupied a central position in light stained area of chromatin material. Chandra (1964) reported the origin of ureteric bud in goat embryos between 13 mm and 17 mm CRL, while Patten (1949) in pig and Arey (1962) in man described its origin from caudal part of mesonephric duct in embryos of 5 mm CRL. With little change in histomorphology, the ureteric bud in goat embryos of 14 mm CRL (24 days) elongated cranio-caudally at ampullated end to differentiate into 2 primary branches by 18 mm CRL (30 days) as forerunners of intra-renal duct system. The epithelium lining these ducts resembled to that of the earlier stages in cellular and nuclear details. The terminal end of each branch was surrounded by nephrogenic blastema (Fig.2).



Figs 1-7. **1.** Photomicrograph of ureteric bud showing its dilatation at distal end capped by nephrogenic blastema and its continuation with mesonephric duct (M) in goat embryo of 12 mm CRL. H & E ×70. **2** Photomicrograph showing cranial and caudal primary branches of ureteric bud in goat embryos of 18 mm CRL. H & E × 28. **3.** Photomicrograph showing dichotomous branching pattern and course of ducts of various orders in goat embryo of 24 mm CRL. H & E × 28. **4.** Photomicrograph showing papillary ducts (D) and their course in the cortex as straight collecting ducts in goat embryo of 160 mm CRL. H & E × 70. **5.** Photomicrograph of a straight collecting duct lined with simple columnar epithelium with nuclei placed in apical part in goat embryo of 160 mm CRL. H & E × 400. **6.** Photomicrograph of a straight collecting duct showing PAS- positive material in basal part of epithelium in goat embryo of 180 mm CRL. PAS stain × 400. **7.** Photomicrograph showing interstitial reticular fibres in goat embryo of 330 mm CRL. Gomoris Reticular stain ×400.

Group 2 (35-60 days)

The duct system of metanephros in goat embryos of this age group showed obvious organizational changes. With progressive shift in position, each primary duct bifurcated at terminal end into successive secondary branches by 22 mm CRL (34 days). The latter ascended towards the cortical area of the gland in embryos of 24 mm CRL (36 days). Their terminal ends were ampullated and wrapped by nephrogenic blastema (Fig.3). These were lined with a stratified epithelium of about 13.5 μm height. The cytoplasm of epithelial cells was distinctly eosinophilic with a little more density in supranuclear zone as compared to the branches of the previous order. The nuclei were generally elongated and showed uniform distribution of chromatin material. The arrangement of blastema around their terminal ends at cortico-medullary junction imparted pseudo-lobulated appearance to the gland in these embryos. Goscicka and Tomasik (1979) described the multilobular appearance of the metanephros in sheep embryos of early ages and its change

later into unipolar gland due to structural integration of metanephric components and alteration in position of inter lobular arteries. The subsequent division of intra-renal ducts into branches of higher order (3rd, 4th and 5th) became evident in goat embryos of 35 mm CRL (46 days). These formed network of channels throughout the cortex of gland by 45 mm CRL (50 days) with little more concentration in the juxtamedullary and intermediate zones than in the subcapsular zone. The renal pelvis was typically basin shaped and lined with transitional type of epithelium. Osathanondh and Potter (1966) stated that the renal pelvis in human embryos arose from first 3-5 branches of ureteric bud. Langman (1966), however, described that the branches of ureteric bud of 5th and subsequent orders gave rise to definite intra-renal collecting ducts in human embryos. The ducts of medullary area showed tendency to be organized into definite columns in goat embryos of 70 mm CRL (56 days). Their number in subcapsular and intermediate cortical zones appeared relatively less than in juxtamedullary zone by 85 mm CRL (63 days).

Group 3 (65-90 days)

With progressive increase in age of the embryos the intra-renal ducts showed colonization as medullary rays in the juxtamedullary zone of cortex by 120 mm CRL (74 days). These were lined with simple cuboidal epithelium (6.6 μm height) and ranged from 38.3 μm to 50.5 μm in diameter. Through gradual shift in position in embryos of 130 mm CRL (76 days), they established definite columns in the intermediate zone with ampullary extension into the subcapsular area by 145 mm CRL (84 days). Canfield (1980) noticed medullary rays in bovine embryos of 12 cm CRL. In goat embryos of 160 mm CRL (89 days) many of the ducts coursed upward as straight collecting ducts and rebranched at their terminal ends into the arched collecting ducts (Fig.4). These were lined with simple columnar epithelium, the nuclei being placed apically in them (Fig.5). The epithelial cytoplasm in ducts of all orders possessed PAS-positive granules with a little more aggregation in the infranuclear part of ducts of medullary area.

Group 4 (95-120 days)

The intra-renal duct system showed certain changes in internal structure in embryos of this age group over the preceding group. The epithelial cells of larger ducts, both in the cortical and medullary areas, showed uniform sudanophilia and heavy concentration of PAS-positive material in the infranuclear part from 180 mm CRL (96 days) onwards (Fig.6). The interstitial fibroblasts aligned along larger ducts as forerunner of their basement membrane, showed distinct argyrophilia at 230 mm CRL (107 days). The relative staining affinity however, increased gradually both in cortical and medullary areas of the gland from 250 mm CRL (112 days) onwards.

Group 5 (125-150 days)

The structural details of duct system and interstitium did not change much in embryos of this age group over the preceding one. However, the ducts of all orders showed gradual increase in staining intensity of PAS positive material and sudanophilic fat in goat embryos of 290 mm CRL (130 days) and beyond. The large PAS- positive granules were noticed in infranuclear zone of epithelial cells lining the collecting ducts. Wake *et al.* (1974) described occurrence of glycogen granules in the infranuclear cytoplasm of epithelial cells lining the collecting ducts in kidney of rat. The interstitial cells showed increase in the cortical area of the gland in embryos of 300 mm CRL (135 days) to 350 mm CRL (150 days). Argyrophilic reticular fibers could be noticed throughout in the inerstitial tissue by this stage of development (Fig.7).

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Indian Journal of Animal Sciences 68 (11) : 1135-1138, November 1998

Histopathological studies on the synovial membrane of donkey with induced chronic arthritis following ultrasound therapy and antiarthritic drug treatment

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Received : 4 December 1997; Accepted : 7 May 1998

ABSTRACT

The chronic arthritis was induced in the left intercarpal joint of 12 clinically healthy donkeys, 5-7years old, to assess the efficacy of ultrasound therapy and antiarthritic drug treatment. They were divided into groups A, B and C of 4 animals each. Groups B and C were further treated with antiarthritic herbal drug (ART) and ultrasound therapy (UST), respectively. The tissue samples of the synovial membranes were subjected to histological examination.

The synovial membrane of group A animals having induced chronic arthritis with no treatment showed loss of synoviocytes, and fibrosis of subintima and the joint capsule. The treatment with ART in group B evidenced the intimal proliferation and subintimal regenerative changes leading to nearly normal synovial membrane. In group C following the UST @ 1.5 w/cm², the intimal proliferations and the mesenchymal tissue were localised but patches of degenerative changes were present. It was concluded that chronic arthritis responds to both the ART and UST treatments, but the former showed better regenerative changes.

Key words : Antiarthritic herbal drug (ART), Chronic arthritis, Donkey, Histology, Synovial membrane

The histopatholoIgical studies on the synovial membrane in chronic arthritis following ultrasound therapy were reported in dogs and calves (Bhatia 1990, Bhatia *et al.* 1992). In equines similar studies on joint capsule were conducted in donkey following antiarthritic herbal drug (ART) and ultrasound treatment (UST) of acute arthritis (Singh 1994). No such literature was available on chronic arthritis in donkey and hence the present work was undertaken to assess efficacy of different therapies.

MATERIALS AND METHODS

Clinically healthy donkeys (12), 5- 7-year-old, weighing 80-105 kg, were used with due standard experimental treatments. The chronic arthritis was induced by injecting 0.2 ml turpentine-oil mixed with 0.2 ml gentamycin into the left intercarpal joint after collection of 1 ml of synovial fluid. This stage was considered as pre-treatment i.e. induction period 0 day normal. The procedure was repeated on 14 day. After 42 day of first injection, development of chronic arthritis was confirmed clinically and radiographically. This stage was designated as 0 day of treatment. For further experimentation the animals with induced chronic arthritis were randomly divided into 3 groups of 4 animals each, viz. Group A - control group, group B - ART group, and group C - UST group.

The treatment schedule included : (i) no further treatment in group A animals, (ii) administration of 20 g ART daily for 25 days starting after 0 day of treatment in the group B animals, (iii) application of pulsed therapeutic ultrasound (UST) on the affected joints @ 1.5 watt/cm² for 10 min daily for 10 consecutive days after 0 day of treatment using standard procedure in group C animals.

All the animals of groups A, B and C were sacrificed 42 days after the development of chronic arthritis. The affected carpus and contralateral (normal) carpus were harvested by cutting the bones proximal and distal to the carpus. The joints were deskinned and the intercarpal joints of both affected and normal joints were opened carefully and were stored in 10% Neutral buffered formalin. The synovial membrane samples were collected. After washing in running water for 24 hr, the tissues were processed to obtain 5 μm thick paraffin sections. The sections were stained with Mayer's - Haematoxylin and Eosin stain for routine histomorphological studies, alcian blue/

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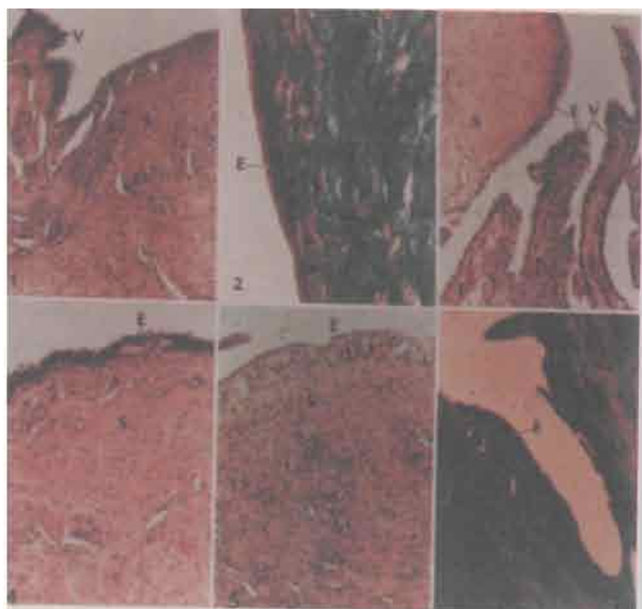


Figs **1-6.** Synovial membrane from intercarpal joint : **1.** of group A showing short villi (v), highly fibrosed subintima (S), Haematoxylin and Eosin stain × 27; **2.** of group A showing proliferative intima (E), abundance of fibroblasts in the subintima and outer fibrous capsule (F), Masson's Trichrome stain × 67; **3.** of group B animal showing well-developed villi (v), intimal proliferations (E) and mesenchymal tissue in the subintima (S), Haematoxylin and Eosin stain × 67; **4.** of another animal of group B showing intimal proliferation (E) and mesenchymal regenerative tissue in the subintima (S) with increased vascularity, Haematoxylin and Eosin stain × 67; **5.** of the same specimen as in Fig.3 showing one of the areas with superficial intimal necrosis (E) and highly vascularized regenerative tissue in subintima (S), Haematoxylin and Eosin stain × 67, **6.** of the same specimen as in Fig. 4 showing almost normal intimal (E) and subintimal (S) staining and lack of light green staining in more outer fibrous connective tissue (arrows), Masson's Trichrome stain × 27.

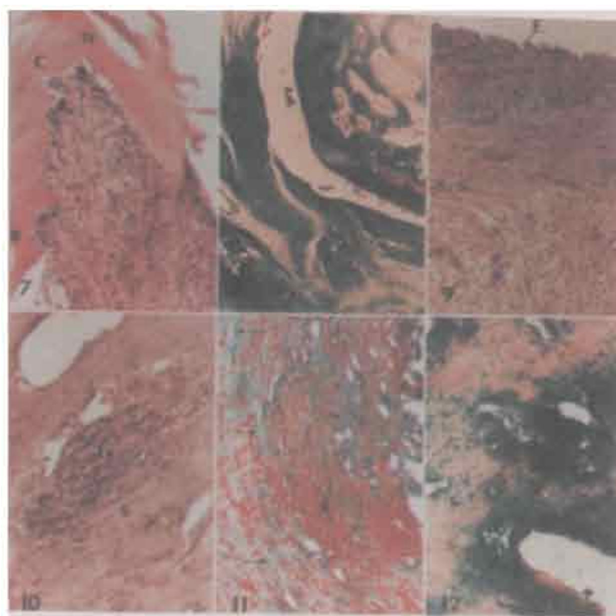
periodic Acid Schiff (pH 2.5) stain for mucopolysaccharides and Masson's trichrome stain for connective tissue (Luna 1968).

RESULTS AND DISCUSSION

In group A animals, the synovial membrane had short villi with proliferative synoviocytes (Fig.1). The subintima was highly fibrosed with abundant fibroblasts and few macrophages and lymphocytes. The fibrous capsule had dense collagenous tissue in it (Fig.2).

In group B the intimal proliferation was common and mesenchymal regenerative tissue with increased vascularity was apparently wide spread in the subintima of the synovial membrane including those of well developed villi (Figs 3,4). Certain areas of the synovial membrane showed superficial intimal necrosis with underlying highly vascularised regenerating tissue (Fig.5). With Masson's trichrome staining the intima and subintima appeared almost normal except that in more outer layers the fibrous tissue lacked affinity for light green stain (Fig.6) indicating the reduced collagen. In the subchondral connective tissue, multinucleated cells could be seen peripherally (Fig.7). This subchondral connective tissue and the subintima of the synovial membrane in general showed more or less uniform alcianophilia (Fig.8).

In group C initimal proliferations were seen at places. The subintima was highly cellular. The vascularity was increased, mesenchymal tissue was prominent (Fig.9). In some cases there was increased cellularity in subintima. Occasional occurrence of localized aggregation of lymphoreticular tissue generally closer to blood vessels was noted (Fig.10). At places villi showed prominent degenerative changes (localized areas), vascularity increased and mesenchymal tissue was seen in patches (arrows in Fig.9). With Masson's trichrome stain, in one of the animals, the necrosed (red in Fig.11) connective tissue of the synovial membrane was penetrated with mesenchymal tissue in patches (green in Fig.11) and numerous blood vessels. In AB-PAS, the connective tissue of the



Figs 7-12. **7.** Articular cartilage of intercarpal joint of group B animal showing non-calcified (artefactually broken) cartilage (N), calcified cartilage (C), underlying subchondral bony tissue (B) and multinucleated cells (arrows) in the subchondral connective tissue in the most peripheral part of the joint, Haematoxylin and Eosin stain × 67; **8.** Intercarpal joint of group B showing alcianophilia in the subintima (S), non-calcified cartilage (N) and subchondral connective tissue in the peripheral area of the joint (between arrows) and subchondral bony tissue (B) and PAS- positive calcified layer of cartilage (C), Alcian blue-Periodic acid Schiff (pH 2.5) stain × 13; Synovial membrane from intercarpal joints of group C showing changes (see text) in intima (E), subintima (S), patches of mesenchymal tissue penetrating into necrosed tissue (arrows) and localized aggregation of lymphoreticular tissue (Ly), **9.** Haematoxylin and Eosin stain × 67; **10.** Haematoxylin and Eosin stain × 133, **11.** Masson's Trichrome stain. × 133; **12.** Alcian blue Periodic Acid Schiff stain ×67.

synovial membrane was moderate to strong AB positive with weak to moderate PAS positive patches (Fig.12).

The above observations revealed that in control animals the synovial membrane was histologically characterised by loss of synoviocytes, fibrosis of subintima and the fibrous capsule. In ARTtreated animals, the intimal proliferation and subintimal regenerative changes led to nearly normal synovial membrane. In ultrasound treated animals, although the intimal proliferations and the mesenchymal tissue were localized, at places the patches of degenerative changes were present. These observations indicated that although both ART and UST proved effective in the treatment of chronic arthritis in donkeys, the former appeared better as compared to latter so far as healing of the synovial membrane was concerned. Also, Singh (1994) concluded better joint capsule healing with ART treatment as compared to UST @ 1 watt/cm² in acute arthritis of donkeys. Bhatia (1990) and Bhatia *et al.* (1992) observed proliferation of the synovial lining cells and marked regeneration in chronic arthritis of dogs and calves in which ultrasound therapy was administered @ 1.5 watt/cm² and 3 watt/cm², respectively.

Our results indicate that both ART and UST @ 1.5 watt/cm² are beneficial in the treatment of chronic arthritis in donkeys. On the basis of histological and histochemical observations of this study, it is concluded that chronic arthritis does respond to ART and UST favourably, but ART showed better regenerative changes.

ACKNOWLEDGEMENTS

Authors thank the Dean, College of Veterinary Sciences, for providing facilities, and to Dr R S Sethi, for his help from time to time.

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Indian Journal of Animal Sciences 68 (11) : 1138-1139, November 1998

Alpha-2 agonists with diazepam as preanaesthetic to ketamine anaesthesia in goats : Haemodynamic effects

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Received : 20 October 1997; Accepted : 15 May 1998

ABSTRACT

Ketamine was used for induction of anaesthesia after premedication of diazepam-xylazine (group A), diazepam medetomidine (group B), diazepam-romifidine (group C) and diazepam alone (group D) in atropinised goats. Gradual hypertension was followed by hypotension E in groups A, B and C. Central venous pressure initially increased and fell gradually in groups A, B and C. No significant ($P>0.05$) change in arterial and central venous pressures were seen in the animals of group D. Changes in electro-cardiogram were minimal. Atrioventricular block and ventricular premature complex was seen in animals of groups B and C respectively.

Key words : Anaesthesia, Haemodynamic effects, Diazepam, Goats, Preanaesthetic

In this study haemodynamic effects of diazepam with and without α-2 agonists like xylazine, medetomidine and romifidine as preanaesthetic to ketamine anaesthesia in goats were studied.

MATERIALS AND METHODS

Adult goats (24), divided into 4 groups of 6 animals each, were premedicated with atropine sulphate (0.5 mg/kg SC). After 15 min, diazepam (0.25 mg/kg IM) alongwith xylazine (25 µg/kg IV) or medetomidine (5µg/kg IV) or romifidine (12 µg/kg IV) and diazepam alone were given in groups A, B, C and D, respectively and 10 min later, ketamine (IV) was administered in each group till the anaesthetic effect.

Arterial blood pressure (ABP), central venous pressure (CVP) and electrodiographic (ECG) readings were taken just before and 5, 10 min after atropine sulphate administration, then at 5 and 10 min after premedication with diazepam and α-2 agonists and then at 5, 10, 20, 30, 45, 60, 75, 90, 105, 135, 150, 165 and 180 min after induction of ketamine anaesthesia. CVP and ABP were recorded till 120 min ECG recordings were made up to 180 min.

RESULTS AND DISCUSSION

Mean values of ABP rose in all the groups after premedication (94.00±06.929 to 120.6±12.426 mm Hg). Although ABP increased further (up to 125.33±15.070 mm Hg) at 5 to 10 min after ketamine anaesthesia in group D, there was a gradual fall (up to 77.33±2.00 mg Hg) during first 30 min in groups A, B and C. The values were then maintained at a slightly low levels in groups A, B and C when compared with base values. This biophysical response of ABP might be due to direct α adrenoreceptors stimulation of peripheral vessels leading to hypertension and subsequent hypotension due to depressed sympathetic out put from central nervous system. Similar biphasic response for ABP was recorded with xylazine in dogs (Hsu *et al.* 1985) and calves (Lin *et al.* 1991) with medetomidine in goats (Pawde *et al.* 1996), sheep (Caulkett *et al.* 1994) and with romifidine in horses (Clarke *et al.* 1991). Increase in blood pressure immediately after ketamine administration may be due to its cardiostimulatory action. The desensitization of baroreceptors or vagal blockade which block the negative feed mechanism on the vasomotor centre might have resulted in hypertension and tachycardia (Pathak *et al.* 1982). Increased cardiac output and direct vasoconstriction might also have contributed to hypertension (Levinson *et al.* 1973). CVP increased in groups A (1.50±0.57-0.66±2.771 cm H_2O), B (1.66±0.72-10.33±7.25 cm H_2O) and C (7.83±3.66-12.16±2.20 cm H_2O) after premedication but decreased slowly to return to the base after ketamine anaesthesia. Similar fall in CVP was seen in buffaloes after xylazine-thiopental anaesthesia (Kumar and Sharma 1986). However, CVP increased with medetomidine in goats (Pawde *et al.* 1996) and diazepam-xylazine-ketamine in calves (More *et al.* 1993). It did not differ significantly between different groups at various intervals, however, the values of group C (12.16±2.204 cm H_2O) at 10 min after atropine were significantly higher ($P<0.05$) as compared to that of group A (5.00±3.50 cm H_2O). In CVP initial rise may be due to increas in systemic vascular resistance pro-

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duced by these drugs. Such increase in systemic vascular resistance was also reported after medetomidine-ketamine administration in Yucatan mini pigs (Vainio *et al.* 1992) and laboratory beagles (Serteyn *et al.* 1993). However, in group D, not much change in CVP was recorded.

Electrocardiogram of groups A, B and C showed sinus bradycardia at 5 to 10 min of administration of diazepam and α-2 adrenoceptor agonist drugs whereas, in group D sinus tachycardia existed throughout the course of observations. *In group A, initial tachycardia was followed by bradycardia in* later half of the study. In group B, second degree AV block was seen after 10 min of premedication. Second degree AV blocks were reported after medetomidine and medetomidine-butorphanol administration in pigs (Sakaguchi *et al.* 1993), medetomidine-ketamine in goats (Hugar 1993), romifidine in horses (Diamond *et al.* 1993) and xylazine in dogs (Lele and Bhokre 1985). In group C, bradycardia was seen except in animal where tachycardia was apparent. Bradycardia and atrioventricular block occurred probably from increased vagal activity due to vasopressure effect to xylazine (Knight 1980). Increased vagal tone may arise as a reflex action from the baroreceptors of the carotid sinus in response to initial hypertension leading to bradycarida and atrioventricular block (Gasthuys *et al.* 1990). In group D, sinus tachycardia accompanied by sinus arrhythmia existed between 20 and 45 min in 2 animals. In one animal initial sinus tachycardia was followed by sinus arrhythmia and bradycardia from 60 to 120 min. However, sinus tachycardia was again recorded in subsequent intervals up to the end.

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Indian Journal of Animal Sciences 68 (11) : 1140-1142, November 1998

Medetomidine-ketamine anaesthesia in canine and its reversal by atipamezole

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Received : 30 December 1997; Accepted : 15 April 1998

ABSTRACT

Intramuscular administration of medetomidine @ 20μg/kg and ketamine @ 50,mg/kg IV in atropine sulphate (0.02 mg/kg IM) premedicated 36 healthy dogs resulted in onset of anaesthesia in 1.98±0.26 min, duration of anaesthesia of 42.86±2.80 min with complete recovery in 94.14±6.18 min. Transient respiratory depression, increased heart rate followed by decrease and slight hypothermia were observed. The sedative and physiological effects were completely antagonized by IV administration of atipamezole (@ 20μg/kg IV) with mean walking time of 2.36±0.18 min. Thus atropine-medetomidine-ketamine combination can safely be used in dogs as it caused rapid onset, prolonged anaesthesia with smooth and uneventful recovery, and its total reversibility permits optimum control of anaesthesia.

Key words: Clinico-physiological effects, Dogs, Ketamine, Medetomidine, Surgical effects

Medetomidine, [4-1-(2, 3-dimethyl phenyl) ethyl -1H-imidazole], a potent sedative and analgesic α2-adrenergic drug (Verstegan *et al.* 1989), along with medetomidine, gives longer duration of anaesthesia, desired muscle relaxation, absence of convulsions and minimum cardiopulmonary depression (Vainio and Palmu 1989, Moens and Fargetton 1990). Use of medetomidine-ketamine combination in clinical case of dogs is not well documented. Therefore, the present study was undertaken to elucidate the clinico-physiological, haematological and surgical effects of this combination and its reversal by atipamezole in canine surgical patients.

MATERIALS AND METHODS

Dogs (36) of either sex, 1-2-year-old, weighing 12 to 24 kg, were kept off feed for 24 hr, and water for 12 hr before the administration of anaesthesia. Atropine sulphate was given IM @ 0.04mg/kg and 15 min later medetomidine was injected @ 20μg/kg IM. After 15 min ketamine was injected intravenously @ 5mg/kg in all the groups of animals.

The study was conducted in 3 phases. In the first phase 12 animals were subjected to clinical, physiological and haematological studies. Clinical parameters like onset, duration, complete recovery, extent of muscle relaxation,

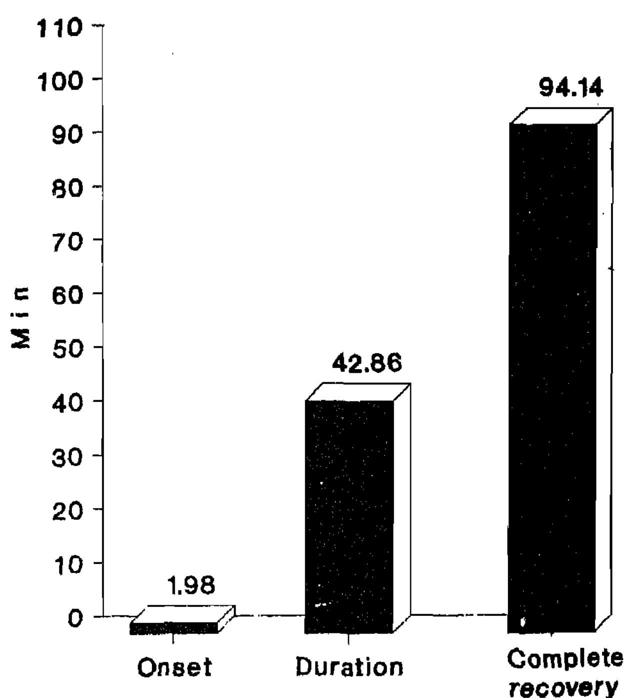

Fig. 1. Onset, duration and complete recovery after medetomidine-ketamine anaesthesia in dogs.

presence or absence of corneal, palpeberal and conjunctival reflex were studied. Physiological parameters included measurement of rectal temperature, heart rate and respiration rate before and 5, 15, 30, 45, 60, 75, 90 and 120 min post-injection of ketamine. Haematological parameters, viz. haemoglobin, packed-cell volume, total erythrocytic count and total

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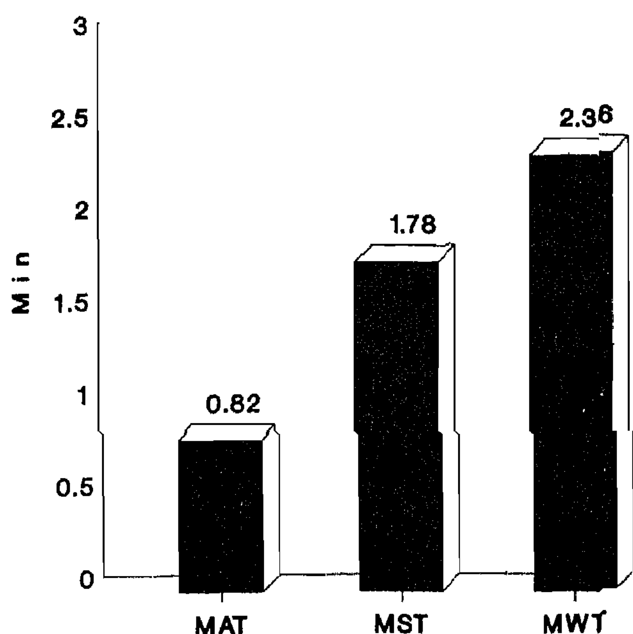

Fig. 2. Mean arousal time (MAT), mean standard time (MST) and mean walking time (MWT) after injection of atipamezole in medetomidine-ketamine anaesthetized dogs.

leukocytic count were measured before and after 30, 60 and 120 min of ketamine administration (Schalm *et al.* 1975). In the second phase 12 animals were subjected to reversal studies. Atipamezole @20μg/kg was injected intravenously 60 min after medetomidine-ketamine anaesthesia and the reversal parameters included measurement of mean arousal time (MAT), mean standing time (MST) and mean walking time (MWT). In the third phase, 12 animals on abdomino-pelvic regions were subjected to various surgical operations, viz. gastrotomy, enterotomy, enterectomyu urethrotomy, ovario-hysterectomy and cystotomy to determine the extent of anaesthesia and muscle relaxation. Data were subjected to paired 'T-test' for comparison of means (Mead *et al.* 1993).

RESULTS AND DISCUSSION

The administration of medetomidine-ketamine caused onset of anaesthesia in 1.98±0.26 min, duration of anaesthesia for 42.86±2.80 min while the complete recovery occurred in 94.14±6.18 min (Fig. 1). The paedal corneal, conjunctival and palpebral reflex were sluggish but remained intact during entire period of anaesthesia. Mild salivation and diuresis was also observed. There was desired muscle relaxation. The recovery was smooth and uncomplicated. These findings are in accordance with the observations of Raiha *et al.* (1989) in dogs.

There was a significant ($P<0.01$) increase in heart rate between 5 and 30 min followed by a significant ($P<0.05$) decrease up to 90 min. Heart rate initially increase might be due to sympathetic stimulant action of ketamine and subsequent decrease might be due to medetomidine which causes inhibition of sympathetic tone from the central nervous system (Scheinin and Mac Donald 1989). Respiration rate decreased significantly ($P<0.05$) between 5 and 75 min. It might be because of direct depressive effect of medetomidine on central nervous system and respiratory centre. It simulates the findings of Short (1992) in dogs. The rectal temperature showed a nonsignificant decrease throughout the period of anaesthesia which might be because of reduced metabolic rate and inhibition of skeletal muscle movement. Young and Jones (1990) reported similar observations after medetomidine-ketamine anaesthesia in cats.

The haematological parameters showed nonsignificant changes throughout the period of anaesthesia and their values ranged from 12.80±0.16 to 13.04±0.12 g/dl for haemoglobin, 42.60±0.40 to 44.80±0.26 % for PCV, 5.50±0.12 to 6.24±0.24 thousand/cumm for TEC and 9.64±0.28 to 10.62±0.16 millions/cumm for TLC.

Intravenous administration of atipamezole completely antagonized the sedative effects of medetomidine with mean arousal time (MAT) of 0.82±0.12 min, mean standing time (MST) of 1.78±0.10 min and mean walking time (MWT) of 2.36±0.18 min (Fig. 2). The animals were able to walk within 2 to 4 min after reversal without any signs of resedation or hyperexcitability The heart rate and respiration rate were also increased following atipamezole administration. Similar observations were reported by Verstegen and Petcho (1993) following reversal of medetomidine butorphanol-midazolam anaesthesia by atipamezole in dogs.

Gastrotomy, enterotomy, enterectomy, urethrotomy, ovario-hysterectomy and cystotomy were successfully performed under medetomidine-ketamine anaesthesia. There was desired muscle relaxation and longer duration of aneasthesia to complete all the surgical procedure. The recovery from anaesthesia was smooth and uneventful.

It is, therefore, concluded that atropine-medetomidine-ketamine combination is safe for aneasthesia in dogs as it produces excellent sedation, analgesia and muscle relaxation of longer duration with minimum alteration in physiological profiles. The side effects of medetomidine and ketamine were antagonized when these drugs were used in combination

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Indian Journal of Animal Sciences **68** (11) : 1143-1146, November 1998

Evaluation of epidural detomidine with and without lignocaine and its reversal by atipamezole in Sahiwal calves

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Received : 31 December 1997; Accepted : 15 April 1998

ABSTRACT

Epidural administration of detomidine (@50μg/kg) with and without lignocaine (0.22 mg/kg) in 20 healthy Sahiwal calves caused quick onset and longer duration of analgesia (138.00±4 26 versus 90.00±6.45 min) compared to detomidine alone. The transient bradycardia, respiratory depression, ruminal stasis were compensated within 2 hr after administration of drugs. There were nonsignificant changes in haematological profiles. Serum glucose was significantly ($P<0.05$) increased and it persisted up to 2 hr in all the animals. Administration of atipamezole (@ 50μg/kg iv) caused early arousal from sleep (1.25± 0.12 min) and animals were able to walk by 4.00 ±0.14 min. Atipamezole also reversed the bradycardia, respiratory depression and ruminal stasis induced by epidural detomidine alone or with lignocaine. Thus epidural detomidine with and without lignocaine can safely be used for undertaking surgical manoeuvres of abdomino-pelvic region in Sahiwal cows.

Key words : Anaesthesia, Atipamezole, Detomidine, Ligonocaine, Sahiwal calves

Chemical restraint combining quick but deep sedation and analgesia, are increasingly in demand in veterinary practice. Administration of detomidine (4E (5) - (2-3-dimethylbenzyl) imidazole, a new and effective sedative-analgesic drug effective in cattle (Jedruch and Gajewski 1986, Peshin *et al.* 1991), produces effective perineal analgesia, minimum recumbency, longer duration of analgesia with distinct post-operative analgesia (Scheinin and Mac Donald 1989). It has synergistic effect when combined with lignocaine and given epidurally (Caulkett *et al.* 1993). In Sahiwal cows, no reports could be traced out regarding epidural use of detomidine with and without lignocaine and its reversal by atipamezole. Therefore, the present experiment was designed to evaluate the clinico-physiological and haematobiochemical effects of epidural detomidine with and without lignocaine and its reversal in Sahiwal cows.

MATERIALS AND METHODS

Healthy Sahiwal calves (15) aging between 6 months and 1 year were selected and randomly divided into 3 groups of 5 animals each. All the animals were administered with atropine sulphate @ 0.04 mg/kg, 15 min prior to start of the treatment. The animals were subjected to the following 3 treatments.

G1 - Detomidine (@50μg/kg) inter-coccygeal epidurally after diluting it with normal saline to make a total volume of 7.0 ml.

G2 - Detomidine as in group 1 + lignocaine (@0.22 mg/kg) were diluted to make a total volume up to 7.0 ml and given by inter-coccygeal epidural route.

G3 - By inter-coccygeal epidural route detomidine (@50μg/kg) was followed 30 min later by intravenous injection of atipamezole (@50μg/kg) to reverse the effects of epidural detomidine.

Parameters studied included

Clinical : Onset, duration of sedation and analgesia, complete recovery, salivation time, frequency of urination, cranial spread of analgesia degree, of sedation and muscle relaxation and side effects if any.

Physiological : Rectal temperature, heart rate, respiration rate and rumen motility were recorded before and 5, 15, 30, 45, 60, 75, 90 and 120 min post-injection.

Haematological: It included estimation of Hb, PCV, TEC, TLC, DLC, MCH, MCV and MCHC before and 30, 60 and 120 min post-injection by standard procedure (Schalm *et al.* 1975).

Biochemical: Serum glucose (Folin and Wu 1920) and serum total proteins (Oser 1979) were estimated before and 30, 60 and 120 min post-injection.

Reversal : It included measurement of mean arousal time (MAT) and mean walking time (MWT), heart rate, respiration rate, rectal temperature and rumen motility before and after injection of atipamezole up to 120 min.

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Table 1. Effect on rectal temperature, heart rate, respiration rate and rumen motility in different treatments at various intervals in Sahiwal calves

Parameters	Group	Time interval (min)								
		0	5	15	30	45	60	75	90	120
Rectal temperature (°C)	G1	39.70 ±0.17	39.75 ±0.11	39.58 ±0.10	39.45 ±0.20	39.39 ±0.15	39.35 ±0.20	39.32 ±0.10	39.30 ±0.12	39.26 ±0.18
	G2	39.29 ±0.12	39.35 ±0.14	39.40 ±0.10	39.46 ±0.15	39.50 ±0.15	39.53 ±0.12	39.56 ±0.16	39.59 ±0.15	39.60 ±0.18
Heart rate (beats/min)	G1	89.00 ±2.88	68.50 ±4.43	59.00 ±4.12	53.75 ±1.58	57.50 ±1.73	63.00 ±1.00	66.25 ±1.34	66.50 ±2.50	71.00 ±1.29
	G2	83.50 ±5.18	71.50 ±6.65	58.00 ±2.70	54.00 ±2.70	60.00 ±4.32	67.00 ±3.87	72.00 ±2.95	77.00 ±2.70	78.50 ±1.50
Respiration rate/min	G1	28.50 ±1.70	24.00 ±1.04	21.00 ±0.58	21.00 ±1.29	23.25 ±1.50	23.00 ±0.57	24.75 ±1.37	25.25 ±0.88	26.00 ±0.82
	G2	26.50 ±1.67	23.50 ±1.76	20.50 ±1.26	18.00 ±1.41	19.75 ±1.75	21.50 ±2.06	24.50 ±2.63	25.50 ±2.75	26.50 ±2.21
Rumen motility (contraction/ 2 min)	G1	4.50 ±0.28	3.25 ±0.42	2.25 ±0.25	1.50 ±0.28	1.75 ±0.25	2.25 ±0.25	3.00 ±0.10	3.50 ±0.28	4.25 ±0.25
	G2	5.00 ±0.37	3.00 ±0.41	2.00 ±0.46	1.25 ±0.25	1.50 ±0.28	1.75 ±0.25	2.50 ±0.28	3.50 ±0.29	5.00 ±0.41

*P<0.05.

Statistical analysis

The data regarding induction and duration of analgesia were analysed using simple 2 factorial random block design and analysis of variance as per the standard procedures outlined by Mead *et al.* (1993).

RESULTS AND DISCUSSION

Clinical effects

The onset of analgesia was observed in 5.40±0.24, and 3.62±0.18 min in group 1 and 2 respectively. The onset was quicker in group 2 where epidural detomidine was mixed with lignocaine. The duration of analgesia was 90.00±6.45 min in group 1 which was significantly (P<0.01) increased to 138.00±4.26 min in group 2. Complete recovery occurred in 123.75±6.54 min in epidural detomidine treated animals whereas it took 153.75±8.46 min in epidural detomidine plus lignocaine treated animals. The salivation was induced in 33.25±2.22 min in group 1 and in 17.50±1.80 min in group 2 where mixture of detomidine and lignocaine was used. The frequency of urination from the time of injection of detomidine till complete recovery was 4 to 5 times in group 1 and 2 to 3 times in group 2. The cranial spread of the analgesia was up to 12th thoracic vertebra when detomidine alone was used whereas it went cranially up to 10th thoracic vertebra when epidural detomidine was combined with lignocaine. The animals showed marked sedation and muscle relaxation, drooping of head, ptosis of upper eyelids, salivation, suppression of palapebral reflexes, ruminal stasis and diuresis during sedation period but animals remained in standing position. Detomidine also have local anaesthetic effect in addition to having sedative and muscle relaxant property (Klide *et al.* 1975). Addition of local anaesthetics with α2-agonists potentiated the local analgesic effects and reduced the dose requirement of detomidine as also reported by Grubb *et al.* (1992). Scheinin and MacDonald (1989) reported that detomidine attenuate sympathoadrenal responses to noxious stimuli encountered during anaesthesia/surgery and provide haemodynamic, metabolic and hormonal stability. Caulkett *et al.* (1993) performed caesarean section under epidural xylazine (@ 0.07 mg/kg) in 29 cattle and found that the onset is quick and duration of analgesia lasted for 120 min with distinct post-operative analgesia which is lacking with other anaesthetic technique. In this experiment the dose of detomidine was intentionally kept constant to know the extent of potentiation of analgesia by addition of local anaesthetic.

Physiological effects

The rectal temperature remained unaffected in both the groups and it showed a fluctuating trend throughout the analgesia. The heart rate showed a significant (P<0.05) decrease at 5 min after injection of epidural detomidine alone or detomidine-lignocaine mixture which persisted up to 50 min in group 1 and 75 min in group 2 (Table 1). The respiration rate also showed a significant (P<0.05) decrease at 5 min in both the groups which remained low up to 75 min in group 1 and 60 min in group 2 (Table 1). Thereafter, the values returned to near normal level by 120 min.

Rumen motility significantly (P<0.05) decreased at 5 min in both the groups which persisted up to 75 min in group 1 and 90 min in group 2 (Table 1). However, it returned to near normal by 120 min.

A significant bradycardia caused by epidural detomidine may be attributed to decrease sympathetic outflow from CNS and involvement of baroreceptor reflex induced by α_2-agonists

Table 2. Effect on rectal temperature, heart rate, respiration rate and ruminal movement after detomidine followed by reversal with atipamezole in Sahiwal calves

Parameters	Pre-reversal (min)				Post-reversal (min)			
	0	5	15	30	5	10	15	30
Rectal Temperature (°C)	39.00 ±0.15	38.95 ±0.14	39.00 ±0.14	38.90 ±0.18	38.85 ±0.12	38.85 ±0.14	38.90 ±0.21	38.80 ±0.20
Heart rate (beats/min)	69.50 ±3.30	60.00 ±3.36	53.00 ±2.62	47.75 ±2.33	55.25 ±2.28	62.00 ±2.12	67.25 ±2.33	70.00 ±1.21
Respiration rate (per min)	25.00 ±1.29	21.50 ±1.25	19.50 ±1.28	17.50 ±1.26	19.75 ±1.03	21.75 ±1.04	24.00 ±0 82	25.75 ±0.63
Rumen motility (contraction/2min)	4.50 ±0.28	2.50 ±0.29	1.50 ±0.24	1.00 ±0.10	1.75 ±0.25	2.75 ±0.22	3.50 ±0.38	4.75 ±0.42

*P<0.05.

(Sarazen *et al.* 1989). Similar observations were also reported after systemic detomidine administration in cattle (Chiavassa *et al.* 1992). The decreased respiratory rate could be due to direct depressant action of α_2-agonists on CNS in general and respiratory centre in particular. It simulates the findings of Peshin *et al.* (1991) in cattle after detomidine administration. Rectal temperature showed a nonsignificant and fluctuating trend throughout analgesia. However, Skarda and Muir (1994) reported hypothermia after epidural detomidine in mares. The significant decrease in ruminal movements after epidural detomidine may be attributed to the phenomenon that detomidine after quick absorption gets bound to α_2-adrenergic receptors in the CNS and or forestomach muscle, thereby inhibiting reticulo-ruminal contractions as also observed by Ruckebusch and Allal (1987).

Haematological effects

The haematological parameters, viz. Hb, PCV, TEC, TLC, DLC and haematological indices were nonsignificantly affected in both the groups of animals and their values ranged within normal physiological limits. However, a transient decrease was reported by Koichev *et al.* (1988) after injection of detomidine in cattle.

Biochemical effects

A significant hyperglycaemia (P<0.05) was observed in both the groups of animals with peak increase of 5.30±0.80 m mol/litre from the control value 4.80±0.16 m mol/litres which persisted up to 120 min. It may be attributed to an α_2-adrenergic inhibition of insulin released from pancreatic β cells or to an increased production in the liver as also observed by Peshin *et al.* (1991) in buffaloes.

Serum total proteins showed a nonsignificant decrease throughout the analgesia in both the groups of animals and their values ranged within physiological limits. Similar observations were reported after detomidine administration in cattle (Peshin *et al.* 1991) and it might be because of haemodilution.

Reversal effects

In the group 3 (reversal group) atipamezole given @50μg/kg IV 30 min after detomidine sedation caused complete and effective reversal of sedative effects of detomidine with the MAT of 1.25±0.12 min and MWT of 4.00±0.14 min. Atipamezole caused significant (P<0.05) increase in heart rate, respiration rate and rumen motility which returned to near normal level by 30 min after injection of atipamezole (Table 2). However, the rectal temperature remained unaffected even after administration of reversal agent.

Similar increase in heart rate and respiration rate were reported after atipamezole administration in detomidine induced sedation in dogs (Jarvis and England 1991) and medetomidine-ketamine sedation in calves (Thompson *et al.* 1991).

It might be attributed to blocking of inhibition of central α_2-adrenoceptors resulting into sympathetic outflow and vagal outflow (Goldberg and Robertson 1983). Atipamezole induced quick reversal of ruminal movements which might be due to inhibition of central α_2-adrenoceptor effects leading to reversal of reticulo-ruminal contractions and thereby suggesting an α_2-adrenoceptor mediation of the inhibition of cyclic motor activity of the reticulo-rumen as also reported by Ruckebusch and Allal (1987).

Thus epidural injection of detomidine with and without lignocaine produced excellent sedation with desired analgesia of tail, perineum inguinal and flank region for a longer period than lignocaine alone. Since the motor control remains unaffected, operations of abdomino-pelvic region can be performed in standing position. This combination induced transient physiological and haematobiochemical changes which were compensated within 2 hr. Further, the sedative effect of detomidine could be effectively and completely antagonized by atipamezole administration thus increasing the margin of safety. Hence this combination can safely be used epidurally for surgical manoeuvres of abdomino pelvic region in cattle.

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Indian Journal of Animal Sciences 68 (11) : 1147-1149, November 1998

Variations in liver and kidney functions after epidural use of centbucridine in dogs*

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Received : 25 September 1997; Accepted : 27 April 1998

ABSTRACT

The epidural administration of centbucridine @ 1.5 and 1.75 mg/kg body weight with and without triflupromazine or diazepam @ 2 mg/kg body weight as preanaesthetics in 6 dogs caused significant ($P<0.05$) increase in blood glucose between 3 to 72 hr, serum alkaline phosphatase between 6 and 48 hr and icteric index between 3 and 6 hr. Centbucridine caused insignificant rise in serum glutamic pyruvic transaminase (SGPT), blood urea nitrogen (BUN) and blood creatinine and the values remained within normal limits.

Key words : Centbucridine, Epidural, Kidney, Liver functions

Centbucridine during the process of its destruction in the liver causes marked congestion and dilatation of sinusoids with scanty mononuclear infiltration in the liver parenchyma with no change either in blood glucose, SGPT, BUN or blood protein in rats and monkeys (Nityanand *et al.* 1982). However, its effects on the liver and kidney are not very well established in dogs. In this study, therefore, its effects on liver and kidney functions with and without diazepam and triflupromazine were studied.

MATERIALS AND METHODS

Apparently healthy normal dogs (6) weighing 12 to 16 kg, were kept under observation for 8 days and vaccination against rabies and deworming with albendazole @ 5 mg/kg body weight was done. Blood samples (5) were collected from each dog and analyzed for different biochemical attributes before experiment and at 3, 6, 12, 24, 48, 72 and 96 hr after atropine, diazepam, triflupromazine and centbucridine administration. It was analyzed for blood glucose (Folin and Wu 1926), serum glutamic pyruvic transaminase (SGPT) (Yatzidis 1968), serum alkaline phosphatase (SAP) (Oser 1969), icteric index (Jain 1986), blood creatinine (Henry *et al.* 1953) and blood urea nitrogen (BUN) (Wildman 1974). The animals were kept under uniform diet and management and starved 24 hr before start of the treatment.

Each animal received following 6 treatments at an interval of 8 days.

Treatment 1 : Centbucridine hydrochloride @ 1.5 mg/kg body weight.

Treatment 2 : Centbucridine hydrochloride @ 1.75 mg/kg body weight.

Treatment 3 : Triflupromazine @ 2 mg/kg iv + centbucridine hydrochloride @ 1.5 mg/kg body weight.

Treatment 4 : Triflupromazine @ 2 mg/kg iv + centbucridine hydrochloride @ 1.75 mg/kg body weight.

Treatment 5 : Diazepam @ 2 mg/kg iv + centbucridine hydrochloride @ 1.5 mg/kg body weight.

Treatment 6 : Diazepam @ 2 mg/kg iv + centbucridine hydrochloride @ 1.75 mg/kg body weight.

Data were analyzed using the analysis of variance (Snedecor and Cochran 1980).

RESULTS AND DISCUSSION

A significant increase in blood glucose at various intervals after centbucridine was noticed in different groups of animals (Table 1). This may be attributed to the increased levels of adrenocorticoid hormones due to stress induced by epidural anaesthesia alone and alongwith preanaesthetics. An increase in blood glucose was also observed after regional blockades i.e. after visceral procaine blockade by Hussain and Kumar (1984 a,b,c), visceral procaine blockade, lumbar epidural and local infiltration with procaine by Malek *et al.* (1988), after epidural administration of procaine and lidocaine (Kumar *et*

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Table 1. Significant variations (mean±SE) in blood glucose (mg/dl), alkaline phosphatase (Bodansky unit) and icteric index (icteric index units) in blood at different time intervals following 6 treatments in dogs

Time 6 (hr)	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6
			Blood glucose			
0	81.3±2.1	82.3±1.8	82.5±3.6	82.5±2.9	78.8±3.2	81.7±2.8
3	101.3±5.0	109.6*±6.3	12.3*±12.0	120.4*±60.	125.8*±1.5	118.3*±10.7
6	125.0*±7.1	129.6*±9.1	138.*±9.6	145.8*±4.3	122.1*±9.1	144.6*±8.6
12	122.5*±7.0	126.7*±11.5	114.2*±4.4	124.6*±7.5	117.1*±5.2	125.4*±14.3
24	109.2*±10.0	116.7*±8.3	11.1*±5.1	111.3*±5.2	103.8*±5.5	108.3*±13.6
48	99.2±6.3	112.5*±7.7	107.1*±6.3	107.9*±3.3	97.5±4.1	102.5±8.1
72	94.2±2.5	110.4*±7.3	104.2*±6.4	88.7±3.0	97.5±5.8	90.4±4.6
			Alkaline phosphatase			
0	1.4±0.6	1.4±0.7	1.4±0.6	1.3±0.2	1.6±0.1	1.1±0.3
3	1.7±0.5	1.7±0.5	2.5±0.6	3.1±0.6	3.2±0.7	1.9±0.5
6	2.1±0.6	4.7*±0.6	5.5*±1.4	4.9*±1.7	4.6*±0.4	4.5*±0.9
12	3.1±0.4	3.2±0.5	4.7*±1.0	4.3*±0.8	4.8*±0.6	3.8*±0.6
24	3.8±0.7	2.3±1.2	3.3±0.5	4.0*±0.9	4.3*±0.4	3.7*±0.6
48	3.5±0.6	1.9±0.3	3.1±0.6	3.6*±0.9	3.3±0.4	3.6*±0.5
72	3.4±0.6	2.2±0.3	3.1±0.7	3.3±0.6	2.5±0.2	2.4±0.4
			Icteric index			
0	6.4±0.3	6.0±1.1	5.2±0.4	5.2±0.6	5.3±0.6	5.7±1.0
3	9.9±0.8	10.0*±1.5	8.7*±1.0	11.6*±0.9	9.0*±0.5	9.5*±0.6
6	12.0*±1.3	11.3*±1.7	8.3*±0.6	9.8*±0.9	8.8*±0.7	11.7*±1.1
12	9.8±1.1	8.5*±0.8	8.2*±0.7	8.4*±1.4	7.0±0.7	9.3*±0.9
24	8.4±1.1	8.3±0.6	7.7±0.3	8.0*±2.0	5.8±0.4	8.0*±1.0
48	7.9±1.6	8.1±1.0	7.0±0.6	7.8±0.9	5.0±0.4	7.0±0.7
72	7.2±1.0	7.3±0.8	6.9±0.7	7.2±1.0	5.2±0.4	6.3±0.6

Treatments : 1. Centrbucridine hydrochloride @ 1.5 mg/kg weight. 2. Centbucridine hydrochloride @ 1.75 mg/kg; body weight. 3. Triflupromazine @ 2 mg/kg + centbucridine hydrochloride @ 1.5 mg/kg body weight. 4. Triflupromziae @ 2 mg/kg + Centburcridine hydrochloride @ 1.75 mg/kg body weight. 5. Diazepam Centbucridine hydrochloride @ 1.5 mg/kg body weight. 6. Diazepam + Centbucridine hydrochloride.

al. 1993), with stellate ganglionic blockade (Jadon *et al.* 1995) and after epidural administration of bupivacaine (Tiwari *et al.* 1989) (Table I).

Lee Femine *et al.* (1987) reported a fair rise in adrenocortical hormones during ananesthesia. Cortisone induced gluconeogenesis plays an important role in enhancing the blood glucose levels following stress (Strawitz *et al.* 1961).

A nonsignificant increase in the levels of SGPT in all the group of animals of the present experiment is in accordance with the findings of Hussain and Kumar (1984a) and Malek *et al.* (1988) after visceral procaine blockade. A significant increase in alkaline phosphatase after centbucridine in all the treatments except I was noticed. The plasma icteric index also *increased significantly ($P<0.05$) in all the treatments (Table 1).*

Centbucridine is destroyed in the liver and causes liver oedema, dilation of sinusoids and degenerative changes in the liver cells (Nityanand *et al.* 1982). The disruption in the liver parenchyma cells increased cell membrane permeability leading to elevation of SGPT levels in the blood. Similarly, the change in production and excretion of bile increased activity of alkaline phosphatase (Kelly 1979). The simultaneous increase in the icteric index would have been because of increased concentration of bile pigments in circulation (Jain 1986) due to liver damage. The prolonged and increased variation in the SGPT, SAP and icteric index values in triflupromazine and diazepam premedicated animals reflect to a greater disruption to the liver parenchyma cells and the increased cell membrane permeability and is in conformity with the observations of Vickers *et al.* (1984).

Moderate increase in BUN was noticed in all the treatments. Similarly, a nonsignificant increase in the blood creatinine was seen. The values of both the parameters fluctuated within the normal range (Table 1). Since there is no significant rise in the levels of BUN and creatinine, it is presumed that administration of centbucridine alone and alongwith preanaesthetic did not alter the glomerular filtration rate.

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Indian Journal of Animal Sciences 68 (11) : 1150-1153, November 1998

Disposition kinetics of gentamicin following intramuscular (IM) administration in kidney damaged goats

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Received: 30 September 1997; Accepted: 6 May 1998

ABSTRACT

Disposition kinetics of gentamicin following single dose (5 mg/kg bw) intramuscular (IM) administration in kidney damaged (renal-impaired)goats was investigated. Kidney in goats was damaged by injecting single IV dose (5 mg/kg bw) of uranyl nitrate. The extent of damage was measured as serum creatinine and blood urea nitrogen levels.

A one- compartment model adequately described the plasma concentration time profile. The absorption of gentamicin, following IM administration was rapid, with peak plasma concentration achieved within 30-60 min in renal-impaired goats. Therapeutic concentration (5 µg/ml) was maintained up to 18hr. An elimination half-life of 482min was observed. Volume of distribution (Vd/F) was 198.4 ml/kg. Clearance value was 0.26 ml/kg/min. Based on the pharmacokinetic data, dosage regimen in renal-impaired goats was calculated.

Key words : Disposition kinetics, Gentamicin, Renal-impaired goats

Nephrotoxicity of aminoglycosides has been well documented in several species of animals. A concentration higher than 12 µg/ml of gentamicin may cause ototoxicity and nephrotoxicity. As there is a narrow therapeutic index of gentamicin, care must be exercised in the selection of dose and frequency of administration specially in patients with impaired renal function (Kaye *et al.* 1974).

It is now established that there is a relationship between chemical half life and the duration of the pharmacological effect of most drugs or their active metabolites. In patients with renal insufficiency, impaired drug clearance may lead to excessive drug accumulation giving rise to toxic effects. Drugs cleared predominantly by the kidneys and that are toxic at high circulating levels, dose adjustment is essential for such drugs in patients with renal insufficiency (Welling *et al.* 1975). This report describes the effect of renal impairment on pharmacokinetic parameters of gentamicin.

MATERIALS AND METHODS

Eight female goats (11.5 ± 0.8 kg) were used in the experiment. Primicin injections containing 40 mg/ml of gentamicin sulphate was used in the study.

Renal impairment was induced in goats by injecting uranyl nitrate as single dose (5 mg/kg bw) intravenously (IV). The damage to kidney was assessed on the basis of serum creatinine (SC) (Folin and Wu 1919) and blood urea nitrogen (BUN) (Richter and Lapointe 1962) over a period of 10 days. However, for pharmacokinetic study, a single dose of gentamicin was injected at a dose rate of 5 mg/kg bw in gluteal muscles on 7th day of administration of uranyl nitrate.

Blood samples (2-3 ml, with Ca EDTA as anticoagulant) were collected each day for 10 days for estimation of serum creatinine and blood urea nitrogen. The blood samples for measurement of gentamicin were collected from jugular vein at 5, 10, 15 and 30 min and at 1,2,4,8,12,24,30 and 36 hr following its administration. The blood samples were allowed to clot at room temperature (28°-30°C) for 4-6 hr. The serum was centrifuged and the supernatant stored at –20°C until analyzed.

Gentamicin serum assay was performed microbiologically 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 coefficient of variation were 6.8 and 7.9% respectively. The linear correlation of gentamicin concentration and zone of inhibition was 0.90.

The statistical analysis of concentration of gentamicin at various time intervals between normal and renal-impaired goats and increase in concentration of SC and BUN compared to their zero time concentrations, respectively, was done by analysis of variance. However, the pharmacokinetic data were compared by non-parametric Wilcoxon's rank sum test (Steel and Torrie 1967). The pharmacokinetic analysis of gentamicin data for each goat was performed with the aid of a non-linear curve fitting programme.

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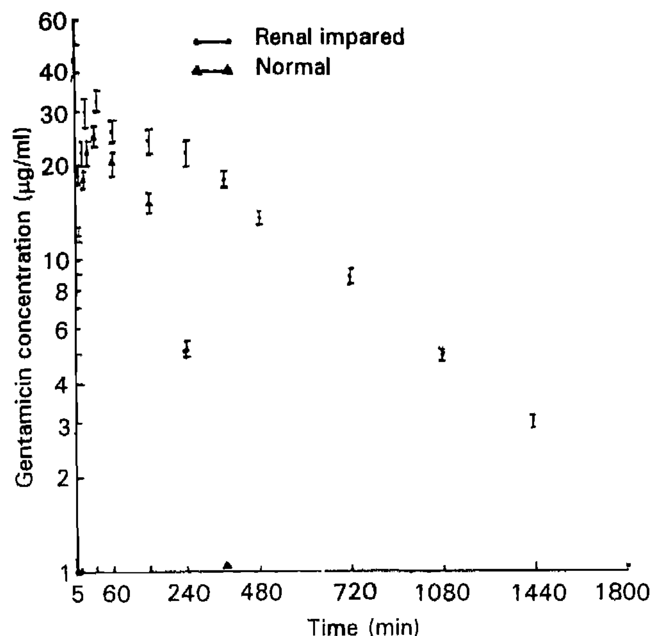

Fig. 1. Serum concentration time profile (mean ± SE) of gentamicin in goats following single dose (5 mg/kg) intramuscular administration in normal (n=8) and renal-impaired goats (n=6).

Table 1 SC and BUN level of goats (n=6) following IV single dose administration of uranyl nitrate (5 mg/kg bw)

Days	Serum creatinine (mg/dl)	Blood urea nitrogen (mg/dl)
0	1.5 ± 0.29	17.6 ± 4.1
1	1.8 ± 0.2	23.7 ± 6.0
2	3.0 ± 0.5	34 5 ± 4.7
3	4.3 ± 0.6	44.7 ± 8.0
4	5.3 ± 0.8*	53.5 ± 6.1
5	6.4 ± 0.8*	57.9 ± 5.2*
6	7.2 ± 0.7**	80.3 ±10 4**
7	7.5 ± 0.8**	97.0 ±13.0**
8	7.0 ± 1.0*	77.4 ±15.5*
9	5.4 ± 0.6	77.3 ±16.5*
10	4.6 ± 0.3	51.2 ± 9.0

* P <0.05, ** P <0.01.

RESULTS

Six days before injecting gentamicin for the pharmacokinetic studies, renal impairment was done by IV injection of uranyl nitrate (5 mg/kg). A significant (P<0.05) increase in SC and BUN level compared to 0 day was observed from 5 to 8 and 6 to 9 day respectively (Table 1).

The concentration of gentamicin at various time intervals in renal-impaired goats remained higher compared to normal ones (Fig. 1). The excretion of gentamicin in renal-impaired goats was very slow and it could be detected in serum up to 30 hr, whereas, in normal ones it was only up to 6 hr. Peak serum concentration was achieved at 30 min following IM administration both in renal-impaired and normal goats. In renal-impaired goats the therapeutic concentration (5 µg/ml) was maintained up to 18 hr, as against 4 hr in respect of normal goats.

A one-compartment open model adequately described plasma concentration time data for each goat both in renal-impaired and normal ones and the correlation coefficients for the fits were 0.86 and 0.96 respectively.

No significant change was observed in absorption half-life of renal-impaired goats compared to normal ones (Table 2). However, a significant (P<0.01) increase in elimination half-life of renal-impaired goats (482 min) was observed compared to normal ones (89 min). Volume of distribution (VD/F) neglecting the absorption phase did not show significant change in 2 groups of animals.

Clearance (Cl/F) of drug from serum neglecting the absorption phase was significantly (P<0.01) lower in renal - impaired ones (0.26 ml/min/kg) compared to normal goats (1.3 ml/min.kg). Area under the curve (AUC) was significantly (P<0.01) higher in renal-impaired goats (18940 µg.min/ml) in compared to normal ones (3795 µg.min/ml). Dosage regimen

Table 2. Pharmacokinetic values (ranges and median) of gentamicin in normal@ (n=8) and renal-impaired (n=6) goats following single IM dose (5 mg/kg)

Parameters	Uni	Normal goats			Renal- impaired goats		
		Min	Max	Median	Min	Max	Median
A	µg/ml	20.3	64.2	31.65	20.4	39.5	25.2*
Ke	min^{-1}	0.005	0.008	0.0075	0.0009	0.0021	0.002*
Ka	min^{-1}	0.055	0.12	0.092	0.07	0.02	0.11
$t^{1/2}$ Ke	min	79.1	133.9	88.75	337.5	740.5	481.9**
$t^{1/2}$ Ka	min	2.1	12.6	7.55	3.5	9.9	6.3
Vd/F	ml/kg	77.9	246.3	158.0	126.6	245.1	198.4
Cl/F	ml/min.kg	0.7	2.1	1.3	0.2	0.34	0.26**
AUC	µg.min./ml	2372.2	7416	3795.25	14540	24510	18940**

**P < 0.01, * P <0.05, @ Data from Ahmad and Sharma (1997).

A, Zero time plasma drug concentration, coefficient of absorption phase; Ke, elimination rate constant; Ka, absorption rate constant; $t^{1/2}$ (ka), absorption half life; $t^{1/2}$ (ke), elimination half life; Vd/F, apparent volume of distribution when the fraction of drug absorbed F is not known; Cl/F, clearance of drug 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.

Table 3. Dosage regimen (IM) of gentamicin in renal-impaired goats based on the median kinetic data

Dosage regimens	Expected serum concentration (mg/ml)		
(mg/kg/bw 24 hr)	C_{ss}	C^{ss}_{max}	C^{ss}_{max}
1.5	4.0	8.3	0.7
2.0	5.3	1.0	0.9
3.0	8.0	16.6	1.4

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

of gentamicin in renal-impaired goats following im administration is depicted in Table 3.

DISCUSSION

Pharmacokinetics of gentamicin is well documented in normal animals, however, scant literature is available in the diseased animals. It is being assumed that the pharmacokinetic values obtained in normal animals will hold true in the diseased ones also. However, various disease states including renal dysfunction can have pronounced effect on the pharmacokinetic and pharmacodynamic characteristics of a drug (Fabre and Balant 1976).

Renal dysfunction can be produced by various methods such as (i) administration of various nephrotoxic agents like dichromate, certain pigments, mercurials, glycerol and uranyl nitrate, and (ii) temporary occlusion of renal artery or intra-renal infusion of vasoconstrictors, ureteral ligation and partial nephroctomy. In this study uranyl nitrate was selected to produce renal dysfunction as it does not affect liver function (Kathleen *et al.* 1981).

Serum creatinine and blood urea nitrogen were selected as indices of renal dysfunction in this study. There was a significant rise in the level of serum creatinine (P<0.05) and blood urea nitrogen (P<0.01) from 5 to 8 and 6 to 9 day, respectively, following uranyl nitrate injection. BUN follows a curve identical to that of serum creatinine concentration in nephrotoxicity (Gilbert *et al.* 1979). Moreover, serum creatinine levels are superior to creatinine clearance for the detection of abnormal glomerular function and of changes in glomerular function in patients with chronic renal disease (Wheeler and Scheiner 1979).

The absorption of gentamicin following im administration was rapid. Peak concentration was achieved within 30 min both in normal and renal-impaired goats.

There was no significant difference in the volume of distribution of gentamicin in normal and renal-impaired goats. In some patients volume of distribution of aminoglycoside increased due to the expansion of extra cellular fluid volume during evolution of nephrotoxic glomerular nephritis. However, the changes may not be observed in other models of renal failure (Riviere *et al.* 1981).

Half-life of elimination for drugs excreted primarily by kidney is inversely proportional to the glomerular filtration rate (Kunin *et al.* 1959). For such drugs the half-life increases slowly until the glomerular filtration reaches its maximum, but with more severe degree of renal dysfunction, the half life markedly increases. In this study the elimination half-life increased significantly (P<0.01) in renal impaired goats compared to that of normal ones.

The clearance value of gentamicin in this study decreased significantly (P<0.01) in renal-impaired goats compared to that in normal ones. However, the clearance value varied widely in nephrectomized dogs (Frazier *et al.* 1988). The variability in pharmacokinetic disposition of drug associated with different diseases emphasizes the need for dosage adjustment, otherwise accumulation of toxic drug concentration could result (Frazier *et al.* 1988), if dose calculated for normal animals are used in animals with renal dysfunction.

When renal function is impaired, elimination of drugs which are excreted mainly by kidney is decreased and this necessitates rescheduling of dosage regimen (Baggot 1980). Based on these findings a dose of 2 mg/kg bw (im) every 24 hr with an average therapeutic concentration can be recommended. The dose size was reduced and time interval for administration increased compared to the normal ones (3.5 mg/kg bw(im) every 8 hr). In renal dysfunction, dose adjustments are made on the basis of serum creatinine concentration. If serum creatinine concentration increases, either the dose size be reduced or dosage interval be increased.

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Indian Journal of Animal Sciences **68** (11) : 1154-1156, November 1998

Blood biochemical profile in normal cyclical and anoestrus cows

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Received : 10 October 1997; Accepted : 20 April 1998

ABSTRACT

Blood serum biochemical constituents were analyzed in 6 normal cyclical cows and 6 anoestrus Jersey crossbred cows. Total protein, albumin, globulins, cholesterol, glucose, calcium, inorganic phosphorus, sodium, chloride, potassium, alkaline phosphatases and SGOT were significantly ($P < 0.01$) lower in anoestrus cows than that of normal cyclical cows. The possible role of these blood serum constituents in reproduction is discussed.

Key words : Anoestrus, Biochemical profile, Blood, Cows

Nutrients play an important role in fertility management in dairy cattle and have specific requirements in reproductive tissue. Variation in certain blood constituents during different reproductive phases alter the proper function of the reproductive organs (Rowlands 1977). Nutrient deficiency may impair the enzymatic function and cellular metabolism of reproductive organs and induced concomitant reproductive disorders i.e. anoestrum and suboestrum (Dutta *et al.* 1988, Shemesh *et al.* 1984, Vohra *et al.* 1995) which in turn affect the reproductive efficiency, and thus an economic loss to dairy farmers. The present study was undertaken to elucidate the relationship between biochemical constituents and anoestrus condition in dairy cows.

MATERIALS AND METHODS

Apparently healthy Jersey crossbred cows (12) of clinical gynaecological cases of Madras Veterinary College, TANUVAS, were selected. All the cows were between the age group of 4 and 6 years and weighed of 200-250 kg. After 2 consecutive rectal examination at an interval of 10 days the animals were grouped into cyclical and anoestrus. Group 1 (normal cyclical cows) consisted of 6 cows with regular oestrous cycle (21 ± 1 days) and no detectable abnormalities on gynaecological examination. Group 2 (anoestrus cows) consisted of 6 cows with the history of absence of oestrus signs for 5 months, with no palpable structures in both the ovaries and no detectable genital abnormalities on repeated rectal examinations. In group 1 the venous blood samples (10-15 ml) were collected at oestrum and in group 2 on the day of second rectal examination. For glucose estimation blood was collected in separated sodium fluoride vials. Serum was separated by standard procedure and stored at –20°C till biochemical analysis. Blood urea nitrogen (BUN), creatinine, total protein, globulins, cholesterol, glucose, calcium, phosphorus-inorganic, sodium, chloride, potassium, alkaline phosphatase (AKP) and serum glutamic oxaloacetic transaminase (SGOT) were analyzed by using commercially available kits and BTS 320 photometer. The data were analyzed by student 't' test (Snedecor and Cochran 1967).

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RESULTS AND DISCUSSION

The serum biochemical constituents are given in Table 1. The concentration of total protein, albumin, globulins, cholesterol, glucose, calcium, phosphorus, sodium, chloride, potassium, alkaline phosphatase and SGOT were significantly ($P < 0.01$) higher in normal cyclical cows than in anoestrus cows. No significant differences were observed in BUN and creatinine level between cyclical and anoestrus cows.

Total protein, albumin and globulins

The normal cyclical cows had significantly ($P < 0.01$) higher level of total protein, albumin and globulins than that of anoestrus cows. Chetty and Roa (1986), Dutta *et al.* (1988) and Vohra *et al.* (1995) also reported similar values. Patil and Desphande (1979) reported that a certain amount of optimal protein level is necessary for the expression of oestrus signs in cows. Bearcten and Fuquary (1992) suggested that deficiency in protein induced weak expression of oestrum or cessation of oestrum. May be the low level of serum protein caused deficiency of certain amino acid required for synthesis of gonadotropins (Vohra *et al.* 1995). The decreased level of

Table 1. Serum biochemical values in normal cyclical and anoestrus cows (ME±SE)

Biochemical constituents	Normal cyclical cows	Anoestrous cows
BUN mg%	3.15±0.36	2.93±0.21 NS
Creatinine mg%	0.58±0.10	0.56±0.50 NS
Total protein mg%	7.45±0.39	4.80±0.53**
Albumin mg%	3.65±0.25	3.11±0 29**
Globulins mg%	3.80±0.16	1.69±0.47**
Cholesterol mg%	188.16±11.11	140.16±5.73**
Glucose mg%	64.50±2.47	41.50±3.45**
Calcium mg%	10.71±0.36	8.98±0.38**
Phosphorus (i) mg%	5.36±0.09	2.58±0.27**
Sodium m.Eq/ml	83.33±2.88	63.50±2.35**
Chloride m.Eq/ml	93.50±3.18	74.66±3.15**
Potassium m.Eq/ml	2.55±0.25	1.91±0.24**
AKP KAU	12.83±0.57	7.11±0.41**
SGOT IU	107.83±5.64	67.00±4.09**

NS, Nonsignificant, ** ($P < 0.01$).

total protein, albumin and globulins in the present study might have affected the biosynthesis of gonadotropins and gonadal hormones in anoestrus cows.

Glucose

The blood glucose concentration was significantly ($P<0.01$) lower in anoestrus cows and in agreement to Dutta *et al.* (1988). The blood glucose concentration is affected by energy status and reproductive efficiency of the animal. The high blood glucose level increased the progesterone production directly by increasing LH pulses and mean concentration of LH (Richards *et al.* 1989) and indirectly by increasing blood insulin levels which stimulated progesterone secretion from luteal cells (Mc Ardle and Holtfort 1989). Howland *et al.* (1966) reported that hypoglycaemia caused loss of ovarian activity by affecting the hypothalamus and release of gonadotropins from hypophysis. The low level of blood glucose in the present investigation indicates the poor energy status of the cows and its role in impaired hypothalamic hypophyseal ovarian axis signal transmission in anoestrus cows.

Cholesterol

The serum cholesterol is significantly ($P <0.01$) lower in anoestrus cows than in normal cyclical cows. Our findings are in consent with the observations of Dutta *et al.* (1989), Paul *et al.* (1991) and Vohra *et al.* (1995). Cholesterol is one of the precursors for synthesis of steroid hormones such as androstenidione, progesterone and oestrogen. Velhanker (1973) found a positive correlation between cholesterol concentration and energy status in animals. Pereek and Deen (1985) stated that subnormal energy status induce hypocholestemia which in turn lead to improper out put of steroids. The high level of cholesterol in cyclic animals is indicative of more secretion of steroids during oestrus due to increased ovarian activity (Dutta *et al.* 1989). The declined level of cholesterol in present study suggests the reduced steroidogenesis in the ovaries of anoestrus cows.

Calcium and phosphorus

The concentration of serum calcium and phosphorus were significantly ($P < 0.01$) lower in anoestrus cows than that of normal cyclical cows. Our findings are in concurrence with the studies of Dutta *et al.* (1988). The altered dietary calcium : phosphorus ratio affects the reproductive performance of animals (Pugh *et al.* 1985). Calcium dependent mechanism is involved in steroid biosynthesis in ovaries (Shemesh *et al.* 1984). Hurley and Doane (1989) stated that GnRH stimulation of LH release from pituitary cells involved in calcium dependent manner, and the LH was not released in the absence of certain calcium concentration (or) in the presence of calcium blocking agents. Phosphorus is often associated with reproductive abnormalities in cattle and its deficiency induce anoestrum and reduced ovarian activity (Pugh *et al.* 1989). The hypophosphoteamia affects the most of the cell functions, as phosphorus is an integral component of nucleic acid, nucleotides, phospholipids and some proteins. Phosphorus is essential for transfer and utilization of energy, normal phospholipid metabolism and large number of coenzyme activation (Hurley and Doane 1989). The role of calcium and phosphorus in anoestrus cows may influence the level of coenzyme system in the synthesis of hormones involved in reproduction.

Sodium, potassium and chloride

In this study anoestrus cows had significantly ($P < 0.01$) low concentration of sodium, potassium and chloride. These minerals play an intermediate role in the action of hormones and enzymes at subcellular levels. These minerals act in integrated fashion in the synthesis of reproductive hormones, action of such hormones on reproductive organs and initiation of oestrus in animals.

SGOT and AKP

Significantly ($P < 0.01$) low levels of SGOT and AKP were observed in anoestrus cows. Our results are in accordance with the findings of Stallcupp *et al.* (1967) and Paul *et al.* (1991). Hormonal imbalance and deranged enzymatic action affect the normal reproductive behaviour of the animal and cause physiological alteration (Paul *et al.* 1991). The concentration of both these enzymes are indicative of level of physiological activity of the tissues. In this investigation, the diminished concentration of SGOT and AKP might be associated with reduced physiological activity of reproductive organs in anoestrus cows.

The present study indicated that anoestrus cows have lower concentration of serum total protein, albumin, globulins, glucose, cholesterol, calcium, phosphorus, sodium, potassium, chloride, AKP and SGOT, than normal cyclical cows, thus

these blood constituents affect the fertility of cows. Profile of these constituents can be used as more precise guideline for correcting anoestrum of nutritional origin.

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Indian Journal of Animal Sciences 68 (11) : 1157-1159, November 1998

Real time ultrasound evaluation of changes in follicular populations during oestrous cycle in buffalo

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Received: 5 December 1997; Accepted : 29 April 1998

ABSTRACT

Changes in ovarian follicular populations during oestrous cycle were examined by real time ultrasonography in buffaloes. The endpoints used were number of follicles 3-4 mm, 5-6 mm, 7-10 mm, ≥ 11 mm and the diameters of the largest and the second largest follicles, between day 0 and day 15 of oestrous cycle. The profile of the diameter of the second largest, but not the largest follicle was bimodal. There was a significant difference among different days in the diameters of both the largest and the second largest follicle. There was a significant difference in the numbers of follicles 5-6 mm and 7-10 mm in diameter but not in the numbers of follicles 3-4 mm and 11 mm, and the total number of follicles 3 mm in diameter among different days. Our results suggest that the follicular populations exhibit dynamic changes during oestrous cycle in buffaloes.

Key words: Buffalo, Follicle, Oestrous cycle, Ultrasonography

High variability and unpredictability in the superovulatory responses is a major limiting factor in embryo transfer programme in buffalo (Madan *et al.* 1996). Real-time ultrasonography has been widely used for studying the ovarian follicular dynamics during unstimulated oestrous cycles and during superovulation in cattle (Fortune 1993, Adams 1994). Detailed information on follicular populations during unstimulated oestrous cycle, which can be very useful in improving superovulation results is, however, very limited in buffalo (Baruselli *et al.* 1997). The objective of this study was to examine the follicular populations during oestrous cycle in Murrah buffalo.

MATERIALS AND METHODS

Animals

Nonlactating, sexually mature, multiparous buffaloes (5), between 6 and 7 years of age, weighing 450 to 550 kg, which had shown at least 1 normal oestrous cycle previously were maintained under general herd managemental conditions in the institute. The study was completed during winter (January to February).

Ultrasonography

The animals were examined by transrectal ultrasonographic imaging with a real-time B-mode instrument with a 5.0 MHz linear array transducer. Ultrasonographic examinations were performed daily between 1000 and 1200 hr starting on the day of natural oestrus and ending on the day of ovulation at the end of the oestrous cycle. The animals were restrained by making them stand in an animal crate. No chemical method was used to restrain the animals during ultrasonography. The transducer was inserted after evacuating the rectum and ultrasonography was performed (Manik *et al.* 1994a). The antra of follicles ≥ 10 mm were measured with the built-in callipers after freezing the ultrasound image, whereas the diameters of smaller follicles were measured against the in-built centimeter scale displayed on the screen alongside the ultrasound image. This was done to minimize the errors during freezing of image. The diameter of nonspherical follicles was calculated by taking the average of the longest and widest measured points of the follicle. The follicles were classified on the basis of diameter as 3-4 mm, 5-6 mm, 7-10 mm and 11 mm. The day of ovulation was determined by the acute disappearance of a follicle 11 mm in diameter. The day of ovulation at the beginning of the interovulatory interval was designated as day 0.

Statistical analyses

The data were analyzed for the period between day 0 and day 15 of oestrous cycle. The endpoints used were number of follicles 3-4 mm, 5-6 mm, 7-10 mm, ≥ 11 mm and the diameters of the largest and the second largest follicles. The data on the number of follicles of various size categories at different days of the oestrous cycle was log transformed prior to analysis. The data were analyzed by statistical procedures using

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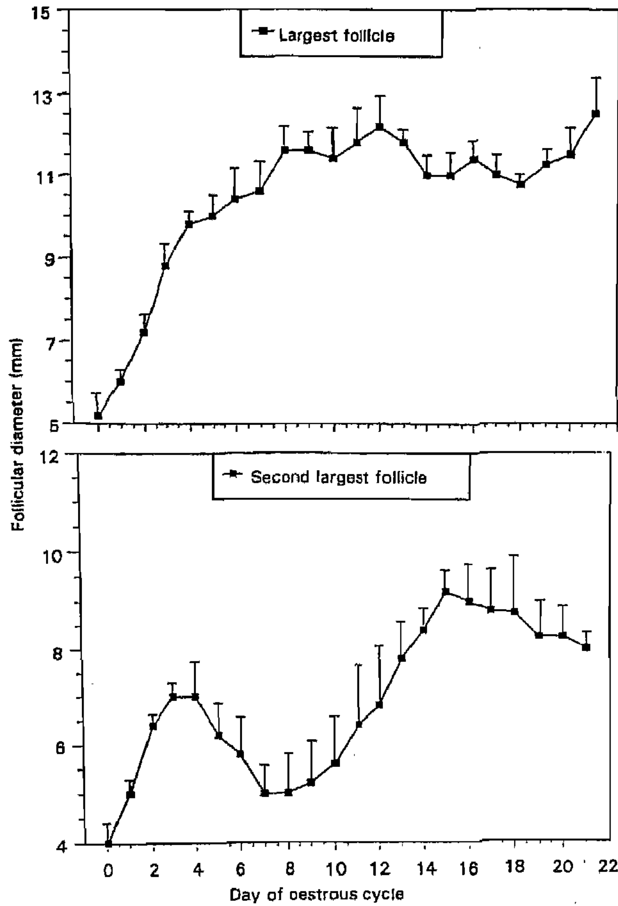

Fig. 1. Mean (± SEM) diameters of the largest and second largest follicles during oestrous cycle in buffaloes (day 0, day of ovulation).

SYSTAT univariate and multivariate analysis of variance (ANOVA) with repeated measures.

RESULTS AND DISCUSSION

The profile of the diameter of the second largest follicle was bimodal, with minimum diameters on days 0 (4.00 ± 0.40 mm) and 7 (5.00 ± 0.57 mm) and maximum diameter on days 3 (7.00 ± 0.28 mm) and 15 (9.20 ± 0.28 mm) (Fig. 1). The profile of the diameter of the largest follicle was, however, not bimodal. There was a significant difference among different days in the diameters of both the largest ($P<0.005$) and the second largest follicle ($P<0.016$). The diameters of largest and second largest follicles were not correlated with each other. The diameter of the largest follicle was negatively correlated ($r = -0.46$, $P<0.001$) with the number of follicles 5-6 mm in diameter, but not with the number of follicles of all other size categories. The diameter of the second largest follicle was positively correlated ($r = 0.57$, $P<0.001$) with the number of follicles 7-10 mm in diameter and negatively correlated with the number of follicles 3-4 mm in diameter ($r = -0.21$, $P<0.05$). There was a significant difference in the numbers of follicles 5-6 mm ($P<0.03$) and 7-10 mm in diameter ($P<0.004$) but not in the numbers of follicles 3-4 mm and ≥ 11 mm, and the total number of follicles 3 mm in diameter among different days (Fig.2). The number of follicles 7-10 mm in diameter was negatively correlated with the number of follicles 3-4 mm ($r = -0.21$, $P<0.05$) and ≥ 11 mm in diameter ($r = -0.41$, $P<0.001$), but not with the number of follicles 5-6 mm in diameter. The number of follicles ≥ 11 mm in diameter were

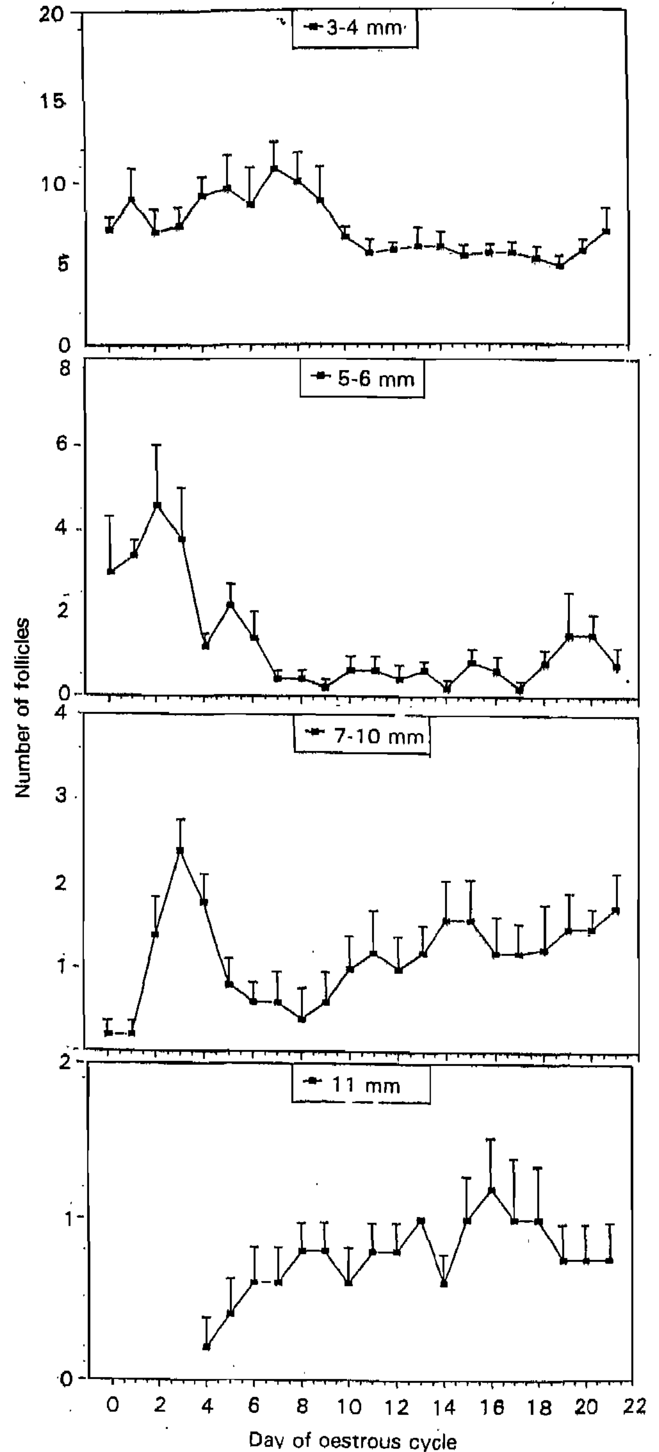

Fig.2. Mean (± SEM) numbers of follicles of various size categories during oestrous cycle in buffaloes (day 0, day of ovulation).

negatively correlated with the number of follicles 5-6 mm in diameter ($r = -0.41$, $P<0.001$).

Our results suggest that the number of follicles of various size categories was not constant throughout the oestrous cycle. These results are in agreement with those of Pierson and Ginther (1987a) in cattle in terms of a bimodal pattern of the second largest follicle, and highly significant differences in the diameters of the largest and the second largest follicle and the numbers of follicles 5-6 and 7-10 mm in diameter among different days of oestrous cycle. Lack of a bimodal pattern for the largest follicle and follicles of 3-4 mm category, as observed in this study differs from observations in cattle (Pierson and Ginther 1987a) and buffalo (Baruselli *et al.* 1997) in which bimodal patterns were also obtained for largest follicle and/or numbers of follicles of various size categories during oestrous cycle. This discrepancy could partly be due to the fact that follicular turnover, occurred in a wave-like pattern during oestrous cycle, may follow α 2- or 3-wave pattern in cattle (Pierson and Ginther 1988, Sirois and Fortune 1988) and buffalo (Manik *et al.* 1994b, Baruselli *et al.* 1997) and partly due to high variability among individual animals. Nevertheless, the pattern emerged indicates that the period between day 0 and day 8, which is marked by the growth and development of the largest follicle is associated with an initial increase of short duration, and a subsequent decline in the size of the second largest follicle and the numbers of follicles 5-6 and 7-10 mm in diameter. The largest follicle generally representing the morphologically and/or functionally dominant follicle is reported to adversely affect the growth and development of subordinate and smaller follicles leading to induction of atresia and degeneration of the latter (Fortune 1993). A fresh wave of follicular growth commences around day 10 of oestrous cycle as indicated by the pattern of growth of the second largest follicle and follicles of 7-10 mm category in this study. The temporal relationships among follicles of various size categories as observed in this study indicate constant turnover of follicles during the oestrous cycle.

The number of follicles ≥ 3 mm in diameter is similar to that reported by Manik *et al.* (1994a) and Baruselli *et al.* (1997) but is lower than that reported in cattle (Pierson and Ginther 1987 a,b, Desaulniers *et al.* 1995). A primary cause for this could be lower number of primordial follicles in buffalo (12 000 - 19 000; Samad and Nasseri 1979) compared to over 150 000 in cattle (Erickson 1966). The magnitude of superovulatory response is reported to be dependent upon the number of follicles 3-6 mm (Romero *et al.* 1991) and <5 mm in diameter at the initiation of superovulation (Van der Schans *et al.* 1991). Administration of exogenous gonadotropins triggers follicle recruitment from follicles smaller in size than those with endogenous levels of gonadotropins. All healthy follicles >1.7 mm in diameter are mobilized following exogenous gonadotropin administration (Monniaux *et al.* 1983). The number of such follicles was reported to range from 1 to 5 in buffaloes compared to 17-32 in cattle (Le Van *et al.* 1989). A lower population of follicles could, therefore, be a major determinant to lower superovulatory responses in buffalo.

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Indian Journal of Animal Sciences 68 (11) : 1160-1163, November 1998

Changes in lipid profile of corpus luteum throughout the estrous cycle in buffalo

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Received : 9 January 1998; Accepted : 5 June 1998

ABSTRACT

A biochemical study has been made on qualitative changes in lipids of corpora lutea during the estrous cycle in buffalo. The total lipid content increased up to day 11 (30.17±3.52 mg/g) and then decreased. The phospholipids (4.64±2.43 μg/100 mg) and cholesterol content (5.65±1.45 μg/100 mg) as well as phospholipid/cholesterol ratio (0.82) were minimum in day 13 to 17 corpora lutea. Regressing corpora lutea depicted maximum glycolipid (14.97 μg/mg) and free fatty acid (0.61 μg/100 mg) content. Triglycerides were maximum in the day 1 to day 15 corpora lutea (84.95±0.45 μg/100 mg). All these lipid changes shall be discussed in relation to the metabolic status of corpora lutea vis-a-vis hormonal titre and ultrastructural changes.

Key words : Buffalo, Corpus luteum, Lipid

Endocrine functions of corpus luteum are under dynamic influence of several hormones and regulatory factors (Billig *et al.* 1989). This steroidogenic tissue reveals variations in size, vascularity and innervation during different phases of estrous cycle (Sangha and Guraya 1989, Guraya *et al.* 1991, Sharma and Sharma 1997). Small and large steroidogenic cell populations of corpus luteum depict changes in their cytochemical and biochemical characteristics during different days of the estrous cycle (Billig *et al.* 1989). Accumulation of lipid droplets demonstrated in the bovine corpora lutea both histochemically (Boss *et al.* 1984, Fields *et al.* 1985, Hubbard and Erickson 1989) and biochemically (Gemmell *et al.* 1976, Parry *et al.* 1980). The luteal lipid concentrations vary with the stage of estrous cycle (Weinhouse and Brewer 1942). Luteal progesterone production is supported by flux rates of cholesterol between free and esterified forms (Christie *et al.* 1979, Veldhuis *et al.* 1985). Membrane polar lipids and cholesterol and phospholipid proportions affect membrane integrity and function (Thompson and Huang 1980). In ewes neutral lipids increased and then decreased during luteal regression (Waterman 1988). But no such report is available on buffalo, so in the present study attempt has been made to document the changes in various lipids in the corpora lutea of buffaloes during the estrous cycle.

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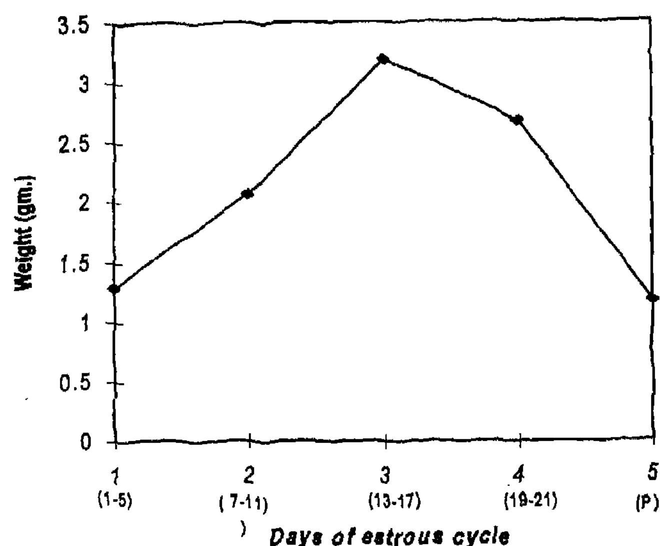

Fig. 1. Weight of corpus luteum (g)

MATERIALS AND METHODS

Ovaries of buffaloes were collected from the slaughterhouse at Delhi (28°-38' N, 77°-12' E). Corpora lutea were dissected from the ovaries and were categorized on the basis of their size, structure, texture and vascularity into 5 categories and each category included 3 to 5 corpora lutea. Total lipids were extracted using Folch *et al.* (1957) method. A homogenous paste of the sample was prepared in presence of anhydrous sodium sulphate. Total lipids were extracted from the paste with chloroform : methanol (2:1 v/v) in ratio of 1:20 (w/v) by intermittent shaking for 12 hr. Extent was filtered through G-3

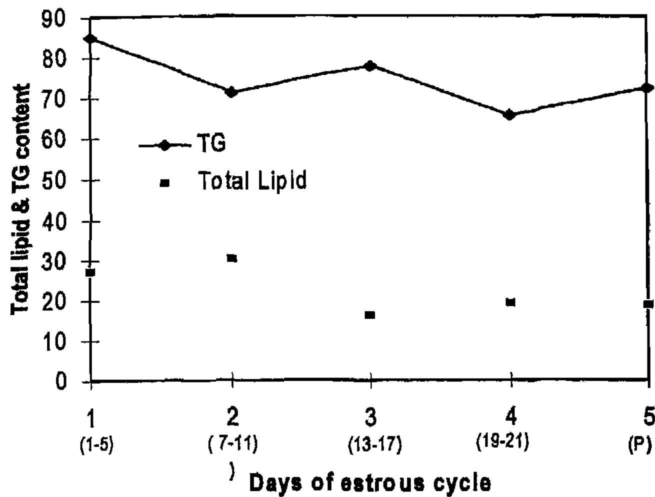

Fig. 2. Changes in total lipid content (mg/g of tissue) and triglycerides (µg/100 mg of lipid)

sintered funnel. The filtrate was given a folch washing with 0.9% saline (5:1 v/v). Total lipids were obtained in a crucible of known weight where evaporation of chloroform : methanol took place and then they were dissolved in cholesterol and used for quantitative analyses. Estimation of cholesterol was done by the method of Stadman (1957). Method given by Roughan and Batt (1968) was followed to determine the glycolipid content. Total phospholipids were quantitiated by Ames (1966) method. Free fatty acids were analysed by Lowry and Tinsley (1976) methods. The data was statistically analysed (Zar 1984).

RESULTS AND DISCUSSION

The gravimatric data on corpus luteum revealed a gradual increase from day 1 to day 17, and from day 18 to 21 a decline was observed (Table 1, Fig. 1). These changes correspond to the luteal and follicular phases of the estrous cycle in buffalo (Guraya 1998). The elevated weight of corpus luteum on day 18corresponds to the development of ultrastructural complexities required for the enhanced steriodogenesis. The decline in the weight observed thereafter corresponds to the lysis of corpus luteum induced by the prostaglandins synthesized and secreted by the uterus (Hixon *et al.* 1983). The total lipid content was heighest 30.17±3.52 mg/g on day 7 to 11 and a minimum of 16.26±8.07 in the days 13 to 17 (Table 1, Fig. 2). The increase in lipid content corresponds to the gradual development and accumulation of abundant lipids (diffused by distributed lipoproteins) and smooth endoplasmic reticulum (Guraya 1998). The degree of differentiation or development of such structures in corpus luteum attain a maxima on day 10 (Hansel *et al.* 1973, Guraya 1998). The cholesterol, glycolipids and phospholipid content were minimum in day 11 to 17 corpora lutea and same was depicted by phospholipid/cholesterol ratios. (Table 1, Figs. 3, 4). The decline is attributed to the enhanced hormone production (progesterone) during the luteal phase (Guraya 1998). The luteal phase concentrations of progesterone in cattle average about 4 ηg/ml (Ireland and Roche 1992, Walters *et al.* 1984) with a pulse amplitude and mean level peak at about day 11. The quantity of progesterone was heighest (2-10 ng/ml) during days 8 to 18 of the estrous cycle in cattle (Glencross *et al.* 1973, Rancheb *et al.* 1976, Schams *et al.* 1977, Kotwica and Williams 1982). The buffalo corpora lutea resembles that of cattle in terms of high progesterone production from day 12 to day 17 as evidenced by low titre of corpora lutea cholesterol, phospholipids and glycolipids. The variations observed in total triglyceride content and free fatty acids (Table 1, Figs 2, 4) corresponds to the variations in progesterone production and lipid droplet accumulations (Cunningham *et al.* 1975, Gemmel *et al.* 1976, Parry *et al.* 1980, Waterman 1988, Guraya 1998). During the luteal regression, a luteal lipoprotein lipase is extremely selective

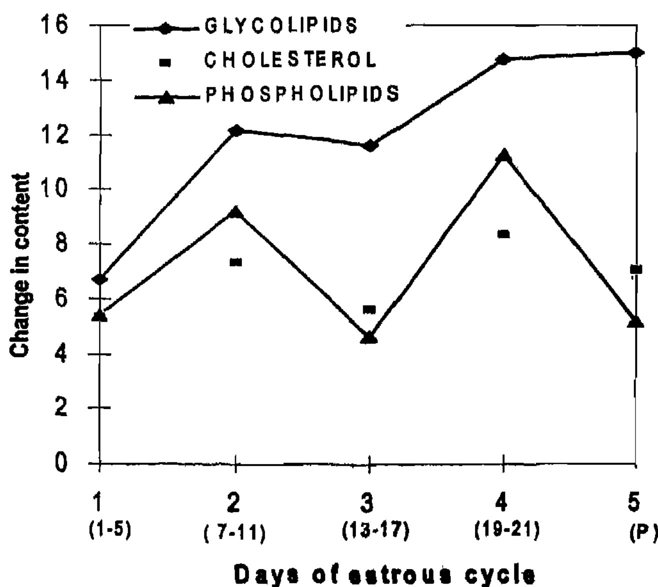

Fig. 3. Pattern of changes in glycolipids, cholesterol, phospholipid (µg/100 mg of lipid).

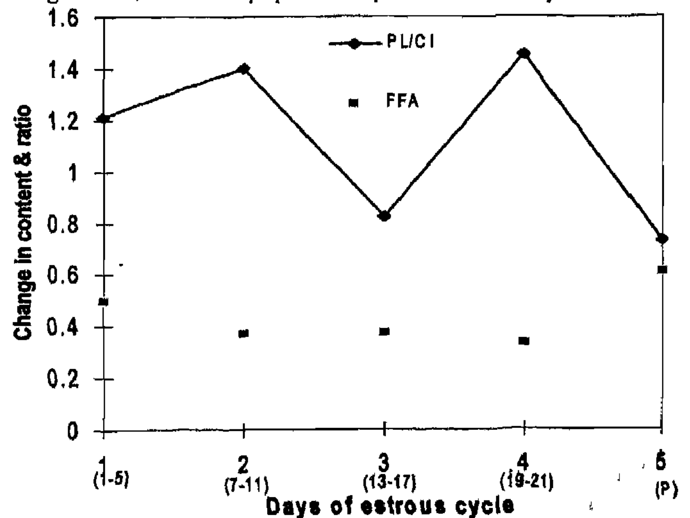

Fig. 4. Changes in free fatty acids and phospholipids/cholesterol ratio.

Table 1. Changes in weight of corpus luteum (g), amount of total lipid (mg/g), chelsterol, glycolipids, phospholipids, free fatty acids and triglycerides (μg/100 mg), phospholipid/cholesterol ratio in corpus luteum during different days of estrous cycle in buffalo

Days of *estrous cycle*	Weight of *CL (g)*	Total lipid (mg/g)	Glycoplipds (μg/100 mg of lipid)	Cholesterol (μg/100 mg)	Phospholipids	Free fatty acids	TG	PL/Cl
1-5	1029*±0.25	27.31±5.71	6.71*±2.71	5.37±1.35	5.37*±0.49	0.50*±0.05	84.95*±0.45	1.21±0.05
7-11	2.06*±0.29	30.17*±3.52	12.13±0.80	7.34±1.15	9.17*±1.09	0.37±0.06	71.25±1.07	1.40±0.16
13-17	3.20*±0.48	16.26±8.07	11.63±3.03	5.65±1.45	4.64*±2.43	0.37±0.04	77.69±5.99	.82±0.32
19-21	2.67±0.25	19.32±1.56	14.73±1.98	8.35±2.13	11.25*±0.31	0.33±0.02	65.33±3.79	1.45±0.40
P (Previous cycle)	1.17	18.72	14.97	7.04	5.16*	0.61	72.22	0.73

*P<0.05.

and sequestring triglycerids and fatty acids or other fatty acid sources must be contributing in luteal lipid accumulations (Garton and Duncan 1964). Organelle membrane integrities and enzymes are sensitive to phospholipid orientations within the lipid bilayer structure and are susceptible to localized alterations in phospholipid metabolism (Thompson and Huang 1980, Bell *et al.* 1981). The changes observed in corpus luteum of previous cycle in fatty acids percentages indicate a breakdonw in intracellular compartmentation. Luteal phospholipids and triglycerids, but not cholesterol, are involved in fatty acids metabolism in regressing corpora lutea (Waterman 1984).

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Indian Journal of Animal Sciences 68 (11) : 1164-1166, November 1998

Temporal relationship between plasma progesterone and oxytocin profiles in relation to ovarian activity in sheep

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Received: 17 November 1997; Accepted : 10 May 1998

ABSTRACT

The relationship between progesterone and oxytocin profiles during the oestrous cycle, early pregnancy, ovarian inactivity and after $PGF_2\alpha$ injection in sheep, was examined. A parallelism between progesterone and oxytocin levels during the estrous cycle ($r = 0.846$, $P < 0.1$) was observed. Early pregnant ewes and those having inactive ovaries had significant low oxytocin levels. Injection of cloprostenol induced peak levels of oxytocin after 2 hr from injection ($r = 0.366$, $P < 0.05$).

Key words: *Oxytocin, Ovarian activity, Progesterone, Sheep*

Oxytocin had previously been known to associate with milk ejection and uterine contraction during labour. Corpora lutea of a number of mammals including sheep contain large amounts of oxytocin (Cann *et al.* 1994) which was implicated in many aspects of reproduction and controlling of ovarian function in farm animals (Carter 1992, Goff *et al.* 1996). Anoestrous ewes have low oxytocin level and receptors (Cann *et al.* 1994). Injection of $PGF_2\alpha$ or its synthetic analogues induce high concentration of oxytocin (Silvia *et al.* 1991). During early stages of pregnancy, low levels of oxytocin were reported in ewes (McCracken *et al.* 1981). This study examine the temporal relationship between plasma progesterone and oxytocin profiles in sheep with emphasis on changes during the oestrous cycle, early pregnancy, ovarian inactivity and $PGF_2\alpha$ injection.

MATERIALS AND METHODS

Crossbred mature Egyptian ewes (14, 30-40 kg live body weight) were raised under routine managemental conditions practised at the National Research Centre Experimental Farm. Two proven rams were used for oestrous detection and mating. Ewes were checked for oestrus depending on ram marks and plasma progesterone levels. The ewes showing no evidence of ovarian activity during the non-breeding season (May to August) were considered to have inactive ovaries. During the breeding season (September to March) oestrous cycle was synchronized using $PGF_2\alpha$ analogue (Cloprostenol), 125 mg IM/head, 2 doses, 9 days apart. Each ewe was followed up for at least 2 oestrous cycles (17-18 days) before subjecting for another experiment. On day 8 of the cycle (day of heat = day 0), 6 ewes were injected with cloprostenol and another 6 ewes were injected with saline as a control group. Rams were allowed to join ewes for mating and pregnancy was confirmed by absence of heat signs and progesterone levels. Blood samples were collected weekly during the non-breeding season as well as during days 0, 3, 10 after oestrous and daily from day 16 until the next oestrous during the breeding season. For cloprostenol experiment, samples were collected on day 8, before and every hour for 4-times after injection of $PGF_2\alpha$. Samples were also collected weekly during the first month after mating followed by fortnight collections thereafter from the assumed pregnant animals. Blood samples were cooled during transportation, centrifuged (1500 g for 15 min) within 1 hr and plasma was stored at –20°C. Progesterone (Abraham 1981) and oxytocin (Dwenger 1984) levels were determined by RIA technique. Samples were extracted by 1% trifluoroacetic acid and 60% acetonitrile for oxytocin measurements. Antioxytocin antibodies were raised in rabbit against synthetic ovine oxytocin conjugated to bovine serum albumin. The sensitivity of assays was 0.02 ng/ml and 1 pg/ml for progesterone and oxytocin, respectively. A single assay was performed for each hormone with intra-assay coefficients of variation of 6.45 and 12.6% for progesterone and oxytocin, respectively. Data were statistically analyzed (Snedecore and Cochran 1976).

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RESULTS

Breeding season

In cycling ewes, both progesterone (ng/ml) and oxytocin (pg/ml) levels changed ($P < 0.01$) among the different phases of the oestrous cycle. The respective values were 0.13±0.03

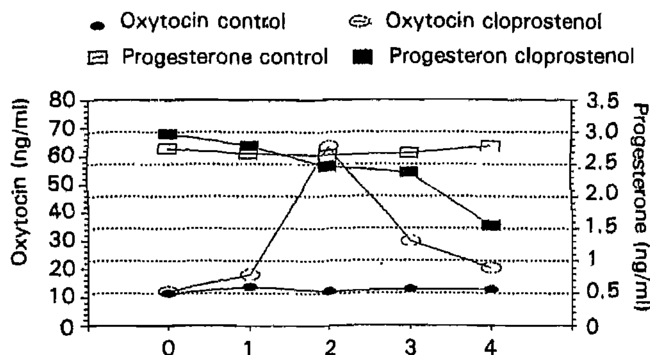

Fig. 1. Effect of cloprostenol injection on the temporal relationship between plasma progesterone and oxytocin in crossbred Egyptian ewes.

and 9.20±0.81 during oestrous, 3.76±0.19 and 14.00±0.89 during metoestrous, 7.82±0.49 and 26.60±1.06 during dioestrous and 0.71±0.08 and 10.40±0.86 during pro-oestrous (day -1). Moreover, a significant positive correlation was estimated between the 2 hormones ($r = 0.846$, $P < 0.01$).

Non-breeding season

During the non-breeding season, ewes having inactive ovaries had non-detectable progesterone (< 0.02 ng/ml) and low ($P < 0.01$) oxytocin (7.80±0.58 pg/ml) levels compared to cyclic ewes (overall means = 3.11±0.50 ng/ml and 14.55±1.07 pg/ml respectively) during breeding season with no significant correlation between both hormones.

Early pregnancy

During early pregnancy, progesterone and oxytocin levels did not follow the same trend, whereas, progesterone level was maintained (4.05±0.36 ng/ml) but oxytocin level fell (8.50±0.34 pg/ml, $P < 0.01$) compared with cyclic ewes with no significant correlation between both hormones.

Effect of prostaglandin injection

Analysis of variance indicated that injection of ewes on day 8 of the cycle with cloprostenol induced significant changes in both progesterone ($P < 0.05$) and oxytocin ($P < 0.01$) levels (Fig. 1). Progesterone level approximately halved after 4 hr from $PGF_2\alpha$ injection and reached a non-detectable value 24-48 hr later on (data are not shown). On the other hand, peak level of oxytocin was attained after 2 hr from $PGF_2\alpha$ injection, thereafter, the level gradually decreased (Fig. 1). A positive significant relationship was computed between both hormones after $PGF_2\alpha$ injection ($r = 0.366$, $P < 0.05$).

DISCUSSION

Data presented here indicated the presence of a significant positive relationship between both plasma profiles of oxytocin and progesterone during the estrous cycle of sheep. This finding was similar to those of Wathes (1984), Jenkin *et al.* (1991) and Cann *et al.* (1994) in sheep who concluded that variations of oxytocin levels during the oestrous cycle depend upon the presence of corpora lutea and parallel that of the secretory pattern of progesterone. Moreover, this finding was confirmed herein, as during the non-breeding season, anoestrous ewes revealed low ($P < 0.01$) oxytocin level. Similarly, Cann *et al.* (1994) found that anoestrous ewes had low plasma oxytocin levels despite the high uterine oxytocin receptors which became high after 6 days treatment with progesterone or oestradiol. Moreover, Webb *et al.* (1981) reported that uterine $PGF_2\alpha$ was dramatically promoted following administration of estradiol 17ß in anoestrous ewes.

In this study, the maintaining of progesterone and declining of oxytocin levels during pregnancy comparing with cyclic animals were in line with the finding of Webb *et al.* (1981) who found that oxytocin level started to fall at day 15 postmating in pregnant ewes. Moreover, McCracken *et al.* (1981) demonstrated that endometrial oxytocin receptors concentration was obviously low in early pregnant ewes as a protective mechanism to prevent luteolysis. Also, the role of the early embryonic factors could not be denied (Webb *et al.* 1981).

In this study, injection of $PGF_2\alpha$ during luteal phase induced significant decrease in progesterone (after 4 hr) and increase in oxytocin (after 2 hr) levels compared to control. Silvia *et al.* (1991) found that $PGF_2\alpha$ promote the additional release of neurohypophysial oxytocin indicating the existence of positive feed back between oxytocin and $PGF_2\alpha$ in sheep. During leuteolysis, oxytocin binds to specific endometrial receptors and activates phospholipase C to hydrolyze phosphoionositol in the presence of high estrogen level, which subsequently stimulate leuteolytic endogenous $PGF_2\alpha$ release (Prince *et al.* 1995). Also immunization against oxytocin (Wathes 1984) or administration of oxytocin receptors antagonists (Jenkin *et al.* 1991) delays luteolysis in ewes. On the other hand, the decrease of plasma oxytocin level after 2 hr from $PGF_2\alpha$ injection may be due to cloprostenol induced depletion of ovarian oxytocin (Flint and Sheldrick 1982). The present observations strongly implicate luteal oxytocin in the process of luteolysis in sheep.

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Indian Journal of Animal Sciences 68 (11) . 1167-1168, November 1998

Purification of intraerythrocytic piroplasms of *Theileria annulata* from infected bovine blood

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Received : 10 November 1997; Accepted 6 May 1998

Key words : Cellulose powder CF-11, Piroplasm, *Theileria annulata*

Availability of deoxyribonucleic acid of *Theileria annulata*, free from contaminating DNA of cattle is the basic requirement for the development of specific nucleic acid probe for the diagnosis of bovine tropical theileriosis. This note communicates a simplified technique for purification of intraerythrocytic piroplasms of *T. annulata* from bovine blood, for use in the extraction of parasite DNA.

Experimental infection of *T. annulata* was set up in 4 - to 6 -week-old crossbred bovine calves by subcutaneous inoculation of ground tick tissue sporozoites equivalent to 5 adult *Hyalomma (a.) anatolicum*. Blood (100 ml) was collected with heparin (5 IU/ml of blood) from the jugular vein of the calf on day 15 post-infection at the height of erythrocytic parasitaemia (50% or more). Blood was diluted with phosphate buffer saline (pH 7.2) and centrifuged at 700g for 10 min at 4°C. The process was repeated for 4 times and the supernatant alongwith the buffy coat was discarded after each centrifugation. Packed cell volume (25 ml) was diluted with double volume of phosphate buffer saline (pH 7.2) and the suspension was passed through a glass column having 2 cm internal diameter, packed to a depth of 15 cm with dry fibrous cellulose powder CF-11. The suspension was filtered through the cellulose powder by gravity without applying any external pressure. The elute was centrifuged at 700g for 10 min and the absence of bovine leucocytes was ensured by optical microscopy. Packed infected erythrocytes (10 ml) were then suspended in 90 ml of 0.83% aqueous solution of ammonium chloride and allowed to lyse for 10 min at 37°C. The lysate was centrifuged at 100g for 15 min at 4°C to separate any unlysed erythrocytes. The supernatant was then centrifuged at 10 000g at 4°C for 30 min. The pellet containing the free piroplasms and erythrocytes stroma was passed through 13 mm diameter filter holder (Millipore) The filtrate was distributed in 500 µl microfuge tubes and the piroplasms were pelleted by centrifugation at 10 000g for 30 min at 4°C. The piroplasms were stored at –20°C.

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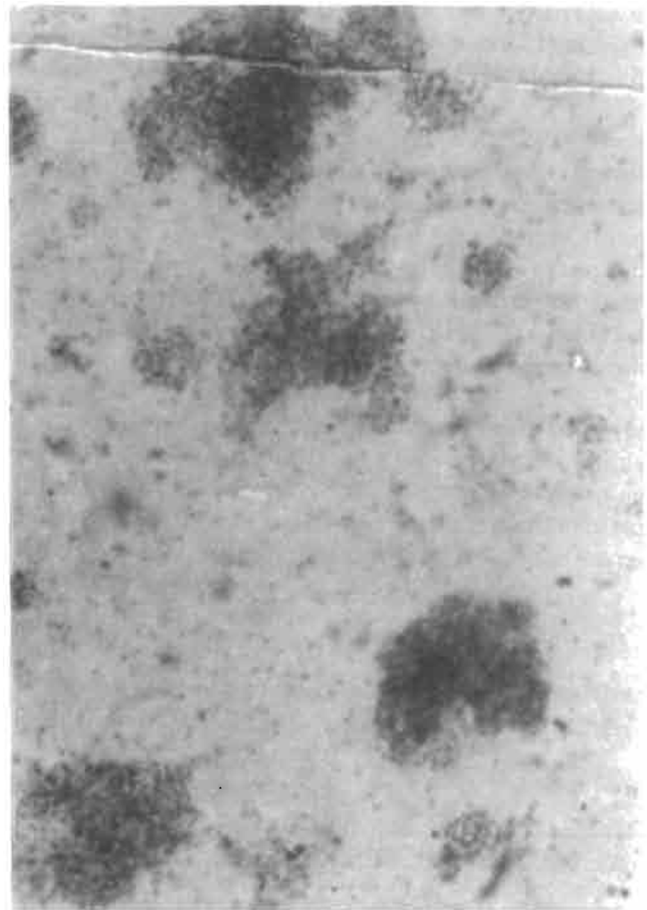

Fig. 1. Purified piroplasms of *Theileria annulata*

The protocol was found suitable for obtaining suspension of free piroplasms of *T. annulata* (Fig. 1). Ambrosio *et al.* (1986) advocated water lysis of bovine erythrocytes infected with *Babesia* sp. prior to passing through cellulose powder. However, in this study, the filtration of the intact cells through

cellulose powder was achieved at a faster rate as the lysates often clogged the passage. The filtration of the pellet of piroplasms through 8μ filter without using a prefilter ensured complete removal of cellular debris. Fujisaki *et al.* (1989) reported contamination of free piroplasms of *T. sergenti* with intact erythrocytes and cell debris in their attempt to purify the piroplasms from a sample of clotted infected blood.

ACKNOWLEDGEMENT

We thank Director, Indian Veterinary Research Institute, Izatnagar, for providing facilities.

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Indian Journal of Animal Sciences 68 (11) : 1169-1170, November 1998

Serodiagnosis of hydatidosis in sheep by counterimmunoelectrophoresis in Chennai, India*

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Key words: Counterimmunoelectrophoresis, Hydatidosis, Serodiagnosis, Sheep

Hydatidosis one of the serious helminthic zoonoses caused by cystic stage of the dog tapeworm *Echinococcus granulosus* is causing considerable economic losses in food animals in developing countries including India. Also indirectly it contributes to human infection. Hydatidosis in food animals is diagnosed only at postmortem. With the advent of powerful drugs against cystic stages of cestodes, the prospect for their control by therapy is bright if diagnosed early by a sensitive test. Indirect haemagglutination test (IHA), and counterimmunoelectrophoresis (CIEP) have been used extensively in various countries with fairly high sensitivity and specificity. Of these CIEP is relatively simple to perform, cost effective, with reasonably high sensitivity and specificity (Shariff and Parija 1991). Hence an attempt was made to evaluate the usefulness of CIEP in detecting ovine hydatidosis. The findings are presented in the paper.

Blood samples were collected from 40 randomly selected adult sheep at the slaughter for 10 days. Sera separated were used for CIEP screening (Ardehali *et al*. 1977) using barbitone buffer (pH 8.6). Dialyzed ovine hydatid cyst fluid antigen was prepared (Aykol 1987) with a protein content of 9.0 mg. Hyperimmune serum raised using the antigen in rabbits and foetal calf serum served as positive and negative serum controls respectively. The electrophoresis tank is of indigenous make and the amperage used is 8 mA per slide. The results were read after 45 min.

Of the 40 serum samples screened by CIEP, 4 proved positive. On routine examination of carcasses only 3 showed, the presence of hydatid cysts and these 3 were also seropositive. Thus a prevalence rate of 7.5% (3 out of 40) was observed in sheep. Of this, multiple cysts (3-4 cysts) were seen in lungs and liver with each cyst measuring 9 cm × 8 cm (Table 1). Prasad and Mandel (1982) in Bihar and Janardhan Pillai *et al.* (1986) in Tirupati reported similar results.

The sensitivity and specificity of the CIEP test were arrived by the ratio of number of false negative and false positive cases to the total number of serum samples screened and were 75% and 97.5% respectively. This is in conformity with the observations similarly made by Aykol (1987) and Sekar *et al.* (1989) in sheep.

ACKNOWLEDGEMENT

The authors wish to thank the Dean, Madras Veterinary College and Chennai Corporation Slaughterhouse, Chennai for the facilities provided for the present study.

Table 1. Prevalence and organ-wise involvement of hydatidosis

Total no. of serum samples screened	No. of negative cases	No. of positive cases		Organ-wise involvement		
		Postive	Rate of	Kind of	No. of	Percentage
Sheep sera (random samples) 40	37	3	7.5%	Liver	1	25.0
				Lungs	2	75.0
				Total	3	100.0

*Paper presented by the first author in the National Symposium on Rapid Diagnosis of Infectious Diseases held during November 21-23, 1997 at AIIMS, New Delhi.

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Indian Journal of Animal Sciences **68** (11) 1171-1172, November 1998

Pathology of infectious bursal disease in broiler chickens

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Received 10 December 1997, Accepted 8 May 1998

Key words Broiler, Infectious bursal disease, Pathology

Infectious bursal disease virus infection results in impairment of humoral immune response which predisposes the birds to variety of diseases and vaccine failure causing substantial loss to farmers. This paper describes the gross and histological lesions of infectious bursal disease in broiler chickens.

Carcasses of birds received for routine postmortem examination in the Department of Pathology of the University performed on all carcasses and gross lesions if any, were recorded. In such cases pieces of tissues from bursa of Fabricius, thymus, spleen, kidney, liver, heart, lung, intestine, proventriculus and in few cases brain were collected and preserved in 10% formaldehyde solution. Tissues were processed and embedded in paraffin. Paraffin sections of 5-6 μ thickness were prepared and stained by Haematoxylin and Eosin method.

Out of 1123 carcasses of broiler birds necropsied, 187 showed appreciable gross lesions of IBD. Bursa of Fabricius revealed varying gross lesions The bursa was enlarged, greyish white, edematous and covered by slimy material. On incision, the plicae were edematous (Fig. 1B) covered by a gelatinous exudate. Occasional petechiae and echymoses were observed on the plicae mucosa. In a few cases bursal haemorrhages were marked and the organ appeared dark reddish (Fig. 1A). In few cases the bursae were atrophied and contained caseated material within the lumen. These lesions were similar to those already described by Helmboldt and Garner (1964), Dal Bono *et al.* (1968), Dongoankar *et al* (1979), and Verma *et al* (1990). The kidneys often appeared pale with distended and prominent ureters. In a few cases the kidneys were enlarged haemorrhagic or congested as described by others (Cosgrove 1962, Helmboldt and Garner 1964, Barron *et al* 1966, Lay *et al.* 1983, Okoye and Uzoukwu 1981). The liver often showed enlargement and appeared either pale or dark brownish red. Congestion and

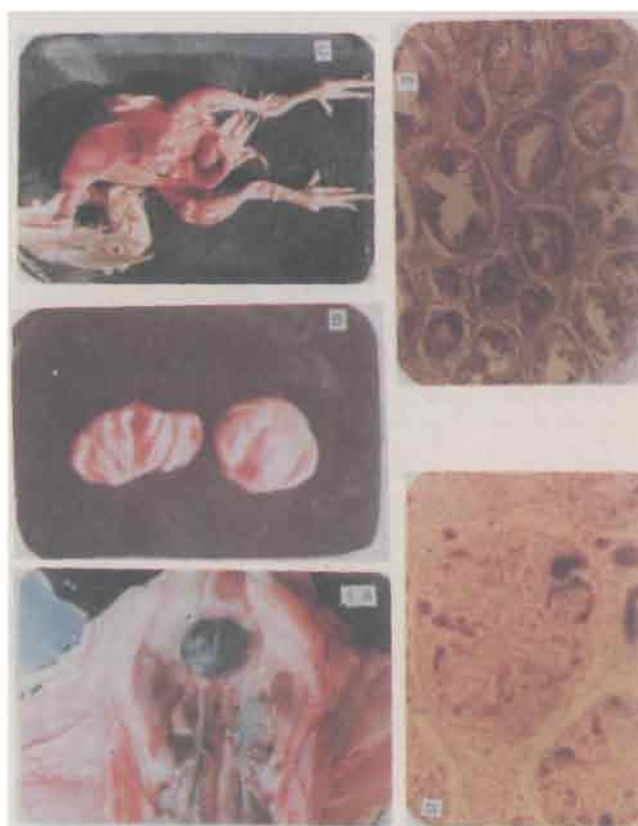

Fig **1A.** Enlarged intensely bluish red bursa of Fabricius. **1B** Enlarged bursa with edematous plicae. **1C.** Haemorrhages in the breast and thigh muscles **1D.** Intrafollicular haemorrhages in the bursa of Fabricius. H E × 10 . **1E.** Glandular transformation of follicles. H E × 10.

haemorrhages of thymus were observed in a number of birds which were similar to some earlier reports (Dal Bono *et al.* 1968, Dongoankar *et al.* 1979). In some cases the lungs were congested and haemorrhagic. Haemorrhagic lesions were also seen at the junction between proventriculus and gizzared in a few birds Similar observation was also made by Bygrove and Farghar (1970), Okoye and Uzoukwu (1981) and Varma *et al.* (1990). Haemorrhages particularly in the thigh and breast muscle (Fig. 1C) were detected in many cases which resembled

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of Cosgrove (1962), Sharma *et al.* (1977) and Ley *et al.* (1983). Congestion and haemorrhages in the intestine and caecal tonsils were also noticed.

Histologically the bursa of Fabricius revealed reduction of lymphoid cells in the follicles, medullary necrosis with accumulation of homogenous eosinophilic masses. Cystic dilatation of follicle, mild to severe infiltration of heterophiles, plasma cells and marked reticular hyperplasia were characteristic. Intrafollicular as well as interfollicular haemorrhage (Fig. 1D) and a glandular transformation (Fig. 1E) were also noticed. These changes are also comparable to those described by several workers (Helmboldt and Garner 1964, Cheville 1967, Dal Bono *et al.* 1968, Ley *et al.* 1983, Okoye and Uzoukwu 1981 and Ray *et al.* 1985). However, in this study inclusion bodies could not be demonstrated in the plical epithelial cells. Degeneration and necrosis of lining tubular epithelium, focal and diffuse haemorrhages with mild heterophilic infiltration in the interstitium of the kidney were noticed which resembled those described by Cosgrove (1962), Helmboldt and Garner (1964) and Del Bono *et al.* (1968). Depletion of lymphoid cells, congestion and diffuse haemorrhages observed in the thymus were also reported by Helmboldt and Garner (1964), Del Bono *et al.* (1968), Dongoankar *et al.* (1979) and Ray *et al.* (1985). Diffuse haemorrhages and congestion were recorded in lungs. Brain revealed congestion of blood vessels, mild gliosis, neuronophagia with satellitosis and mild demyelination.

ACKNOWLEDGEMENT

Authors are thankful to the Dean, Faculty of Veterinary Science, Assam Agricultural University, Khanapara, for providing the necessary facilities and to the Director of Animal Husbandry and Veterinary Department, Government of Nagaland, for providing the fellowship to the senior author to undertake the present study.

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Indian Journal of Animal Sciences **68** (11) : 1173-1174, November 1998

Testicular development during postnatal life of crossbred buck

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Received : 15 December 1997; Accepted :15 May 1998

Key words : Age, Goat, Growth, Testis

Selection of male animal plays an important role in any successful breeding programme. Besides, pedigree records of genetic makeup and other phenotypic characteristics, the testicular measurements and seminal characteristics may give a clue of breeding efficiency in the male animal for effective breeding. The measurement of scrotal circumference (Thompson and Johnson 1995) is an important criterion for selecting male for breeding. During the initial stage of postnatal life the rate of growth of the bovine testis is slow which is followed by rapid growth after puberty. In a study, a rapid testicular growth along with body weight was observed during 3 to 6 months of age in crossbred bucks (0 day to 1 year of age at quarterly intervals), and thereafter a steady growth was observed (Sarmah *et al.* 1997). The aim of the present experiment is to ascertain the exact initial stage of rapid testicular growth alongwith body weight by reducing the age interval gaps.

Crossbred normal, healthy bucks (48; Beetal × Assam local) of different age groups (0 day to 7 months) were observed for testicular measurements like scrotal circumference, length, breadth and thickness of both testicles. The animals were maintained under standard managemental practices in the experimental animal shed, of the university. Animals were divided into 8 age groups, viz. group 1 (0-15 days), group 2(15 days to 1 months), group 3 (1-2 months), group 4 (2-3 months), group 5 (3-4 months), group 6 (4-5 months), group 7 (5-6 months) and group 8 (6-7 months). Body weight of each of the animal was recorded by a spring balance at the time of testicular measurements. Testicular measurements were carried out by procedure described by Hahn *et al.* (1969).

The results obtained were subjected to statistical analysis (Snedecor and Cochran 1967).

Different testicular measurements and body weight between various age groups, differences were statistically significant ($P<0.01$). Critical difference test recorded significantly higher value in both group 7 (7.99± 0.07; 3.63±0.13; 3.86±0.07; 19.17±0.37 and 12.07±0.41) and group 8 (8.08±0.12; 3.78±0.07; 4.01 ± 0.09; 19.60 ± 0.31 and 12.07 ± 0.43) as compared to other groups (2.42± 0.03 to 7.01 ±0.32; 1.20±0.06 to 3.63±0.13; 1.09±0.04 to 3.86 ±0.07; 6.03±0.32 to 19.17±0.37 and 2.42 ±0.16 to 12.07 ± 0.41) in respect of testicular length, breadth, thickness, circumference (cm) and body weight (kg) respectively. Although group 8 (6-7 months of age) recorded highest values in respect of testicular measurements and body weight, no significant difference was observed with group 7 (5-6 months of age). In this study, testicular measurements and body weight increased with the advancement of age which supports the earlier observation of Rao (1994).

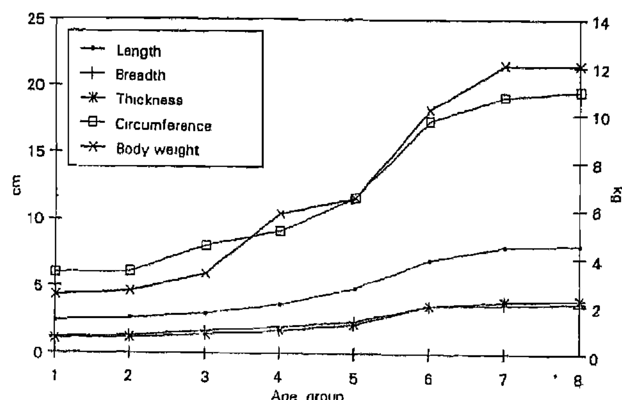

Fig 1. Testicular measurements and body weight in different age groups

The graphical representation of testicular measurements and body weight in different age group (Fig.1) revealed a gradual increase pattern of testicular length, breadth and thickness. The scrotal circumference showed a gradual increase up to 3 months(Group 5) then suddenly increased in group 6 (4-5 months of age). The body weight gain in different age groups showed similar trend as that of the scrotal circumference. This observation finds supports from earlier study on cross bred buck (Sarmah *et al.* 1997) indicating influence of age on body weight and testicular growth, which was rapid during 3 to 6 months of age. Coulter *et al.* (1975) also reported higher testicular growth in young Holstein bulls,

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A rapid testicular growth and body weight gain in the present study were observed at an average age of 4 months which almost continued to 5 months of their age, followed by a steady growth. Rapid testicular growth from 4 months onwards might be due to the fact that the animals were approaching towards the attainment of puberty at this age. As attainment of puberty was associated with the scrotal circumference (Lunstra *et al.* 1978) in turn, the scrotal circumference was highly correlated with testicular growth and increased body weight, which were more closely related with on set of puberty (Hafez 1987). The present findings in crossbred buck may serve as a guide for selection of breeding buck as early as 4 months of their age. Further work is in progress.

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Indian Journal of Animal Sciences **68** (11) : 1175-1176, November 1998

Clinico-biochemical effects of xylazine in yaks

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Received 6 June 1997; Accepted . 8 May 1998

Key words: Clinico-biochemical effects, Xylazine, Yaks

Xylazine has been used for sedation and muscle relaxation in wild ruminants (Kloppel 1969). The present paper deals with sedative and clinico-biochemical effects of xylazine in yaks.

Xylazine hydrochloride was used @ 0.22 mg/kg body wt, intramuscularly in 9 healthy yaks of either sex, aged between 1.5 and 6 years and weighing 163.83±9.68 kg (mean ± SE; range 60 to 250 kg). All these animals were kept in the same managemental conditions prior to experimentation. For sedative and behavioural studies fasting of 24 to 36 hr was observed. The animals were kept in quite place for 30 to 40 min and then the drug was administered, immediately thereafter the rope was loosened and leashed animals were then observed for change in attitude, outlook and posture and time parameters viz. onset time, down time and recovery time. For clinico-biochemical studies the experiments were performed after securing the animal in lateral recumbency. The depth of sedation and analgesia was assesed by monitoring various occular reflexes, pin prick reflexes, relaxation of neck, jaws, tail and anus, salivation, lacrimation, urination and grunting at 0 hr (before administration of drug) and at 5,15, 30, 45 and 60 min post- administration of the drug. Rectal temperature, respiration rate and heart rate were also recorded. Heparinised blood samples were collected from jugular vein and plasma was separated. Plasma sodium and potassium were estimated by flame photometery. Lactate dehydrogenase (LDH), aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALKP), glucose, total proteins, blood urea nitrogen (BUN), creatinine and chloride were analysed using commercially available kits on computerised semi-auto blood analyzer. Statistical analysis was performed using students 't' test. Results were evaluated at 1% and 5% level of significance.

Following administration of xylazine, the average onset time (mean ± SE) was 2.00 ± 0.32 min, down time 4.8 ± 1.07 min and recovery time 49.0 ±2.92 min (standing ataxia) and 64.0 ±4.84 min (normal gait) respectively. Within 2-3 min of drug administration, the first visible reaction was staggering. The loss of body balance and ataxic behaviour is due to depression of cerebellum (Short 1986). Sedation lasted longer than analgesia and animal appears to be cerebrally depressed. Generally there was mild to moderate muscle relaxation with complete relaxation observed only at 15 min interval. Swallowing reflex was absent up to 30 min of study. The muscle relaxing effect of xylazine is attributed to partial synaptic blockade in the CNS (Knight 1980) causing inhibition of intra-neural transmission of impulses in the CNS (Sagner *et al.* 1968). Regurgitation and salivation were seen throughout the period of observation whereas tympany was noticed after 15 min of xylazine administration. Grunting was noticed initially after 5 min of administration and then during recovery period. Recovery was smooth. Hind limbs were first to regain its use There was mild to moderate depression of palpaberal, corneal and photopupillary reflexes after xylazine administration in all the yaks.

Respiration rate decreased throughout the study with a steep fall at 5 min post-administration period which was highly significant ($P<0.01$) and remained so up to 60 min. Depression of respiratory activity observed in yaks was also seen in cattle (Kumar and Singh 1976). Sagner *et al.*. (1968) interpreted this effect as a retention at resting value instead of existence of any specific inhibition of respiratory system Body temperature showed decreasing trends as xylazine depressed the thermoregulation in cats (Pander and Clark 1980). Xylazine caused bradycardia in all animals. A nonsignificant hyperglycemia was seen at 30, 45 and 60 min interval. The other biochemical parameters remained within normal ranges The hyperglycemic effect of xylazine can be attributed to its α_{-2} adreno receptors agonist activity. Similar effects were seen in cattle (Symmonds 1976).

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Indian Journal of Animal Sciences 68 (11) : 1177-1178, November 1998

Cryopreservation of caprine oocytes by 2-step freezing

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Received 6 December 1997; Accepted : 7 May 1998

Key words : Cryopreservation, Goat, Oocytes

Cryopreservation of female gamets (oocytes) besides being a valuable adjunct to embryo transfer, *in vitro* fertilization and micromanipulation studies, is also important for conservation of valuable genetic material and those species/breeds which are in danger of extinction. Very little progress in freezing domestic animal oocytes has been reported (Didion *et al.* 1990, Lim *et al.* 1991, Sulieman *et al.* 1990). The purpose of this study was to examine sensitivity and refractoriness of caprine oocytes to cryopreservation.

Source of oocytes

Caprine ovaries of abattoir origin were used as a source of oocytes. Immature oocytes were recovered by puncturing the follicles with the help of a 18 G hypodermic needle. Only the oocytes enclosed in compact multilayer cumulus cells and with evenly granulated cytoplasm were used.

Cryopreservation of fresh oocytes

Good quality oocytes after 2-3 washings in Dulbecco's PBS enriched with 20% oestrus goat serum were exposed to 1.4 M glycerol in PBS (enriched with 20% goat serum) in step-wise fashion (0.47 M, 0.94 M, 1.4 M, 10 min each). After 10 min equilibration at room temperature the oocytes were loaded to the middle part of 0.25 ml straw in a small medium separated by 2 air bubbles from the same medium filling rest of the straw. Straws after loading were placed in the chamber of freezer and cooled @ 5° C/min to –7° C. After holding straw at –7° C for 5 min, seeding was induced by touching the outside of the straw just above the meniscus with forceps precooled in LN_2. Straws were immediately returned to the chamber and held for another 5 min. Straws were further cooled at 0.5°C/min down to –30°C and then plunged in LN_2. After storage in LN_2 for variable time (1-6 months), straws were rewarmed by direct transfer into water at 35°C. Contents of straws were emptied into a petri-dish and left for 10 min at room temperature before morphological examination.

Table 1. Cryopreservation of fresh oocytes

Items	No
Oocytes	
used for freezing (No of replicates)	245 (22)
lost in cryoprotectant medium before loading	1
frozen	244
thawed	203
recovered	192
recovered oocytes exhibited morphological damage	45
Recovered oocytes examined for nuclear changes	
Germinal vesicle	35
Germinal vesicle break down (GVBD)	9
Metaphase I (met-I)	9
Anaphase (ana-I)	--
Telophase I (telo-I)	--
Metaphase II (met-II)	--
Oocytes without chromatin material	29
Condensed	13
Total	95

Table 2. Cryopreservation of matured oocytes

Items	No
Oocytes	
used for freezing (No. of replicates)	220 (14)
lost in cryoprotectant medium before loading	1
frozen	219
thawed	90
recovered	86
recovered oocytes exhibited morphological damage	10
Recovered oocytes examined for nuclear changes	
Germinal vesicle (GV)	2
Germinal vesicle break down (GVBD)	--
Metaphase I (met-I)	3
Anaphase I (ana-I)	--
Telophase I (telo-I)	1
Metaphase I (met-I)	11
Oocytes without chromatin material	10
Condensed	27
Total	54

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Table 3. *In-vitro* maturation of frozen oocytes

Items	No.
Frozen oocytes cultured	71
Oocytes studied for nuclear changes	40
Stages of meiosis :	
Germinal vesicle (GV)	--
Germinal vesicle break down (GVBD)	--
Metaphase I (met-I)	--
Anaphase I (ana-I)	--
Telophase I (telo-I)	--
Metaphase II (met-II)	19
Oocytes without chromatin material	12
Condensed	9

Cryopreservation of matured oocytes

Fresh oocytes graded as good were incubated in CO_2 incubator maintained at 38°C temperature 95% RH and 5% CO_2 in air. TCM 199 enriched with 20% goat serum, D-glucose and sodium pyruvate was used as culture medium. After 24 hr incubation, oocytes were cryopreserved using the method described above.

Examination of frozen-thawed oocytes

Frozen-thawed oocytes were examined for morphological damages and nuclear changes under stereo-zoom microscope at low magnification. Oocytes were fixed and stained with aceto-orcein to observe nuclear changes.

Cryopreservation of fresh oocytes

Oocytes (245) graded as good were used for freezing by PtC-1013. Number of oocytes lost in cryoprotectant medium before loading, oocytes recovered after thawing and their morphological and nuclear status are shown in Table 1.

Cryopreservation of matured oocytes

Mature oocytes (220) were used for freezing. Number of oocytes lost in cryoprotectant medium before loading, oocytes recovered after thawing and their morphological and nuclear status were studied. The results are shown in Table 2.

In-vitro *maturation of frozen-thawed oocytes*

To check the ability of frozen-oocytes to mature and fertilize *in-vitro*, 71 oocytes were thawed at 35°C water-bath and after step-wise dilution of cryoprotectant were kept for culture in TCM-199 supplemented with 20% oestrus goat serum at 38°C temperature, 95% RH and 5% CO_2 in air in incubator. After 24 hr of culture the oocytes were fixed and stained with aceo-orcein to observe maturational changes (Table 3).

In-vitro *maturation of frozen oocytes stored in LN_2 for more than a year*

Oocytes (96) frozen by 2-step freezing method (biological freezer, PTC-1013) and stored in LN_2 (–196°C) for more than a year were thawed in a water-bath maintained at 35°C. Frozen thawed oocytes were examined for morphological changes. All the 96 (100%) oocytes were recovered of which 5 oocytes exhibited damage in zona pellucida and ooplasm. Morphologically normal looking oocytes (90) were cultured for 24 hr in CO_2 incubator maintaining 38°C temperature, 95% humidity and 5% CO_2 in air. TCM 1999 enriched with 20% goat serum, D-glucose and sodium pyruvate was used as culture medium. Oocytes (24; 27%) reached to metaphase II stage within 24 hr of incubation. The study revealed an adverse effect on oocytes maturation after long storage (24/90) compared to storage for 1-6 months (19/40).

Mammalian oocytes as a single cell entity are characterized by several unique features which make them more vulnerable to environmental changes and also increase their susceptibility to damage during cryopreservation procedure as compared to multicellular pre-implantation embryos. Disassembly of the meiotic spindle due to chilling, cryoprotective agents or freezing may cause chromosomal abnormalities. It may also perturb cytoskeletal arrangements critical to the progression of meiosis and normal fertilization. Structural differences of oocytes probably contribute, to the low developmental potential of frozen oocytes. In our study oocytes at the metaphase II stage (Table 2) are more sensitive to cryoinjuries as compared to the oocytes at the germinal vesicle stage (Tables 1, 3). This is contrary to the findings of Lim *et al.* (1992) in bovine.

ACKNOWLEDGEMENTS

I thank Director of the Institute for providing the facilities. Financial support received from Department of Biotechnology, Ministry of Science and Technology, Government of India is acknowledged.

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Indian Journal of Animal Sciences 68 (11) : 1179-1180, November 1998

Evaluation of canine frozen semen by hypo-osmotic swelling test

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Received : 15 September 1997; Accepted :22 June 1998

Key words : Canine, Cryopreservation, Hypo-osmotic swelling test, Semen

Semen quality assessed by evaluation of physical parameters such as pattern and percentage of sperm motility and abnormalities have been reported to have low correlation and low predictive values to fertility (Kumi-Diaka 1993). The development of freezing protocols for dog semen so far has been based on post-thaw evaluation of sperm motility. Studies by Silva and Verstegen (1995) revealed that post-thaw assessment of sperm motility was not the best technique to evaluate sperm fertility. The spermatozoa could be highly mobile, but non fertile owing to acrosomal damage (Pursel *et al.* 1972). However, hypo-osmotic swelling test can be used for accurate prediction of fertilizing ability of spermatozoa (Correa and Zavos 1994, Rodriguez - Gill *et al.* 1994). In the present study canine semen was frozen and the effect of cryopreservation was assessed by post-thaw motility, live sperm percentage, abnormal sperm percentage and hypo-osmotic swelling test.

Healthy adult mongrel male dogs (5) were taken for the study. Ejaculates (75) of semen were collected by digital manipulation technique (Allen 1991). Characteristics like volume, colour, consistency and motility were recorded. Sperm rich second fraction was extended in tris-citric acid -fructose buffer with 20% egg yolk (Yubi *et al.* 1987) and a buffer pH of 7. A glycerol level of 9 % and equilibration period of 4 hr were used. A slow freezing rate was achieved by placing filled 0.25 ml straws 10 cm above liquid nitrogen level for 20 min. The frozen semen was evaluated by post-thaw motility, live sperm percentage, abnormal morphology and hypo-osmotic swelling test.

Live sperm percentage and abnormal morphology were evaluated by eosin nigorsin staining technique (Hancock 1951). Hypo-osmotic swelling test was performed by mixing 1ml 150 milli-osmol fructose solution with 0.1ml of ejaculate in a test-tube and incubating the mixture for 30 min at 37°C (England and Plummer 1993). After incubation smears were prepared and stained with 3% buffered Rose Bengal stain for 15 min (Tomar 1976). The smears were washed, dried and a minimum number of 200 spermatozoa were counted to estimate the percentage of hypo-osmotically swelled spermatozoa which exhibited tail curling (Figs1-7).

Post - thaw functional characteristics of canine spermatozoa revealing the effect of cryopreservation are expressed as mean percentages ($\pm$SE). Post -thaw motility of 59.67$\pm$0.97%, live sperm percentage of 67.5$\pm$ 0.32, abnormal sperm percentage of 26.92$\pm$0.61 and hypo-osmotic swelling response of 62.02$\pm$0.41 % were recorded.

The average post-thaw motility of 59.67$\pm$0.97 % recorded in the present study is lower compared to 65% recorded for Beagle dogs by Silva and Verstegen (1995) using different extenders and 4% glycerol level. The variation may be due to the influence of extenders or breed variation. Similarly 26.92$\pm$0.61 % abnormal spermatozoa recorded in the present study is higher than 15 % recorded by Silva and Verstegen (1995). Perusal of available literature give no information about post-thaw live sperm percentage of canine semen.

The hypo-osmotic swelling response showed higher value 62.02$\pm$0.41 % in the present study compared to 56.7$\pm$17 % tail curling reported by Kumi -Diaka (1993), for semen of mixed breeds of dogs, frozen with 7% glycerol level. This difference may be due to difference in molality of the hypo-osmotic swelling medium (60 milli-osmol) used, and differences in freezing technology and semen quality.

Correa and Zavos (1994), recorded 48.0 (4.56 %) hypo-osmotic swelling response for frozen bull semen, and Sivaramalingam (1994) reported 66.0 (4.56 %) for frozen ram semen. These results show variation in the results of this bioassay among different species.

England and Plummer (1993) reported that although there was no correlation with motility, the hypo-osmotic swelling of

The article is published from the thesis approved for post-graduate degree by Tamil Nadu Veterinary and Animal Sciences University

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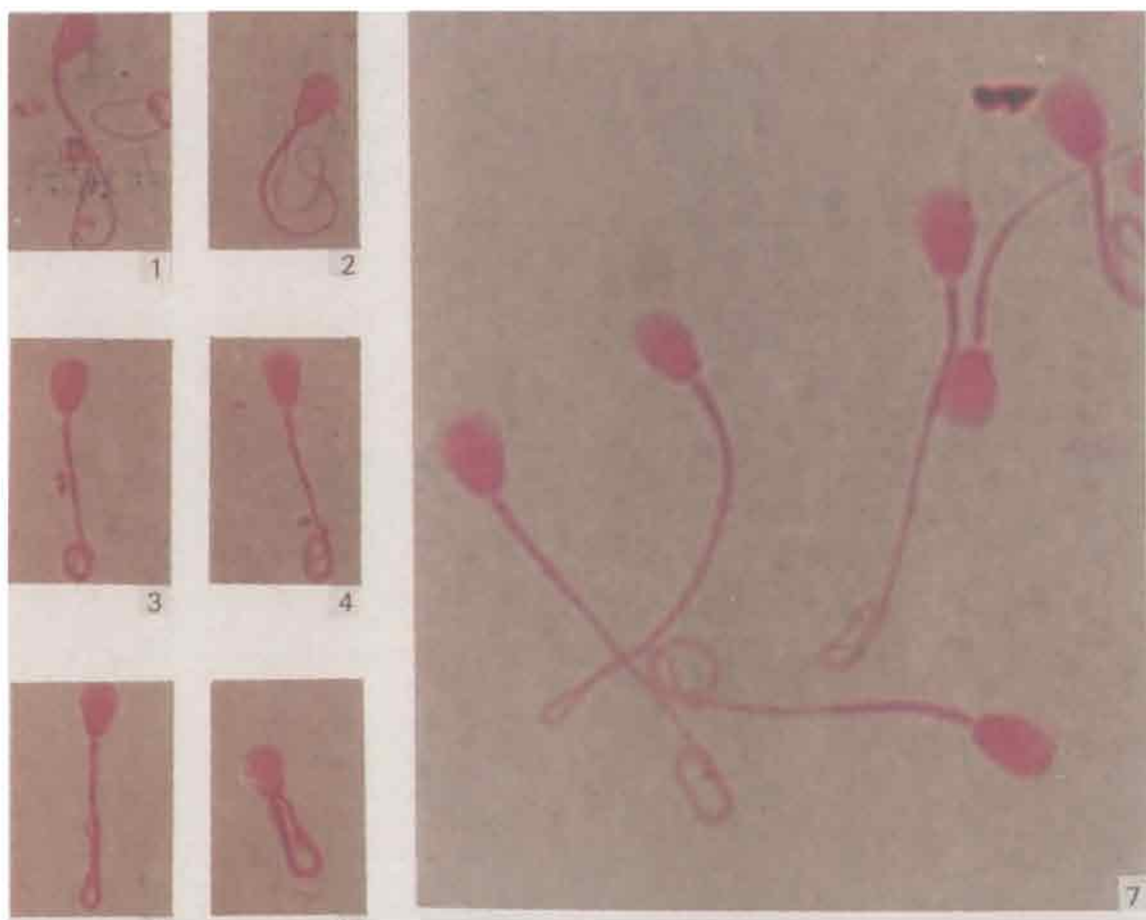

Figs. 1-7. **1-6** Rose Bengal stained smears. Typical tail swellings / curling of spermatozoa following hypo-osmotic swelling test (Host) (1-6) (× 800). Hypo-osmotic swelling response of spermatozoa (×2000).

dog spermatozoa was reliable and repeatable and might be a useful assay for the diagnosis of spermatozoal membrane function. Thus the evaluation of frozen semen based on hypo-osmotic swelling response along with conventional methods like motility estimation give more accurate prediction of fertilizing ability of frozen - thawed semen. The bioassay is more easy and economical for laboratory investigation. It substitutes time consuming and laborious but most accurate method of fertility evaluation by insemination trials.

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Indian Journal of Animal Sciences **68** (11) : 1181-1182, November 1998

Histochemical study on chronic aflatoxicosis in ducks

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Received :23 August 1997; Accepted .16 April 1998

Key words : Aflatoxin, Duck , Histochemistry

Aflatoxin is a well-known hepatotoxin and carcinogenic material and its pathology in ducks is well documented (Mukit 1976, Siamsul *et al.* 1990).

The study was conducted on 3- month-old ducks (72) divided into 3 groups. Groups A and B were fed aflatoxin B1 daily @10 and 20 μg/kg body weight for 3 months and the ducks of group C were kept as control. Six ducks from each group were sacrificed at monthly interval and remaining ducks were reared for another month to see the post-treatment effect.

Cryostat sections of liver and kidney were incubated for alkaline – (AKPase), acid – (ACPase), adenosine triphosphatases (ATPase, Azo dye method); succinate – (SDH), lactate – (LDH) dehydrogenases and cytochrome oxidase (CyO, Nitro BT method; Pearse 1980). The neutral fat and phospholipid were stained by using Sudan B and acid hematin method, respectively (Chayan *et al.* 1973).

The hepatocytes lost the typical perinuclear activity for oxidoreductases (Table1) as recorded in the control ducks. In some hepatocytes, the enzyme positive granules for oxidoreductases decreased and were absent indicating varying degree of degeneration and necrosis in these cells. Gill *et al.* (1980) opined that decrease of oxidative enzymes from the hepatocytes is suggestive of hepatocellular injury due to binding and inhibition of key enzymes and degeneration of the membranes. The proliferating hepatocytes exhibited irregular distribution of enzyme positive granules within the cells. However, regenerating hyperplastic nodule revealed an interesting reactivity for the enzyme understudy. In this area, the histoenzymic activity was strong in the peripheral cells and weak in the cells at the centre of the nodule.

In this study, the AKPase and ACPase activities relatively increased in the degenerating hepatocytes while the ATPase activity was lower compared to normal hepatocytes of the control ducks. The necrosed hepatocytes exhibited variable histoenzymic intensity ranging from negative to moderate reactions. Proliferation of biliary epithelial cells in experimen-

Table 1. Histochemical distribution of phosphatase, oxidoreductases and lipids in the liver of duck due to aflatoxicosis

Enzyme	Hepatocytes	Biliary epithelium	Bile canaliculi	Sinosoids	Kupffer	Blood vessels	Connective tissue
LDH	0/++++	+/+++	NS	NS	NS	NS	NS
SDH	0/+++	+/++	NS	NS	NS	NS	NS
CyO	0/+++	+/+++	NS	NS	NS	NS	NS
AKPase	0/+++	+/++	+/+++	+/+++	0	+/+++	0
ACPase	0/+++	+/++	0/+++	+/+++	0/++	+/+++	0
ATPase	0/+++	+/++	+/+++	+++	0	+++	0
Phospholipid	0/++++	++/+++	NS	NS	NS	NS	NS

Gradation of histochemical reaction · 0, negative, +, weak, ++, moderate, +++, strong, ++++, intense, NS, reaction not significant.

Table 2. Histochemical localization in kidney of ducks

Enzyme	PCT	DCT	Blood vessels	Connective tissue
LDH	0/+++	+/++	0	0
SDH	0/++++	+/++	0	0/+
CyO	0/++++	+/+++	0/+	0
AKPase	0/++	0/+	0	0
ACPase	+/+++	+/++	+/++	+/++
ATPase	0/++	0/+	0	0
Phospholipid	0/+++	+/++	0	0

Gradation of histochemical reaction: 0, negative, +, weak, ++, moderate, +++, strong, ++++, intense.

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tal ducks observed during histopathology exhibited stronger enzymic reactivity than the normal cells. This is probably due to increased metabolic activity in the newly proliferating cells.

Sudanophilic lipid droplets increased within the affected hepatocytes. Accumulation of sudanophilic lipids might be either due to degenerative changes produced by the toxin or due to inhibition of lipid transport system.

In kidney, activities for oxidoreductases reduced in the lining epithelium of degenerating proximal and distal convoluted tubules (Table 2). The AKPase and ACPase were found localized in the proximal convoluted tubular epithelia with variable intensity being relatively increased in treated groups compared to normal ones. Such increase of ACPase and AKpase was also noted by Singh *et al.* (1992) in aflatoxin and ochratoxin affected tubular epithelium. Reduced ATPase activity in the tubular epithelia of intoxicated ducks was in accordance with Gill *et al.* (1986).

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Indian Journal of Animal Sciences **68** (11) : 1183, November 1998

Status of macroelements in acute haemonchosis in Pol Dorset sheep

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Received : 28 October 1997 ;Accepted : 13 May 1998

Key words : Acute haemonchosis, Macroelements, Sheep

There is sparse and inadequate literature on haemonchosis an acute problem in India. The study on status of macroelements and their possible role in Pol Dorset sheep suffering from acute haemonchosis was undertaken.

Pol Dorset sheep (2-year-old), imported from Australia and raised at the Government Sheep Breeding Farm, Mattewara (Ludhiana), were acutely suffering from haemonchosis. Blood samples were collected aseptically in heparinized screw capped glass vials and were kept in the ice after collection. The mineral estimation was carried out on atomic absorption spectrophotometer.

In sheep, suffering from acute clinical haemonchosis significant decline in the plasma levels of sodium (263.4 ± 5.27 mg/dl) but marked hyperkalemia (202.8 ± 13.46 mg/dl) compared to normal values were obtained (Table 1). Hyponatraemia is a frequent clinical finding especially in animals with large losses of intestinal fluid. Animals with gastrointestinal diseases almost always have marginal to severe hyponatraemia. Enteritis and nephritis with large number of adult haemonchus parasites in abomasum (Brar and Kwatra 1992) recorded in acute haemonchosis in sheep may be one of the possible causes of hyponatraemia and hyperkalemia observed in this present study. Kaneko (1980) reported that hyperkalemia is most frequently associated with acidosis, acute renal failure and extensive cellular necrosis.

Calcium is one of the more precisely regulated constituents of plasma. Plasma concentration of calcium (5.78 mg/dl) declined significantly in acute haemonchosis in sheep compared to that in healthy sheep (11.5-12.8 mg/dl). In sheep sudden deprivation of feed with forced exercise may cause marked depression of serum calcium (Pierson and Jensen 1975). Moderate hypocalcemia is a common finding during disease with protein loosing gastro-enteropathies in which it has been shown that hypocalcaemia is due to gastrointestinal loss of Ca^{++} bound to albumin (Kaneko 1980). *Haemonchus contortus* is a blood sucking parasite and leakage of serum albumin in intestinal parasitism leads to hypoproteinemia (Dobson 1965). Moreover, presence of worms appears to interfere with digestion and absorption of proteins (Sood 1981).

In terms of concentration, magnesium is fourth among cations in the body. Cattle and sheep face clinical disorders due to disturbances in Mg^{++} metabolism. However, no significant change in the plasma concentration of magnesium was observed in acute haemonchosis in Pol Dorset sheep.

Table 1 . Effect of acute haemonchosis on plasma macroelements in Pol Dorset sheep

Macroelement	Normal*	Acute haemonchosis
Sodium (mg/dl)	319 7-349.6	263.41±5.27
Potassium (mg/dl)	15.2-21.1	202.77±13.46
Calcium (mg/dl)	11.5-12 8	5.78±0.45
Magnesium (mg/dl)	2.2-2.8	2.83±0 14

Values are mean ± SE of 9 animals; *Kaneko (1980).

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Indian Journal of Animal Sciences 68 (11) : 1184-1186, November 1998

Inheritance of muzzle print pattern of dam to their progeny in cattle *(Bos indicus)*

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Received : 17 November 1997; Accepted . 27 April 1998

ABSTRACT

The muzzle printometry was carried out on 12 pairs of dams and their progenies (including a pair of twin). The prints were obtained on white borosil filter paper by using dermatoglypher and examined by qualitative methods. The percentage of progenies inheriting dam's characters for the muzzle pattern, shape of print and valley was 75 each while for valley pattern it was 83.3. According to the ridge pattern the percentage of progenies inheriting dam's character for group a, b and c was 16.7, 50 0 and 33.3 respectively. The prints of twin were dissimilar in qualitative examination indicating dizygosity

Key words : Cattle, Muzzle print, Progeny

Surface marking of the muzzle presents numerous grooves forming a definite pattern and it may be used for the identification of animal by studying the muzzle prints (Dyce and Wensing 1971). The planum nasolabium is a characteristic feature for an individual animal and remains constant with age. The muzzle pattern is inherited non-lethal anatomical character (Gilmore 1950, Wiener 1954). Hence the present work was carried out to study the muzzle print pattern of dam and their progenies.

Table 1. Qualitative observations of the muzzle pattern in dam and their progenies

Animal print No	Progeny print No	Valley pattern		Shape of the print	Muzzle pattern	Pattern of the ridges (Group)		
		Type	Shape			a	b	c
G-3		Open	Diamond	I	C	3/3	3/2	2/2
	G-26	Open	Diamond	I	C	2/2	3/2	2/2
G-5		Open	Diamond	I	C	3/3	2/2	3/3
	G-38	Open	Diamond	II	C	3/2	2/2	3/2
G-6		Open	Diamond	I	C	3/1	3/2	2/2
	G-25	Open	Diamond	I	C	2/3	2/2	2/2
G-12		Close	Diamond	I	A	3/3	3/3	2/2
	G-23	Open	Diamond	I	C	2/2	2/2	3/3
G-14		Open	Diamond	III	C	3/3	2/3	2/2
	G-13	Open	Diamond	I	C	2/2	3/2	2/3
G-19		Open	Diamond	I	C	3/2	2/2	2/2
	G-48	Open	Diamond	I	C	2/2	2/2	3/3
G-22		Open	Irregular	I	A	2/3	2/3	2/2
	G-49	Open	Diamond	I	C	3/3	2/2	3/3
GH-1		Open	Diamond	I	C	2/3	2/2	3/3
	GH-10	Open	Diamond	I	C	3/3	2/2	3/3
GH-11		Open	Diamond	II	B	2/2	2/3	3/3
	GH-32	Open	Irregular	III	B	2/2	3/2	2/3
GH-18		Open	Diamond	I	C	3/3	2/2	3/3
	GH-29	Open	Diamond	I	C	2/2	2/2	2/2
	*GH-34	Close	Irregular	I	B	2/2	2/3	3/3
	*GH-35	Open	Diamond	I	C	3/3	2/2	3/2
Percentage of progenies inheriting dam's pattern		83 7 (10)	75.0 (9)	75.0 (9)	75.0 (9)	16.7 (2)	50.0 (6)	33.3 (4)

Twin animal No. GH-18

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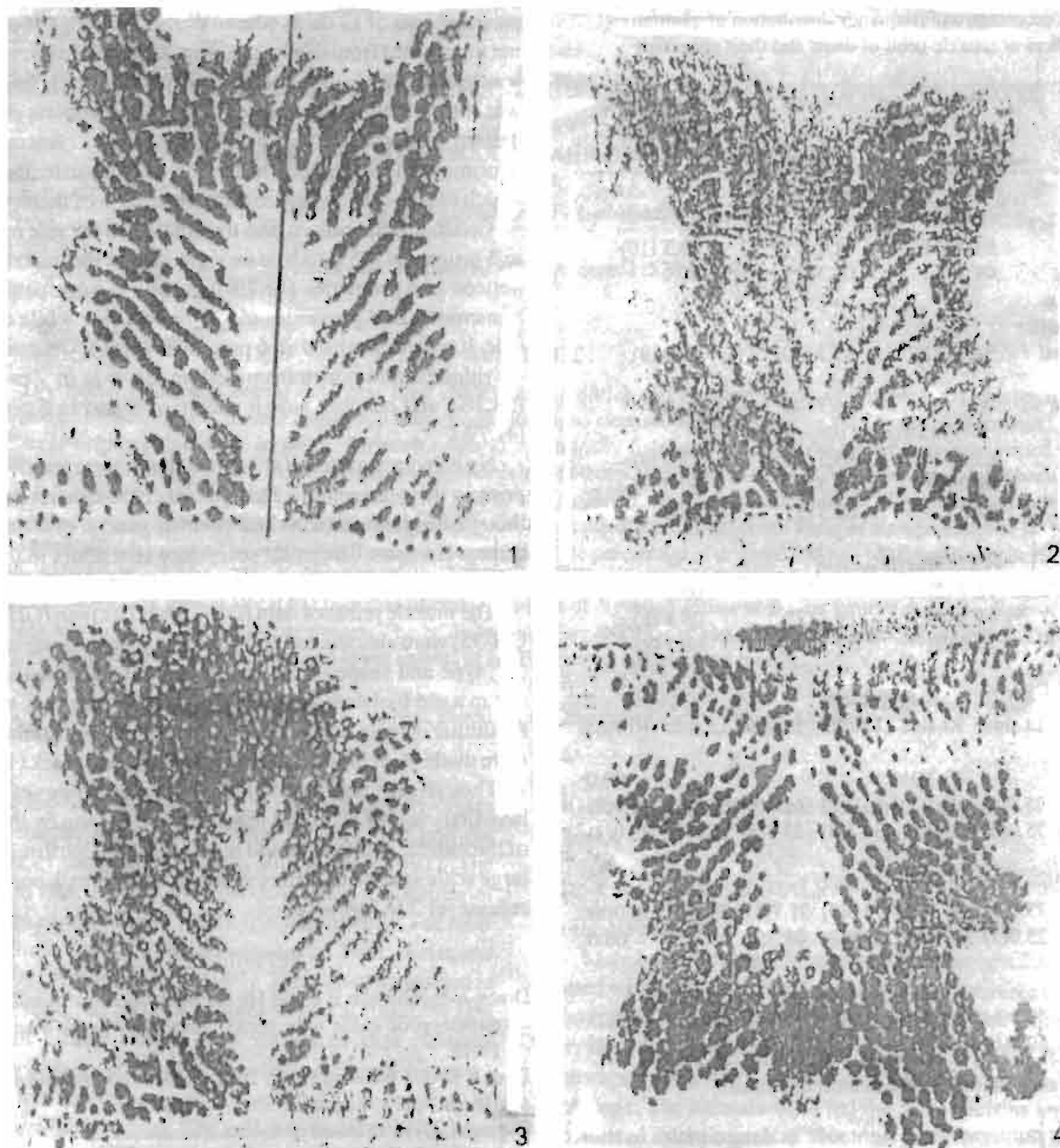

Figs 1-4 . **1.** Muzzle print of animal No. G-5 showing 1. Shape of print - I 2. Muzzle pattern - C 3. Valley type - open 4. Valley shape - Diamond 5. Ridge pattern -3/3 2/2 3/3. **2.** Muzzle print of animal No. G-38 (progeny of G-5) showing 1. Shape of print - II, 2. Muzzle pattern - C , 3. Valley type - Open, 4. Valley shape - Diamond, 5. Ridge pattern - 3/2 2/2 3/2. **3.** Muzzle print of animal No. G-19 showing 1. Shape of print - I, 2. Muzzle pattern - C, 3. Valley type - Open, 4. Valley shape - Diamond, 5. Ridge pattern - 3/2 2/2 2/2. **4.** Muzzle print of animal No. G-48 (progeny of G-19) showing 1. Shape of print - I, 2. Muzzle pattern - C, 3. Valley type - Open, 4. Valley shape - Diamond, 5. Ridge pattern - 2/2 2/2 3/3.

MATERIALS AND METHODS

The present study of muzzle print was carried out on 12 pairs of dams and their progenies (including 1 pair of twin). The animals were selected for the study from cattle breeding farm, Kandivali, Mumbai.

The muzzle of each animal was washed with water and mopped with muslin cloth. Immediately after mopping muzzle surface was painted with stamp pad ink and then the print was obtained on borosil filter paper fixed over dermatoglypher by presssing it on the muzzle surface. The prints thus obtained were allowed to dry and then examined by following qualitative methods (Figs 1 to 4).

Valley pattern : It included types as open at one or both ends and closed and shapes as diamond, oval and irregular.

Shape of the print : It was studied as described by Habu (1935).

Muzzle pattern and pattern of ridges : These were studied as per Hirsch *et al.* (1952). The left side of the print was

Table 2. Percentage and frequency distribution of qualitative characters of muzzle print of dams and their progenies

Parameters	Dam		Progeny		% of progenies inheriting dam's pattern	
Valley pattern						
A. Type of valley						
1. Open	91.7 (11)		91.7 (11)		83.3 (10)	
2. Close	08.3 (1)		08.3 (1)		00.0	
3. Absence	-		-		-	
B. Shape of valley						
1. Diamond	91.7 (11)		83.3 (10)		75.0 (9)	
2. Oval	-		-		-	
3. Irregular	08.3 (1)		16.7 (2)		00.0	
Gross shape of the print						
1.	83.3 (10)		83.3 (10)		75.0 (9)	
2.	08.3 (1)		08.3 (1)		00.0	
3.	08.3 (1)		08.3 (1)		00.0	
4.	-		-		-	
Muzzle pattern						
A.	16.7 (2)		-		00.0	
B.	08.3 (1)		16.7 (2)		08.3 (1)	
C.	75.0 (9)		83.3 (10)		66.7 (8)	
D.	-		-		-	
Ridge pattern						
	Lt.side	Rt.side	Lt.side	Rt.side	Lt.side	Rt.side
Group a						
1.	-	08.3(1)	-	-	-	00.0
2.	25.0(3)	16.7(2)	66.7(8)	66.7(8)	08.3(1)	16.7(2)
3.	75.0(9)	75.0(9)	33.3(4)	33.3(4)	16.7(2)	25.0(3)
Group b						
1.	-	-	-	-	-	-
2.	75.0(9)	66.7(8)	75.0(9)	91.7(11)	58.3(7)	50.0(6)
3.	25.0(3)	33.3(4)	25.0(3)	08.3(1)	08.1(1)	00.0
Group c						
1.	-	-	-	-	-	-
2.	50.0(6)	50.0(6)	41.7(5)	41.7(5)	25.0(3)	16.7(2)
3.	50.0(6)	50.0(6)	58.7(7)	58.7(7)	33.3(4)	25.0(3)

Figures in bracket indicate the number of animals.

designated as numerator and right side as denominator in the formula.

RESULTS AND DISCUSSION

The observations of the qualitative characters of the muzzle prints of dams and their progenies and percentages of transmitting characters are given in Tables 1 and 2 respectively. The open type of valley pattern of 10 dams (83.3%) was transmitted in their progenies out of 11 while closed type valley of dam was not transmitted in its progeny. The diamond shape valley of dam was transmitted in 9 progenies (75%) out of 12 dams. Irregular shape of valley of dam was not transmitted in its progeny while none of the pair showed oval type of valley.

Nine dams (75%) transmitted the fern-shaped print in their progenies out of 12 dams while 'Y' and 'V' shape of print was not transmitted from dam to progeny. 'C' type of muzzle pattern was transmitted in 8 progenies (66.7%) out of 9, 'B' pattern was transmitted in 1 progeny (8.3%), whereas none of the 'A' pattern was transmitted in their progenies. According to Trofimenko (1988), both the parents have a particular pattern which can be transmitted to more than 75% of their progeny.

Group-a ridge pattern was transmitted on left side of muzzle in 3 progenies (25%) while on right side of the muzzle it was noticed in 5 progenies (41.7%);.group - b ridge pattern was transmitted in 8 progenies (66.7) on left side while on right side, it was transmitted in 6 progenies (50%); whereas group - c ridge pattern was transmitted on left side in 7 progenies (58.3%) and on right side it was transmitted in 5 progenies (41.7%).

No dam transmitted 100% qualitative characters of muzzle print in their progenies. Dracy *et al.*(1953) stated that even though the identical twins had a similar general muzzle pattern there were some dissimilar secondary characters in the ridge pattern.

The muzzle prints of dam (GH-18) and its twin (GH-34 and GH-35) were also studied and observed that the muzzle pattern 'C', type and shape of the valley and the ridge pattern from group a and b of dam were similar in the female calf, whereas dissimilar in male calf indicating the dizygosity. Similar views were made by Schmidt and Kliesch (1938) and Haak (1942).

Though the results of the prints studied revealed the hereditary mechanism and identification of twin by the study of the muzzle prints, it would require further confirmation by large scale studies of different breeds of livestock population at least for 2 to 3 generations.

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Indian Journal of Animal Sciences 68 (11) : 1187-1190, November 1998

In situ ruminal digestion kinetics of different forages in buffalo calves

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Received : 20 October 1997; Accepted :5 May 1998

ABSTRACT

Dry matter (DM) and neutral detergent fibre (NDF) digestabilities and rate and extent of legumes, viz. berseem *(Trifolium alexandrinum)*, lucerne *(Medicago sativa)*, cowpea *(Vigna sinensis)* and non-legumes such as maize *(Zea mays)*, millet *(Panicum miliaceum)*, sorghum *(Sorghum vulgare)* in ruminally fistulated male buffalo calves were determined. Nylon bags measuring 13 cm × 21 cm, with an average pore size of 50 mm, were used. Sample (10 g) was weighed into each bag and exposed to the ruminal fermentation for 0, 1, 2, 6, 10, 16, 24, 36, 48 and 96 hr. DM and NDF degradability was measured at 48 hr. Extent of DM and NDF disappearance was determined at each time point. Rates of disappearance of DM and NDF were determined by regressing the natural logarithm of the percentage of original DM and NDF remaining in the bags between 1 and 96 hr. Dry matter digestibility (DMD) of the legume forages when incubated in the rumen of male buffalo calves was greater ($P < 0.05$) than that of grasses. Extent of digestion followed similar pattern as DMD. Rate of DMD was higher in legumes than grasses. The NDF degradability (NDFD) of legumes and grasses did not differ. The legumes had higher ($P < 0.05$) rates and lower extents of NDF digestion compared to grasses.

Key words : Buffalo calves, Digestibility, Digestion kinetics, Forage, Grasses

The rate and extent of digestion of dietary carbohydrates has a tremendous impact on ruminal fermentation and the productivity of the animals (Varga and Whitsel 1991). The fermentation rate of fibre is dependent upon the source of feedstuff (Varga and Hoover 1983), species, maturity, and morphology (Cherney *et al.* 1990, Bowman *et al.* 1991, Weiss and Shockey, 1991, Cherney *et al.* 1993). The rate of digestion is negatively related to NDF of forages stored at different DM (Nocek and Grant 1987). This indicates that ruminal digestibility of NDF is not uniform across different forages.

Information on the rate and extent of fermentation of commonly available forages, FBP and crop residues in Pakistan is limited. The use of feedstuffs of unknown nutritive value to formulate rations may be a wrong approach as it is neither beneficial for the farmers nor for the animals. The latest approach for greater performance is to feed a balance of ingredients of slow and fast digestible carbohydrates to get the maximum out of ruminal microflora (Nikolic *et al.* 1981). This can avoid uncoupling of essential nutrients at the ruminal level which consequently results into an effective and profitable livestock production. The objectives of this study were to determine (i) lag time, rate and extent of NDF digestion, and (ii) slow and fast digestible feedstuffs in the rumen of buffalo calves.

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MATERIALS AND METHODS

Animals and diets

Male buffalo calves (20, average body weight 200 kg ± 5.7) fitted with ruminal cannulae were used to evaluate 6 different fodders *in situ*, in 2 batches. In each batch, the animals were given 10 days of adaptation period to the diets in the start of the experiment followed by 4 days of the *in situ* incubation of the bags. The animals were fed the same diets as were being incubated in their rumen. This was done to avoid the effects of diets on the ruminal fermentation of the feed stuffs (Clark and Davis 1983, Clark *et al.* 1981). In the first batch berseem, lucerne and cowpeas were incubated in the rumen and the animals were fed fresh berseem only twice daily, @ 1% (on DM basis) of their body weight for each meal. In the second batch, maize, millet, and sorghum were incubated in the rumen and the animals were fed fresh grass only twice daily @ 1% (on DM basis) of their body weight for each meal (Table 1). During each experimental period the animals were housed on a concrete floor in separate pens and the water was offered twice daily.

Sample preparation and analysis

The legumes (berseem, lucerne and cowpea) used in this experiment were harvested at mid-bloom stage and the grasses

Table 1. Composition of fodders (% DM basis)

Items	Berseem	Lucerne	Cowpea	Maize	Millet	Sorghum
CP	16.0	15.5	17.0	8.0	7.5	8.0
NDF	52.0	54.0	51.0	72.0	73.0	72.6
ADF	38.0	38.0	37.0	45.0	44.0	42.9
ADL	10.5	11.0	9.5	8.1	8.3	8.0
Cellulose	27.5	27.0	26.5	27.0	35.7	35.1
Hemicellulose	14.0	16.0	14.0	27.0	29.0	29.6

(maize, millet and sorghum) were harvested at early heading stage. The legume and non-legume samples were first chopped in a locally manufactured chopper and then dried in the forced air oven at 55°C. All the feed samples (legumes and non-legumes) were then ground through a Wiley mill (2-mm screen) and analyzed for DM, nitrogen (AOAC 1984), NDF and ADF (Van Soest *et al.* 1991).

Incubation procedure

Nylon bags measuring 13 cm x 21 cm, with an average pore size of 50 mm were used to determine the rate and extent of DM and NDF disappearance *in situ*. For each time point, 10 g of each feed sample was weighed into bags in triplicate. Two bags were used to determine DM and NDF disappearance and the third bag served as blank. The bags were closed and tied with braided nylon fishing line. This provided approximately 18.3 mg/cm^2 bag surface area. To remove soluble and or 50 mm filterable material, the bags were soaked in 4 litres of tap water for 15 min , just before the ruminal incubation. Weight loss due to soaking was expressed as pre-ruminal disappearance of DM. On day 11 of each experiment at 0800 hr, 3 bags for each fermentation time were incubated in the rumen for 0, 1, 2, 4, 6, 10, 16, 24, 36, 48 and 96 hr, in reverse order and removed all at the same time. After removal from the rumen, bags were washed in running tap water until the rinse was clear. The bags were then dried in a forced air oven at 55°C for 48 hr. After equilibration with air for 8 hr, the bags were weighed back and the residues were transferred to 100 ml cups and stored for later analysis of NDF. Digestibility coefficients of DM and NDF were calculated at 48 hr of incubation. Rates of disappearance of potentially digestible NDF from all feedstuffs were determined by regressing the natural logarithm of the percentage of original NDF remaining in bag between 1 and 96 hr of incubation. The slop of the regression is equivalent to the rate of digestion while the lag time was calculated using the following equation:

$$\text{Lag time } (\%/\text{hr}) = \frac{(\text{Ln} \times 100) - \text{intercept}}{\text{Rate of digestion}}$$

Where Ln stands for natural logarithm.

Statistical analysis

The data on each parameter (lag time, rate and extent of digestion of DM and NDF) were analyzed according to completely randomized design. The means were compared using Duncan's Multiple Range test (Steel and Torrie 1981).

RESULTS AND DISCUSSION

Dry matter digestion kinetics

The DM digestibility coefficients of berseem, lucerne and cowpea were higher ($P< 0.05$) than those of maize, millet and sorghum (Table 2). The reduced DMD of maize, millet and sorghum compared to berseem, lucerne and cowpea may be due to higher NDF. Varga and Hoover (1987) showed that the NDF is negatively correlated to the apparent digestibility of the forages. The higher DMD of berseem, lucerne and cowpea than that of maize, millet and sorghum can be attributed to their faster rate of disappearance (as DMD was measured at 48 hr). Glenn *et al.* (1989) reported a higher ($P< 0.05$) DMD of alfalfa hay compared to orchard grass hay.

Extent of digestion, calculated at 96 hr of the fodders followed the same trend as DMD. The greater extent of digestion of berseem, lucerne and cowpea can be attributed to the quality of fodders. Weiss and Shockey (1991) and Slabbert *et al.* (1992) reported that extent of DMD *in vivo* decreased by lowering the quality of the diet fed to feedlot steers.

The rate of DM disappearance was higher ($P< 0.05$) in berseem, lucerne and cowpea than maize, millet and sorghum (Table 2). These results are in agreement with the study of Varga and Hoover (1987). However, *in situ* or *in vitro* digestion rate given no information on residence time in the rumen and

Table 2. Comparative *in situ* dry matter digestibility (DMD), lag time, rate and extent of digestion of leguminous and non-leguminous forages in ruminally cannulated buffalo male calves

Items	Leguminous			Non-leguminous			
	Berseem	Lucerne	Cowpea	Maize	Millet	Sorghum	SE±
DMD, %*	76.69^{a}	74.26^{a}	77.00^{a}	60.46^{b}	54.52^{bc}	52.41^{c}	1.85
Lag, H	1.18^{b}	1.55^{b}	1.40^{b}	2.80	3.01^{c}	3.05^{c}	0.12
Rate, %/hr	6.00^{b}	6.00^{b}	6.25^{b}	4.50^{c}	4.25^{c}	4.50^{c}	0.57
Extent, %**	80.51^{a}	84.10^{a}	78.51^{a}	69.86^{bc}	69.67^{bc}	67.51^{c}	1.88

Means in the same row with different superscripts differ ($P< 0.05$).
*Dry matter digestibility was determined at 48 hr of incubation.
** Extent of digestion was determined at 96 hr of incubation.

Table 3. Comparative *in situ* neutral detergent fibre digestibility (NDFD), lag time, rate and extent of digestion of leguminous and non-leguminous forages in ruminally cannulated buffalo male calves

Items	Leguminous			Non-leguminous			
	Berseem	Lucerne	Cowpea	Maize	Millet	Sorghum	SE±
NDFD %*	52.50^{a}	50.19^{a}	56.49^{a}	52.65^{a}	50.51^{a}	39.57^{b}	1.91
Lag, H	1.84^{b}	1.75^{b}	3.05^{c}	3.19^{c}	3.21^{c}	4.15^{d}	0.12
Rate, %/hr	5.10^{b}	5.50^{b}	4.25^{c}	4.15^{c}	4.22^{c}	3.00^{d}	0.31
Extent, %**	59.70^{a}	58.55^{a}	68.51^{b}	65.50^{b}	67.51^{b}	44.90^{c}	1.76

Means in the same row with different superscripts differ ($P < 0.05$).
* Neutral detergent fibre digestibility was determined at 48 hr of incubation.
** Extent of digestion was determined at 96 hr of incubation.

therefore, provide no direct estimate of nutrient availability (Nocek and Russell 1988).

Neutral detergent fibre disappearance kinetics

The NDF disappearance (NDFD) of berseem, lucerne, cowpea, maize, millet and sorghum were not different (Table 3). The reason for similar NDFD of all the fodders may be the longer incubation period (48 hr), which may have unabled to detect the expected differences due to the quality of different feeds. This notion is confirmed by the greater extent of digestion of maize, millet and sorghum.

The berseem, lucerne and cowpea have higher rate and lower extent of NDF digestion than in maize, millet and sorghum. These results are in agreement with previous reports of Buxton (1989), Fisher *et al.* (1989), Grant and Mertens (1992) and Andrighetto *et al.* (1993) reported that fractional rate of NDF digestion *in situ* was higher and extent was lower in alfalfa than in bromegrass. Generally extent of NDF digestion legume is lower than that of grasses because of lower cell wall content and higher lignification of the former (Jung 1989, Van Soest 1994). Rate of cell wall digestion seems to be related to the anatomical and histological ture of plant tissues (Akin 1979, 1989) or greater microbial colonization of legumes versus grasses (Bowman and Firkins 1993). Firkins *et al.* (1991) concluded that carboxymethylcellulase (CMCase) activity gave a quantitative assessment of mass of cellulolytic bacteria colonizing plant fibre. Using CMCase as an indicator of cellulolytic bacteria, it has been shown that cellulolytic bacteria, as a proportion of total particle associated bacteria, were attached in higher concentration to red clover than grasses (gammagrass and orchardgrass) during early (3-18 hr) incubation (Bowman and Firkins 1993). This difference in colonization rate of forage particle by cellulolytic bacteria may be a reason for more rapid rate of fibre degradation for legumes than in grasses. Huhtanen and Khalili (1992) also reported that the CMCase activity was highly correlated with disappearance rate of NDF *in situ*. This could be a possible explanation of why the legumes have a higher NDF digestion rate than grasses.

The DMD of the leguminous forages were higher than that of non-legumes. However, the NDFD of the forages did not differ among themselves. It can be explained that the slowly degradable grasses had compensated their NDF disappearance due to the longer incubation period (48 hr), as is confirmed by the highest extent of digestion of grasses. An inverse relationship between lag time and low rate of disappearance was observed in all feedstuffs. *In vivo* studies need to be conducted to estimate the nutrient availability of the feedstuffs as *in situ* digestion experiments gives limited information on the residence time of the feedstuffs in the rumen.

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Indian Journal of Animal Sciences 68 (11) : 1191-1192, November 1998

Physicochemical and microbial alterations in rumen liquor during *Lantana camara* toxicity in buffalo calves (*Bubalus bubalis*)*

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Received : 28 December 1997; Accepted : 20 May 1998

ABSTRACT

Rumen liquor analysis of *Lantana camara* intoxicated buffalo calves revealed complete absence of protozoal motility, concentration and idophillic activity with significant ($P < 0.01$) increase in sedimentation activity test and methylene blue reduction time. However the glucose fermentation test value decreased significantly ($P < 0.01$). Significant ($P < 0.01$) increase in rumen pH (8.16±0.18) and ammonia nitrogen concentration (27.33±2.05 mg/dl) was also recorded during the toxicity. Total volatile fatty acid concentration decreased significantly ($P < 0.01$) to 9.06±0.88 m Eq/litre. It was concluded that *Lantana camara* var. *aculeata* caused gross rumen microbial inactivity in buffalo calves.

Key words: Buffalo calves, *Lantana amara* toxicity, Rumen function tests

Lantana camara is one of the most noxious weeds of the world. Field outbreaks of lantana toxicity have frequently been reported in India (Katiyar 1981). Toxic effects of lantana on liver and kidney are well documented, however, its effect on rumen microbes has attracted a little attention. Hence the present study was undertaken to investigate the effect of lantana toxins on rumen liquor in terms of physical, chemical and microbial alterations.

MATERIALS AND METHODS

The toxicity was induced in 6 male healthy buffalo calves (9-12 month old) by oral administration of shade dried *L. camara* var. *aculeata* leaf powder (@ 6 g/kg b. wt. once only). Another group of 6 calves was kept as a control.

About 100 milliliters (ml) of rumen liquor was collected with the help of paraffin lubricated stomach tube, from each calf at 0, 12, 24, 48, 72 and 96 hr and was immediately analyzed for its physical characteristics (Rosenberger 1979), protozoal motility (Mishra and Singh 1974), protozoal concentration and iodophillic activity (Mishra *et al.* 1972), pH (portable pH meter), total volatile fatty acids (Barnett and Reid 1957), ammonia nitrogen by micro-diffusion technique (Conway 1957), sedimentation activity test (SAT) and glucose fermentation test (GFT) (Nicholas and Penn 1958) and methylene blue reduction time (MBRT) (Rosenberger 1979). Statistical analysis (paired t-test) was done as per Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The normal greenish brown colour of rumen liquor changed initially to brownish yellow between 24 and 48 hr and finally to deep brownish by 96 hr of toxicosis. The normal aromatic odour recorded at 0 hr turned to ammonical by 96 hr. These changes in colour and odour might be because of corresponding increase in rumen pH and NH^3·N concentration respectively. The viscous consistency changed initially to watery at 12 hr. However later on, rumen liquor became highly viscous probably due to rumenstasis and dehydration (Pass 1986, Kaushal 1994).

The protozoal motility, concentration and iodophillic activity decreased progressively from +++ to + by 48 hr with complete absence at 96 hr of intoxication. This might be due to significant ($P < 0.01$) increase in rumen pH (8.16±0.18) and non-availability of carbohydrate substrate due to anorexia. In addition, it is highly possible that lantana toxins (lantadenes) might have directly affected the protozoal population which needs substantiation by *in-vitro* studies.

Initial decline in microbial activity was followed by complete absence of rumen fermentation between 48 and 96 hr, post-induction of toxicity as reflected by SAT, MBRT and GFT (Table 1) values. This might be due to either direct effect of lantana toxins on microbial population as caused by other plant toxins like perloline, tannins and some essential oils

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Table 1. Microbial and biochemical changes during lantana toxicity (mean±SE)

Parameters	Time of sampling (hr)					
	0	12	24	48	72	96 or before death
SAT (Min)	12.17±1.06 (11.15±1.13)	23.67**±1.92 (13.17±1.08)	51.00**±3.36 (12.33±1.16)	Nil (9.83±0.98)	Nil (11.83±1.24)	Nil (10.5±1.02)
MBRT (Min)	3.17±0.47 (3.67±0.33)	15.33**±2.17 (2.50±0.22)	32.67**±4.58 (3.17±0.31)	Nil (4.5±0.34)	Nil (3.5±0.22)	Nil (2.83±0.17)
GFT (ml/hr)	1.62±0.12 (1.54±0.09)	0.73**±0.08 (1.46±0.09)	0.48**±0.03 (1.48±0.16)	0.18**±0.06 (1.67±0.14)	Nil (1.52±0.12)	Nil (1.60±0.11)
pH	6.61±0.1 (6.56±0.13)	37.08±0.11 (6.62±0.18)	7.12*±0.13 (6.48±0.12)	7.21*±0.16 (6.64±0.16)	7.64**±0.09 (6.7±0.14)	8.16**±0.18 (6.51±0.09)
NH3-N (mg/dl)	6.72±0.53 (7.05±0.62)	8.02±0.66 (6.78±0.51)	10.67*±0.75 (6.64±0.16)	18.83**±1.16 (7.19±0.62)	21.1**±1.45 (7.09±0.53)	27.33±2.05 (6.96±0.51)
TVFA (mEq/litre)	74.05±5.26 (72.83±3.48)	50.50*±4.17 (68.44±3.52)	24.7**±3.46 (62.57±4.15)	19.58**±3.03 (74.53±4.37)	11.37**±1.39 (69.71±3.58)	9.06**±0.88 (76.33±4.26)

Figures in parentheses, control group; * significant at 5% level ($P < 0.05$); ** significant at 1% level ($P < 0.01$).

(Allison 1977, Keeler *et al.* 1978) or due to indirect effect on account of unfavourable intra-ruminal atmosphere (Hungate 1966, Czerkwaski 1986).

Progressive and significant ($P < 0.01$) increase in rumen pH (Table 1) appears to be because of corresponding increase in NH_3-N concentration and decrease in TVFA concentration (Tsuda *et al.* 1991).

In rumen liquor NH_3-N concentration the gradual increase might be due to microbial digestion of dead bacteria retained in the static rumen combined with decreased utilization of ammonia by rumen bacteria under scarcity of soluble carbohydrate (Warner 1956 a, b, Hungate 1966).

It is concluded that *L. camara* var. *aculeata* - red flower containing lantadene A, lantadene B and reduced lantadene A caused gross rumen microbial inactivity in buffalo calves.

ACKNOWLEDGEMENTS

We thank the ICAR, New Delhi, for providing financial help to carry out the present study.

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Indian Journal of Animal Sciences **68** (11) : 1193-1197, November 1998

Relationship of dietary energy and heat tolerance in sheep

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Received : 10 January 1997; Accepted :21 May 1998

ABSTRACT

Relationship between dietary energy and heat tolerance of Mutton synthetic rams was assessed by feeding them on 100 % of their maintenance energy requirement under shed (G1) and solar exposure (G2) or 115 (G3) and 130 (G4)% of their energy requirement under summer sun. DMI was similar in all the groups as per experimental design whereas water consumption was higher in G3 compared to G1, G2 and G4. The rams exposed to heat had average water consumption of 11.5 % of body weight amounting to 6.23 l/kg DMI. The respiration frequency (RF) registered usual variation increasing by 210 % from morning to peak hot period (PHP) in these heat exposed rams. Such increase in RF was of lower magnitude in animals under protection compared to solar exposed groups and the trends remained same on its expression in terms of unit rise in rectal temperature (RT). The RF in the 2 recordings and its increase for unit °C rise in RT was higher ($P<0.01$) in G4 followed by G3, G2 and G1 in that order. Pulse rate (PR) increased by 24% from morning to PHP. PR was higher in G3 and G4 than in G1 and G2 both in morning and PHP recordings, however, the increase in PR from morning to PHP was higher in G2 than other groups. The rectal temperature (RT) was 38.8 °C in morning and increased to 39.4 °C at PHP. Morning RT was higher in G4 (38.96 °C) and G3 (38.80 °C) than G1 (38.72 °C) and G2 (38.71 °C). The increase in RT from morning to PHP was lower ($P<0.01$) in high energy fed G4 and higher in G1 and G3 in that order whereas highest values were recorded in G2. Sweating rate at PHP was lower in G1 under shed (133 g/m^2/hr) than solar exposed G2, G3 and G4 (227 g/m^2/hr) and among the solar exposed groups it was lower in G4 and G2 than G3. It is concluded that although heat exposed, high energy feed groups exhibited greater respiratory distress, their morning to peak hot period increase in rectal temperature was of lower magnitude indicating better heat tolerance.

Key words: Dietary energy, Heat tolerance, Sheep

Environment for sheep rearing vary from hot arid to sub-arctic conditions in different parts of the world whereas in India majority of sheep populations are localized in hot semi-arid and arid regions. The production traits of these sheep are low and major factors contributing to such low productivity are inadequacy of grazing resources and under nutrition for most part of the year, prevailing high ambient temperature, unorganized management and inadequate prophylactic and curative health coverage.

Elevated temperature depresses appetite centre of hypothalamus (Baile and Forbes 1974) reducing dry matter intake (Guerrini 1981) with concomitant depression in digestive efficiency (Bhattacharya and Hussain 1974) and energy availability. Further, physiological responses elicited by heat exposed sheep during summer to counter the imposed stress (respiratory ventilation and cutaneous evaporation) are dynamic in nature which increase their maintenance energy requirement (Mc Dowell *et al.* 1969).

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Even with efficient thermolysis, the core temperature of the animals remain at a higher plane also adding to maintenance energy requirement due to Van't Hoff's effect (Graham *et al.* 1959). The detailed factors operating together increase the maintenance energy requirement of heat exposed animals (Rogerson 1960, Beede and Collier 1986).

The prevailing agro-meteorological conditions and animal's defence strategies to counter the imposed stress cannot be changed hence it is felt that fortification of diet by increasing energy density of the ration compensating heat mediated depression in DMI and digestive efficiency and enhanced maintenance energy requirement would maintain the animals in positive energy balance and improve heat tolerance and production.

MATERIALS AND METHODS

Mutton synthetic (32: Dorset × Malpura halfbred) rams adopted from institute flock were divided into 4 equal groups. Prior to initiation of the experiment these rams were maintained under extensive range management with *ad lib.* conserved fodder after grazing and 300 g concentrate supplementation during breeding season. One group was

Table 1. Physical and chemical composition of the experimental rations (%)

Rations	1	2	3
Physical composition			
Ground cenchrus hay	55	31	7
Maize	21	50	79
Groundnut-cake	22	17	12
Mineral mixture	1.5	1.5	1.5
Common salt	0.5	0.5	0.5
Chemical composition			
Dry-matter	93.23	91.83	91.10
Crude protein	12.68	13.62	15.75
Ether extract	2.76	2.69	3.72
Crude fibre	20.92	12.19	7.02
Nitrogen free extract	57.37	65.96	69.39
Ash	6.27	5.54	4.12

maintained in side open asbestos roofed animal shed (G1) while rest three groups (G2, G3 and G4) were exposed to summer sun in individual enclosures. Animals in shed (G1) and one solar exposed group (G2) received feed (R1) calculated to meet 100 % of their maintenance energy requirement (Ranjan 1985) while remaining 2 solar exposed groups, G3 and G4, were fed 15 and 30 % above their maintenance energy requirement on R2 and R3 respectively. To ensure uniform dry matter intake (DMI) in all the groups, rations 2 (R2) and 3 (R3) offered to G3 and G4 were computed to contain 15 and 30 % higher energy respectively (Table 1). The animals were watered free choice once daily at 1400 hr and records of water consumption was maintained throughout the experiment. Calculated amounts of feed was offered daily in the morning hours after discarding previous days residues and the offer was adjusted based on weekly body weight changes. The experiment was initiated in mid- March and continued for 120 days till mid - July encompassing the summer season.

The three rations formulated with varying energy density to meet 100, 115 and 130 % of energy requirement of G1 and G2, G3 and G4 respectively were offered in equal quantities to the four groups which was adjusted in weekly intervals on the basis of their body weight changes. Calculated feed offer was 2 % of body weight in all the groups and animals fully consumed the offer without left over. Animals in all the four groups were control fed hence their DMI was similar and the animals consumed their calculated offer without left over. The envisaged experimental variation in energy intake was 100 % in G1 and G2 and 115 and 130 % respectively in G3 and G4. The actual TDN intake in digestibility trial of these animals was 509.9, 460.8, 575.5 and 676.9 g/day respectively in G1, G2, G3 and G4 whereas its expression as unit metabolic body size indicated that TDN intake/day was similar in G1 and G2 and 8 and 19 % higher as against expected 15 and 30 % higher in G3 and G4 respectively (Karim 1991).

Physiological responses, viz. respiration frequency (RF) by counting double heaving of flank movement, pulse rate (PR) by palpation of femoral artery and rectal temperature (RT) by Aplab Multi Channel Tele-thermometer at 0730 and 1430 hr and sweating rate (Schleger and Turner 1965) at 1430 hr were recorded on all the animals in fortnightly intervals. Dry bulb temperature, relative humidity (at 0730 and 1430 hr), minimum and maximum temperature, wind velocity and hours of sunshine were recorded from Institute observatory and solar radiation data were obtained from local meteorological observatory of Indian Meteorological Department, Jaipur. The meteorological data averaged for fortnights are presented in Fig. 1. The generated data were subjected to least squares analysis of variance (Harvey 1990) and significant group differences were compared by Duncan's Multiple Range Test (Duncan 1955).

RESULTS AND DISCUSSION

Water consumption was higher (P<0.01) in G3 compared to G1, G2 and G4 and the trends remained same on expression in terms of DMI (Table 2). Water consumption was lower (P<0.01) in pre-experimental period and gradually increased registering peak values during 11th week followed by slow decline (Fig. 2). Overall average water consumption of rams exposed to hot summer was 11.5 % of body weight whereas under comfortable condition water consumption of such animals is reported to be 5 to 6 % of their body weight (More and Sahani 1978). Higher water consumption in heat exposed sheep was also observed by Singh *et al.* (1983). Further,

Table 2. Physiological responses and water consumption of rams maintained on varying levels of energy under heat exposure

	G1	G2	G3	G4	SE±M
Water intake (ml/kg BW)	114.9	115.5	122.1	111.5	2.06
Water intake (l/kg DMI)	6.237	5.961	6.862	6.445	0.1109
Respiratory frequency/min					
Morning(AM)	30.2^{B}	22.7^{A}	27.3^{A}	35.6^{B}	1.12
After noon(PM)	65.6^{A}	92.3^{B}	94.1^{B}	104.6^{B}	2.51
Increase	35.4	69.6	66.8	69.0	2.56
Increase/Δ°C RT	76.3^{A}	116.8^{B}	156.9^{C}	177.6^{C}	10.9
Pulse rate/min					
AM	52.3^{A}	51.4^{A}	57.2^{B}	66.1^{C}	0.62
PM	65.2^{A}	67.2^{A}	71.1^{B}	78.3^{C}	0.92
Increase	12.9^{A}	15.8^{B}	13.9^{A}	12.2^{A}	0.83
Increase/Δ°C RT	27.1	27.7	29.9	27.3	2.34
Rectal temperature(°C)					
AM	38.72^{A}	38.71^{A}	38.80^{A}	38.96^{B}	0.031
PM	39.29^{A}	39.41^{B}	39.36^{A}	39.42^{B}	0.029
Increase	0.57^{A}	0.70^{B}	0.56^{A}	0.46^{A}	0.032
Increase/10°C AT	0.50^{A}	0.59^{B}	0.51^{A}	0.47^{A}	0.029
Sweating rate (g/m^2/hr)	133.3^{A}	220.2^{B}	252.0^{C}	208.1^{B}	12.11

Unlike superscripts in rows differ significantly: capital letters (P<0.01), small letters (P<0.05).

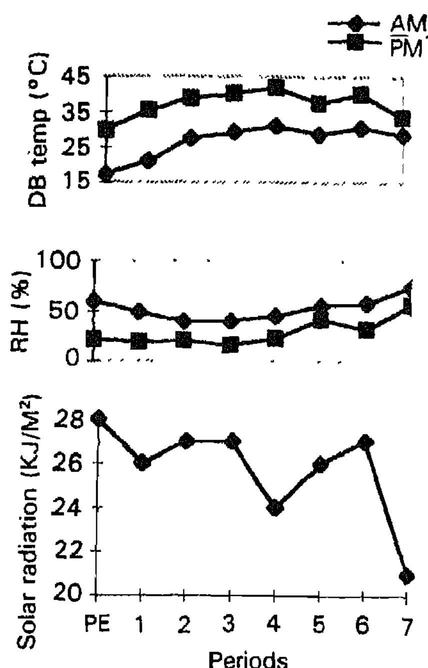

Fig.1. Environmental variables during the experiment.

water intake is proportional to DMI (Mac Farlane 1976) and with similar DMI in all the groups higher water intake in solar exposed groups was obviously used to meet the thermolytic demand (Karim *et al.* 1984, Azamel *et al.* 1987).

Respiration frequencies (RF) with usual variation registered 210 % increase from morning to peak hot period. The RF in morning recording was higher ($P<0.01$) in G4 and G1 as compared to G2 and G3, whereas during peak hot period the G4 animals had highest ($P<0.01$) RF followed by G3 ,G2 and G1 (Table 2). Morning to peak hot period increase in RF was lower ($P<0.01$) in G1 under shed compared to solar exposed G2, G3 and G4. However, morning to peak hot period increase in RF for unit °C rise in RT was higher ($P<0.01$) in solar exposed G3and G4 followed by G2 while it was least in G1 animals under protection. The morning and peak hot period RF gradually increased up to 6 fortnight followed by decline during last fortnight. Similar trends were also observed on expression of RF for unit rise in RT from morning to peak hot period whereas the declining trend was apparent from 5 fortnight. Higher RF in G1 and G4 than G2 and G3 during morning recording was possibly a reflection of their metabolic status. The finding that RF in heat exposed animals increases to greater extent under high plane of feeding is in agreement with the observations of Blaxter *et al.* (1959) in sheep and Berbiger (1983) in cattle. Higher RF during the peak hot period in animals exposed to sun compared to animals in shed was due to the effect of solar radiation in terms of heat component which added to heat load of the animals (Azamel *et al.* 1987). The progressive increase in RF with increasing ambient temperature (AT) during the experiment was a reaction of the animals to prevailing hot conditions while peak RF recorded in 6 fortnight represented high AT as well as high RH. It is established that high RH at higher AT interferes with efficient respiratory and cutaneous evaporative cooling thus increasing respiratory ventilation to a greater extent (Bouvier *et al.* 1974).

The pulse rate (PR) in this study pooled for the 4 groups registered 24 % increase from morning to peak hot period. During exposure to summer heat the morning to peak hot period increase in pulse rate (PR) of animals under shed (G1) was 25 % as against earlier report of 15 % at the same location (Singh *et al.* 1982) while similar rise in G2 was 31 % which declined to 24 and 18 % in high energy fed G3 and G4 respectively. Quantitatively higher PR during morning recording in high energy fed groups could be due to their higher metabolic rate associated with high plane of nutrition (Graham *et al.* 1959) and greater cardiac activity with circulatory orientation towards periphery (Hales 1976) while lesser rise of PR from morning to peak hot period in high energy fed groups was indicative of beneficial effect of high energy feeding under such situation. Over the periods, the PR was higher in phases of higher temperature and low RH as well as comparatively lower temperature and higher RH. Earlier reports also indicate that PR increase with rise in environmental temperature (Hanus 1969) and such response was pronounced in hot humid conditions (Kaushish *et al.* 1995).

Overall average rectal temperature (RT) of the rams exposed to summer condition under stall feeding was 38.8 °C in the morning and increased to 39.4 °C in the peak hot

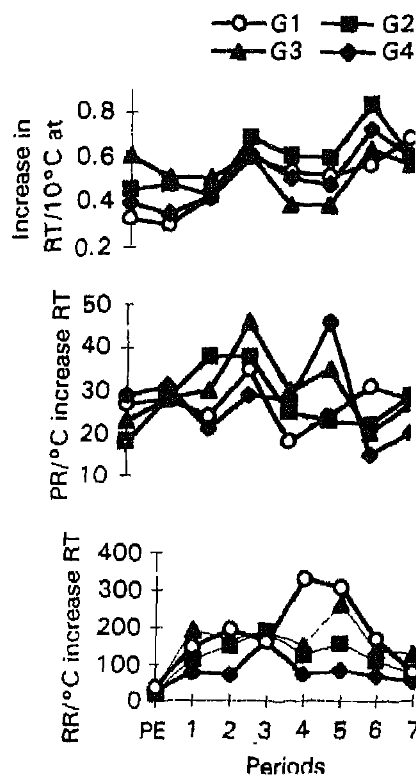

Fig.2. Physiological responses of sheep maintained on varying levels of energy under heat exposure.

period registering 0.6 °C increase between the two recordings which was statistically significant ($P<0.01$). Body temperature of the animals is influenced by environmental variables, plane of nutrition and physical activity (Stonier *et al.* 1984) and reaches equilibrium slowly, therefore, is a good index of true steady state under heat exposure. Morning RT was higher ($P<0.01$) in high energy fed G4 followed by G3 and similar in G1and G2 fed at maintenance level. In the afternoon although the RT was still highest in solar exposed G4, it was followed by G2, G3and was least in G1 under protection. However, the quantum of increase in RT from morning to peak hot period was lowest ($P<0.01$) in high energy fed G4 followed G1, and G3 whereas it was highest in G2. During summer rectal temperature of G1 and G2 animals fed as per their maintenance requirement increased by 0.7 °C from morning to peak hot period due to cyclic oscillation of body temperature as a consequence of metabolic status (Kleitman 1949) and variation in radiant heat load (Bligh and Harthoom 1965). Bligh (1976) also reported that in free ranging sheep such increase in RT was to the tune of 1°C. Among the solar exposed groups although the morning and peak hot period RT was higher in high energy fed G3 and G4 because of involved metabolic process (Blaxter *et al.* 1959), their increase in RT from morning to peak hot period or for every 10°C rise in AT was lower indicating better heat tolerance. Based on RT recordings of morning and peak hot period it was observed that RT increased by 0.5°C for 10°C rise in ambient temperature. The groups exposed directly to sun registered greater rise in RT than to those of under protection due to enhanced heat load of solar radiation impinging on the body of the animal (Johnson 1991). Increased RT in solar exposed compared to animals under protection is a consistent finding in several studies (Azamel *et al.* 1987, Gupta and Acharya 1987). Even with efficient thermolysis (respiratory and cutaneous evaporative cooling) the solar exposed groups were unable to loose the incident and generated body heat effectively resulting in increased core temperature. Over the fortnight periods, the morning RT was similar in all the 4 groups whereas in peak hot period it was higher ($P<0.01$) in fortnight 3,6 and 7. The responses also differed ($P<0.01$) between the groups over the fortnight recordings. Increase in RT for 10°C increase in AT was higher in periods 3 and 6 representing increasing phase of peak ambient temperature and peak RH respectively (Fig. 1).

Overall sweating rate of the sheep exposed to heat during peak hot period of summer was 203 g/m^2/hr. Sweating rate was lower ($P<0.01$) in G1 animals under protection compared to G2, G3 and G4 exposed to solar radiation and among the solar exposed groups it was lower in G4 and G2 compared to G3. The sweating rate gradually increased registering peak values in 6 fortnight, the effect was pronounced in G2 and G3 animals during that fortnight. The higher sweating rate in solar exposed groups compared to group under protection of animal shed is suggestive of their greater sweat gland activity since sweat gland activity is almost twice in field existence compared to those recorded in climatic laboratory (Murray 1966). Sweating rate peaked in 6 forthright representing period of high temperature and highest RH.

Although the solar exposed high energy fed groups exhibited greater respiratory and circulatory distress their body temperature showed lesser morning to peak hot period increase indicating better heat tolerance.

ACKNOWLEDGEMENTS

We are grateful to the Director for facilities and assistance rendered by Mr M Asgar in laboratory analysis is acknowledged.

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Indian Journal of Animal Sciences 68 (11) : 1198-1201, November 1998

Effect of supplemental thiamine, niacin and palm oil on performance of broilers fed maize-groundnut-cake based ration

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Received : 23 August 1997; Accepted : 4 April 1998

ABSTRACT

Commercial chicks (320) were used in a 2 × 2 × 2 factorial treatment experiment conducted to study the effect of dietary levels of thiamine (2.5 mg and 3.0 mg/kg), niacin (35 and 40 mg/kg) and palm oil (2 and 4%) on the performance of broilers from day-old to 56 days of age. The level of supplemental palm oil in the diet affected performance and vitamins requirements for optimum feed utilization and production of desirable broiler carcass quality. Supplementation of the diet with 4% palm oil significantly reduced weight gain and feed intake, and depressed the accumulation of lipid in liver, kidneys and abdominal region, but did not affect the carcass characteristics of broilers. A combination of 3.0 mg thiamine and 35 mg niacin/kg of diet was required in 2% palm oil supplement as compared with a combination of 2.5 mg thiamine and 40 mg niacin required/kg of diet supplemented with 4% palm oil.

Key words: Broiler chicken, Groundnut-cake, Niacin, Palm oil, Thiamine

Requirement of thiamine and niacin for broilers vary widely due to their variation in their bioavailability and utilization in the test diet (Braunlich and Zintzen 1976, Lonza 1984). Involvement of these vitamins in fat synthesis (Scott *et al.* 1982), inhibition of hepatic lipogenesis (Bortz *et al.* 1963), coupled with marked effect on fatty acid composition (Johansson 1956) may influence their requirement in broilers. This paper explains the effect of groundnut-cake based diet supplemented with thiamine, niacin and palm oil in broilers.

MATERIALS AND METHODS

Commercial broiler chicks (320; day-old) were randomly assigned to 8 treatment groups in a 2 × 2 × 2 factorial design. Each treatment group had 2 replicates consisting of 20 chicks each. The dietary factors having 2 levels of thiamine (2.5 and 3.0 mg/kg), niacin (35 and 40 mg/kg) and palm oil (2 and 4%). Experimental rations were made isoproteinic and isocaloric. They differed in the level of palm oil inclusion (Table 1). Chicks were raised in deep litter and fed with respective experimental diet for 56 days. At the end of the feeding trial, 4 replicate samples of experimental chicks were randomly selected from each treatment group, fasted for 6 hr, weighed, slaughtered and dressed for carcass studies including meat to bone ratio.

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Table 1. Composition of basal diets

Items	%	
	Diet 1	Diet 2
Ingredients		
Yellow maize	54.75	50.75
Groundnut-cake	25.00	25.00
Blood-meal	3.00	4.00
Fish-meal	4.00	5.00
Brewer's grain	7.50	5.50
Oyster shell	1.00	2.00
Bone-meal	2.00	3.00
Palm oil	2.00	4.00
Vitamin/mineral premix*	0.25	0.25
Methionine	0.30	0.30
Salt (NaCl)	0.20	0.20
Total	100.00	100.00
Calculated analysis		
Crude protein (%)	22.38	22.95
Ether extract (%)	5.42	7.21
Metabolizable energy, ME (Kcal/kg)	2920.63	2975.53
ME/CP	130.50	129.65
Thiamine (mg/kg)	1.8	1.7
Niacin (mg/kg)	16.1	15.8
Tryptophan (%)	0.25	0.26

*Vitamin/mineral premix supplied per kg of feed: vit. A, 1300 IU, vit. D, 2600 IU; vit. E_1 10 IU; stabilized menadione sodium bisulphite, 2 mg; vit. B_2 5 mg; vit. B_6, 4 mg; biotin, 0.05 mg; calcium d-pentothenate, 10 mg; vit. B_{12}, 0.02 mg; folic acid, 1 mg; choline chloride, 500 mg; manganese, 150 mg; iron, 50 mg; zinc, 45 mg; copper, 2.4 mg; cobalt, 0.2 mg; iodine, 1.4 mg; selenium,

Table 2. Feed utilization and carcass characteristics of broilers fed on maize-groundnut-cake diet supplemented with varying levels of thiamine (B1), niacin (B3) and palm oil (0).

Parameter	2% oil					4% oil					
	2.5 mgB[1]		3 mgB$_1$		Mean1+	2.5mgB$_1$		3 mgB[1]		Mean2+	SEM**
	35 mgB$_3$	40 mgB$_3$	35 mgB$_3$	40 mgB$_3$		35 mgB$_3$	40 mgB$_3$	35 mgB$_3$	40 mgB$_3$		
Feed utilization											
Body weight at 56 days (g)	1390.0^{bc*}	1440.0^{b}	1592.5^{a}	1535.0^{a}	1489.4^{x}	1280.0^{c}	1557.0^{a}	1310.0^{bc}	1560.0^{a}	1426.8^{y}	56 68
Body weight gain (g/bird/day)	24.02^{b}	24.91^{b}	27.63^{a}	27.21^{a}	25.94^{x}	22.05^{b}	27.00^{a}	22.59^{b}	27.05^{a}	24.67y	1.042
Feed intake (g/bird/day)	87.10^{b}	86.96^{b}	89.77^{a}	89.42^{a}	88.31^{x}	80.74^{d}	83.10^{c}	81 04^{d}	83.29^{c}	82.04^{y}	1.683
Feed efficiency (g gain/g feed)	0.28	0.29	0.31	0.30	0.30	0.27	0.32	0.28	0.32	0.308.992E-03	
Carcass characteristics											
Carcass weight (g)	1010.0^{b}	1057.0^{b}	1155.0^{a}	1145.0^{a}	1091.8^{x}	930.7^{c}	1167.8^{a}	980.3^{bc}	1143.3^{a}	1055.5^{y}	42.90
Dressing percentage	72.66	73.40	72.55	74.59	73.30	72.71	75.00	74.83	73.29	73.96	0.480
Total edible meat (g)	655.9^{b}	690.8^{b}	789.4^{a}	787.5^{a}	730.9	633.0^{b}	806.0^{a}	679.1^{b}	791.4^{a}	727.4	33.26
Meat (% carcass weight)	64.94^{b}	65.35^{b}	68.35^{a}	68.78^{a}	66.86	68.01^{a}	69.02^{a}	69.27^{a}	69.22^{a}	68.88	0.812
Total bone (g)	354.1^{a}	366.2^{a}	365.6^{a}	357.5^{a}	360.9^{x}	297.7^{b}	361.8^{a}	301.2^{b}	351.9^{a}	328.2^{y}	13.23
Bone (% carcass weight)	35.06^{a}	34.65^{a}	31.65^{b}	31.22^{b}	33.15	31.99^{b}	30.98^{b}	30.73^{b}	30.78^{b}	31.12	0.812
Meat: bone ratio	1.85	1.89	2.16	2.20	2.03	2.13	2.23	2.25	2.25	2.22	0.075
Abdominal fat pad (g)	43.83^{a}	50.00^{a}	33.4^{c}	31.9^{c}	39.78^{x}	29.8^{c}	32.7^{c}	28.7^{c}	32.13^{c}	30.83^{y}	3.512
Abdominal fat (% carcass weight)	4.34^{a}	4.73^{a}	2.89^{b}	2.79^{b}	3.79	3.69^{b}	3.20^{b}	2.93^{b}	2.81^{b}	2.94	0.362

*Values within a row bearing different subscripts (a-d) are significantly ($P<0.05$) different.

**SEM, Standard error of the treatment mean; 1, average values for 2% palm oil treatment groups: 2, average values for 4% palm oil treatment groups.+, mean values denoted by different subscripts (x, y) for a parameter are significantly ($P<0.05$) different.

The livers and kidneys were excised, drained of fluids with blotting paper, weighed and kept for total lipid determination (Folch *et al.* 1957). The data were analyzed as per Steel and Torrie (1960).

RESULTS AND DISCUSSION

The need for supplementation of practical poultry rations based on actual feed ingredients with thiamine and niacin has been demonstrated and variations in values (i.e., thiamine, 1 - 1.8 mg/kg diet; niacin, 30 - 100 mg/kg diet) reported as their requirements for growth in broilers were attributed to variability in composition of the test diets (Czarnecki *et al.* 1983, NRC 1984, Waldroup *et al.* 1985, Oloyo 1997).

Body weight at 56 days, average daily weight gain and average daily feed intake of experimental chickens were significantly ($P<0.05$) affected by the dietary treatments (Table 2). With 2% oil supplement in the diet, birds given 35 mg or 40 mg niacin/kg diet in addition to 2.5 mg thiamine/kg diet consumed less feed, gained less body weight and had smaller body weight than those given the same levels of niacin supplements in addition to 3 mg thiamine/kg diet. However, no marked difference was observed in growth performance between birds fed on 35 mg or 40 mg niacin/kg diet at either of the 2 levels of thiamine supplementation. Comparison of the feed utilization of birds on the same supplemental niacin level but with 4% oil addition indicated that increasing supplemental thiamine level in the diet from 2.5 mg to 3 mg/kg did not affect significantly ($P>0.05$) the growth performance of the chickens. However, birds given 35 mg niacin / kg of 4% oil supplemented diet consistently had poorer feed utilization than those given 40mg niacin/kg diet at each level of thiamine supplementation. Average body weight at 56 days, daily body weight gain and daily feed intake were also significantly less ($P<0.05$) when diets were supplemented with 4% oil. These results suggested that a combination of 3 mg thiamine and 35 mg niacin/kg of diet supplemented with 2% palm oil or a combination of 2.5 mg thiamine and 40 mg niacin/kg of diet supplemented with 4% palm oil were optimum for broilers.

Increasing the palm oil supplementation from 2 to 4% might have increased the requirement of amino acids, particularly tryptophan (Jensen *et al.* 1970) and further reducing its conversion to niacin (Czarnecki *et al*. 1983). This explains why niacin requirement of birds given 4% oil supplement was higher (40 mg/kg) than those of that given 2% oil supplement (35 mg/kg). Relatively low level of fat and high thiamine in the diet might have stimulated high feed consumption (Mateos *et*

Table 3 Weights and lipid contents of livers and kidneys from broilers fed on varying levels of thiamine (B_1), niacin (B_3) and palm oil (0)

Parameter	2% oil					4% oil					
	2.5 mgB_1		3 mgB_1		Mean1±	2.5mgB_1		3 mgB_1		Mean2±	SEM**
	35 mgB_3	40 mgB_3	35 mgB_3	40 mgB_3	SE	35 mgB_3	40 mgB_3	35 mgB_3	40 mgB_3		
Liver weight (g)	32.53^{a*}	35.10^{a}	26.65^{b}	26.80^{b}	30.27^{x}	22.75^{b}	25.35^{b}	22.79^{b}	26.83^{b}	24.43^{y}	2.046
Liver (% body weight)	2.34^{a}	2.44^{a}	1.67^{b}	1.76^{b}	2.05	1.78^{b}	1.63^{b}	1.74^{b}	1.72^{b}	1.72	0.148
Liver lipid (mg/g)	222.17^{a}	195.64^{a}	168.62^{b}	157.36^{b}	185.95^{x}	113.80^{c}	109.22^{c}	102.02^{c}	97.26^{c}	105.58^{y}	22.083
Kidney weight (g)	13.58^{a}	13.35^{a}	9.18^{b}	9.58^{b}	11.42^{x}	8.80^{b}	9.55^{b}	7.90^{b}	9.36^{b}	8.90^{y}	0.986
Kidney (% body weight)	0.98^{a}	0.93^{a}	0.58^{b}	0.63^{b}	0.78	0.69^{b}	0.61^{b}	0.60^{b}	0.60^{b}	0.63	0.075
Kidney lipid (mg/g)	170.70^{a}	159.08^{a}	118.11^{b}	114.99^{b}	140.72^{x}	86.89^{c}	90.57^{c}	89.47^{c}	92.12^{c}	89.76^{y}	15.428

*Values within a row bearing different superscripts (a-c) are significantly ($P<0.05$) different.
**SEM. Standard error of the treatment mean.
1, Average values for 2% palmoil treatment groups, 2, average values for 4% palmoil treatment groups.
±, Mean values denoted by different superscripts (x, y) for a parameter are significantly ($P<0.05$) different.

al. 1982 and Sure *et al.* 1932) and vis-à-vis nutrients and thus the birds requirement for niacin would have been met. From this the reduction in the amount of thiamine needed for optimum feed utilization due to increase in palm oil supplementation in the present study confirmed the sparing action of fat on the vitamin (Thornton and Shutze 1960).

Data for carcass weight and total edible meat expressed as per cent of carcass weight of experimental chicken (Table 2) followed similar pattern as that of feed utilization. There was no difference in the bone weights of birds fed diets supplemented with 2% oil, whereas those fed diets supplemented with 4% oil, 35 mg niacin/kg had lower weights than those given 40 mg niacin/kg diet at both levels of thiamine supplementation. Bone expressed as per cent of carcass weight was higher in birds given both supplemental levels of niacin in combination with 2.5 mg thiamine/kg in 2% oil supplemented diet. With the exception of carcass weight and bone weight, no significant ($P>0.05$) change in the mean values of other parameters for the carcass characteristics of birds was observed. Both the carcass and bone weights were higher in birds fed on 2% oil supplement. It was thus inferred that with 2% oil supplementation combination of 35 mg niacin and 3 mg thiamine/kg of diet and with 4% oil a combination of 40 mg niacin and 2.5 mg thiamine per kg diet were adequate for good carcass weight. On the other hand, for bone (% carcass weight), a combination of 35 mg niacin and 3mg thiamine/kg diet with 2% oil and 35mg niacin and 2.5 mg thiamine/kg diet with 4% oil seemed adequate.

Results showed that desirable carcass quality required more thiamine as evident by the significant dietary treatment effect on the characteristics. However, the amount needed for this purpose depended on the amount of supplemental oil in the diet, and it was lower (2.5 mg/kg) in 4% oil supplemented diet than in 2% oil diet (3mg/kg). The higher carcass and bone weights of birds fed on 2% oil supplement in this study might be due to the higher mean body weight recorded in the group (Table 2). Results on abdominal fat pad (Table 2) *per se* and expressed as per cent of carcass weight suggested that a combination of 35mg niacin and 3mg thiamine/kg of diet supplemented with 2% oil was adequate. In case of 4% oil supplemented diet, a combination of 35 mg niacin and 2.5 mg thiamine/kg was required.

Liver and kidney weights (g) and proportions of body weights (Table 3) indicated that heavier organs were found in birds fed on both supplemental niacin in combination with 2.5 mg thiamine/kg of 2% oil supplement diet than the rest of the groups. Lipid of both organs were higher in birds fed lower oil supplement (2%). The results suggested that a combination of 3 mg thiamine/kg with 35 or 40 mg niacin/kg marginally decreased the organs' lipids in birds given 2% oil supplement, whereas a combination of 2.5 mg thiamine/kg with 35 or 40 mg niacin/kg diet supplemented with 4% oil reduced lipids.

The significantly lower mean values of liver and kidney weights and lipids in groups of birds fed diets supplemented with 4% oil (Table 3) indicated reduced lipid deposition in these organs. This observation is in agreement with that of Oloyo and Ogunmodede (1992). Thiamine is involved in lipogenesis (McHenry and Cornett 1944) and coenzyme function. The process of liponeogenesis is, however, inhibited by dietary fat supplementation (Bortz *et al.* 1963) and thus spares thiamine (Thornton and Shutze 1960). This explains why lower thiamine level (2.5 mg/kg) is needed to keep at low level lipid deposition in the organs and in the abdominal region of the chicken when fed on 4% palm oil supplemented diet compared to 3 mg thiamine/kg diet required when fed 2% oil supplement.

It may be concluded from this study that level of dietary supplemental palm oil affected performance and vitamins requirements for optimum feed utilization and production of good carcass quality. While a combination of 3 mg thiamine

and 35 mg niacin/kg of diet was required with 2% palm oil supplement, a combination of 2.5 mg thiamine and 40 mg niacin/kg was needed when the diet was supplemented with 4% palm oil.

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Indian Journal of Animal Sciences 68 (11) : 1202-1204, November 1998

Genetic studies on growth, feed conversion, reproduction and production traits in a broiler dam line

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Received : 30 March 1998; Accepted : 10 May 1998

Key words : Broiler, Dam line, Feed conversion, Growth, Production traits, Reproduction traits

Growth rate, the most important trait in broilers, is highly heritable. Breeders have relied on positive genetic correlations with growth rate to improve feed efficiency during selection for high body weight. Though body weight remains the single most important criteria in the selection of broiler, but feed conversion, egg weight and egg production needs to be considered for the selection of dam line broilers.

Broiler chicken (1497) obtained from the mating of 117 sires and 630 dams in 5 hatches taken at weekly intervals from second week of May to second week of June, were maintained at the Poultry Research Centre of this university. The chicks were reared under floor brooding and provided with all standard managemental conditions and feeding as per Bharat Standard Institution Specification. The chicks were transferred to individual cages at the age of 35 days. They were given weighed amount of broiler starter mash daily and the residue was weighed at the end of 2 weeks. Birds were leg banded on age at first egg. Data were recorded on body weight at 1-day and 6-, 8-, 20- and 24- weeks and at sexual maturity (g), feed consumption (g), body weight gain (g) and feed conversion efficiency from 6 to 8 weeks of age, age at sexual maturity (days), first egg weight (g), egg weight at 32- weeks of age (g) and egg production up to 40- weeks of age (Nos). The analysis of variance and covariance were done after adjusting the data for hatch effects (Harvey 1975). The heritability, genetic and phenotypic correlations were calculated as per the methods of King and Henderson (1954) and Becker (1964) respectively.

Means of different body weights and feed conversion traits (Table 1) were well comparable to those reported by Matny (1987). The heritability of day-old weight in the present investigation was in close agreement with the findings of

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Table 1. Means and heritability estimates with standard errors of different traits in broiler dam line

Traits	Mean± SE	Heritability±SE
Body weight at 1 day (X1), g	41.52±0.92	0.42±0.09
Body weight at 6 weeks (X2), g	631.75+3.52	0.66±0.11
Body weight at 8 weeks (X3), g	1025.50±4.22	0.38±0.08
Body weight at 20 weeks (X4), g	1960.65±14.16	0.23±0.20
Body weight at 24 weeks (X5), g	2665.15±20.32	0.29±0.21
Weight at sexual maturity (X6), g	2735.52±21.35	0.12±0.14
Feed consumption 6 to 8 weeks, (X7), g	1250.90±6.62	0.15±0.06
Body weight gain 6 to 8 weeks (X8), g	394.76±2.87	0.07±0.05
Feed conversion efficiency 6 to 8 weeks (X9)	106.59±3.59	0.03±0.04
Age at sexual maturity (X10), days	190.55±4.66	0.08±0.34
First egg weight (X11), g	47.55±6.75	0.09±0.16
Egg weight at 32 weeks (X12), g	59.88±2 42	0.02±0.14
Egg production upto 40 weeks (X13), nos	45.85±7.72	0.18±0.18

Matny (1987), but lower than the reports of Reddy and Patro (1983). This may be due to that the day-old weight is influenced by several non-genetic factors such as egg size and hatchery management. Therefore, the heritability of day-old weight is likely to vary from flock to flock. The heritability of 6- and 8-weeks body weight (0.66±0.11; 0.38±0.08) indicated that there is sufficient amount of additive genetic variability in the flock which can be exploited through selection. The results of present investigation were similar to those reported by Devroy *et al.* (1983). The heritability estimates of 20- and 24- weeks body weight were medium and well comparable with the report of Kupiec *et al.* (1982).

The estimates of heritability for feed consumption, body weight gain and feed conversion efficiency from 6- to 8- week were low. These estimates were in close agreement with the results of Matny (1987) but lower than those reported by Leenstra and Pit (1988). The heritability estimates of age and

Table 2. Genetic (above the diagonal) and phenotypic (below the diagonal) correlations between different traits in broiler dam line

Traits	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13
X1	-	0.07± 0.13	-0.15± 0.15	0.65± 0.36	0.19± 0.54	-0.39± 0.60	0.05± 0.20	-0.36± 0.23	0.41± 0.32	-0.63± 0.58	-0.18± 0.46	-0.07± 0.47	-0.48± 0.92
X2	0.13**± 0.03	-	0.94**± 0.02	0.99**± 0.17	0.71*± 0.18	0.41± 0.37	0.89**± 0.04	0.55*± 0.23	0.24± 0.33	-0.51 0.45	0.09± 0.29	-0.47± 0.81	-0.36± 0.54
X3	0.10**± 0.03	0.65**± 0.02	-	0.76*± 0.18	0.59*± 0.25	0.46± 0.38	0.85**± 0.06	0.33± 0.24	-0.13± 0.39	0.18± 0.64	-0.06± 0.32	0.85**± 0.23	0.38± 0.57
X4	0.07± 0.07	0.25**± 0.07	0.22**± 0.07	-	0.99**± 0.37	0.35± 0.63	0.31± 0.88	-0.55± 0.71	0.22± 0.78	0.08± 0.97	-0.33± 0.42	-0.07± 0.89	-0.78*± 0.35
X5	0.08± 0.07	0.27**± 0.06	0.20**± 0.07	0.73**± 0.05	-	0.45± 0.52	0.54± 0.61	0.26± 0.86	-0.30± 0.66	0.97**± 0.33	-0.18± 0.42	-0.07± 0.96	-0.41± 0.69
X6	0.04± 0.07	0.14*± 0.07	0.19**± 0.07	0.34**± 0.06	0.26**± 0.07	-	0.06± 0.11	0.63 ± 0.70	-0.78*± 0.36	-0.21± 0.97	-0.22± 0.52	-0.08± 0.47	0.24± 0.93
X7	0.07*± 0.03	0.36**± 0.02	0.45**± 0.02	0.18**± 0.07	0.16*± 0.07	0.07± 0.07	-	0.66*± 0.25	0.23± 0.50	0.38± 0.36	-0.92**± 0.12	0.04± 0.96	0.74± 0.54
X8	0.01± 0.03	-0.09**± 0.03	0.70**± 0.02	0.08± 0.07	0.02± 0.07	0.13*± 0.07	0.25**± 0.03	-	-0.98**± 0.11	0.14± 0.39	-0.49± 0.59	0.04± 0.96	0.33± 0.93
X9	0.04± 0.03	0.16**± 0.03	-0.50** ± 0.02	-0.02± 0.07	0.03± 0.07	-0.08± 0.07	0.08**± 0.03	-0.81**± 0.02	-	-0.75± 0.55	-0.27± 0.56	-0.05± 0.92	-0.50± 0.60
X10	0.00	0.01± 0.07	0.00	-0.08± 0.07	-0.03± 0.07	0.17*± 0.07	0.04± 0.07	-0.01± 0.07	-0.04± 0.07	-	-0.54± 0.52	-0.04± 0.69	0.30± 0.36
X11	0.03± 0.07	0.09± 0.07	0.10± 0.07	0.01± 0.07	-0.04± 0.07	0.10± 0.07	0.03± 0.07	0.06± 0.07	0.01± 0.07	-0.05± 0.07	-	0.14± 0.51	0.67± 0.43
X12	0.13*± 0.0	0.02± 0.07	0.02± 0.07	-0.05± 0.07	0.02± 0.07	-0.02± 0.07	-0.03± 0.07	0.01± 0.07	0.01± 0.07	0.04± 0.07	0.20**± 0.07	-	0.10± 0.93
X13	0.00	-0.12± 0.07	-0.10± 0.07	0.02± 0.07	0.08± 0.07	0.06± 0.07	0.07± 0.07	-0.02± 0.07	0.04± 0.07	0.08± 0.07	-0.05± 0.07	0.02± 0.07	-

* P< 0.05 and ** P< 0.01.

weight at sexual maturity were also low and similar to the findings of Aswathanarayana (1978).

The phenotypic correlations between 1- day and 6- and 8-week-body weight were low but significant (Table 2). The positive and significant genetic and phenotypic correlations of 6- week with 8-, 20- and 24- week body weight suggested that these traits are governed by similar set of genes. Improvement in 6- week body weight will also increase the body weight at 8-, 20-, and 24- weeks. Devroy *et al.* (1983) and Matny (1987) also reported similar results. The genetic and phenotypic correlations of 6- and 8- weeks body weight with feed consumption, body weight gain were also positive and significant and in close agreement with the results of Marahrens and Flock (1980) and Matny (1987).

The phenotypic correlation of 8- week body weight with feed conversion efficiency during 6- to 8- weeks was negative and significant. This suggested that better feed conversion efficiency during this period leads to increase in 8- week body weight. These results are well comparable with the report of Matny (1987). Leenstra and Pit (1988) also reported negative phenotypic correlation between 7- week body weight and feed conversion efficiency from 3- to 6- weeks of age.

The genetic and phenotypic correlations of 1 day and 6-week body weight with other production traits were mostly nonsignificant. The genetic correlation between 8-week-body weight and egg weight at 32- week was significant. The phenotypic correlation between 8- week body weight and body weight at sexual maturity was also significant (Table 2). These correlations suggested that birds with higher weight at an early age have better reproduction and production performance. Similar observations were made by Merritt (1968).

The positive genetic and phenotypic correlations between 20- and 24- week-body weight with body weight at sexual maturity confirmed the findings of Merritt (1968) and Rabsztyn (1979). The genetic correlations of feed consumption and feed conversion efficiency with other traits had high standard error. The phenotypic correlation of age at sexual maturity with body weight at sexual maturity was positive and significant. It suggested that late maturing birds attain higher weight. Similar observations were reported by Johari *et al.* (1987).

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Indian Journal of Animal Sciences 68 (11) : 1205-1206, November 1998

Effect of body condition class on important physical and production parameters of crossbred dairy cattle*

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Received : 10 September 1997; Accepted : 25 May 1998

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The relationship between body condition score and important physical and production parameters in crossbred cows, was studied. Crossbred cows (294) belonging to Karan Swiss and Karan Fries breeds in different stages of lactation and dry period of the institute herd were condition scored on a 6-point scale (Prasad 1994). All the animals were scored for the body condition twice within a week by the same scorer and the average condition score of these 2 measurements was used for comparison. Body condition scores were categorized in 3 classes, viz. low (BC score<3.0), medium (BC score>3.0<4.5) and high (BC score>4.5). The body condition class was then related to the physical parameters, viz. body weight (BW), heart girth (HG), abdominal girth (AG), tail thickness at base (TTH) and skin-fold thicknesses over tail head (STTH), pin (STP), back (STB) and rib (STR) regions as also the production traits, viz. yield of 4% FCM (FCMY), fat (FY), solids-not-fat (SNFY), protein (PRY), calories/ kg milk (Cal/kg), peak milk yield (PKY), milk yield obtained over the first 90 days (90d MY) and first 180 days of lactation period (180d MY). For taking the body weight, the animals were weighed on a standard weighing bridge. Body measurements were taken using a metallic measuring tape. Skin-fold thickness measurements were taken using caliper. Fat and SNF in the

Table 1 Body condition class wise least squares means (±SE) of physical and production parameters

Parameters	Body condition class			
	Low (BC score<3.0)	Medium (BCscore>3.0<4.5)	High (BC score>4.5)	Overall
Physical parameters				
Body condition score	2.70 ± 0.11^a	3.98 ± 0.03^b	5.11 ± 0.05^c	3.93 ± 0.04^b
Body weight (kg)	340.13 ± 15.92^a	396.95 ± 4.23^b	455.57 ± 6.32^c	397.55 ± 6.03^b
Heart girth (cm)	158.84 ± 3.53^a	167.19 ± 1.01^b	176.15 ± 1.50^c	167.39 ± 1.35^b
Abdominal girth (cm)	171.06 ± 4.20^a	181.66 ± 1.21^b	191.50 ± 1.79^c	181.41 ± 1.60^b
Tail thickness (cm)	20.44 ± 0.41^a	21.54 ± 0.12^b	22.81 ± 0.17^c	21.60 ± 0.16^b
Skin thickness over tail head (cm)	1.213 ± 0.51^a	1.683 ± 0.16^b	2.543 ± 0.11^c	1.811 ± 0.06^b
Skin thickness over pins (cm)	1.022 ± 0.10^a	1.270 ± 0.29^b	1.692 ± 0.04^c	1.810 ± 0.06^b
Skin thickness over ribs (cm)	1.071 ± 0.06^a	1.142 ± 0.02^{ab}	1.571 ± 0.03^b	1.354 ± 0.11^c
Skin thickness over back (cm)	1.122 ± 0.07^a	1.231 ± 0.02^{ac}	1.431 ± 0.03^b	1.26 ± 0.03^c
Production traits				
FCM yield	9.68±0.95	9.92±0.25	8.89±0.43	9.46±0.36
Fat yield	0.41±0.04	0.41±0.01	0.36±0.02	0.39±0.02
SNF yield	0.79 ± 0.09^{ac}	0.81 ± 0.02^a	0.75 ± 0.04^{bc}	0.79 ± 0.30^c
Protein yield	0.21±0.04	0.19±0.01	0.19±0.02	0.20±0.16
Calories/kg milk	749.8±52	740.8±13.3	713.4±22.4	738.0±19.5
Peak milk yield	14.03±1.12	15.55±0.34	14.84±0.51	14.77±0.49
First 90d yield	878.0±99.1	1057.25±26.	1068.8±39.4	1001.4±37.7
First 180d yield	1785.7±201.0	2026.6±51.4	2031.9±78 5	1948.1±17.6

*Parameter means bearing common or no superscripts do not differ significantly.

*Part of the Ph.D. Thesis of the first author.

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milk were estimated using milk tester and lactometer, using standard procedure. Energy content of milk was estimated as per Tyrell and Reid (1965).

Data were analyzed by least-squares analysis of variance procedure (Harvey 1976). Data were also subjected to regression analysis using simple linear regression procedure.

As the body condition of the animal improved from low to medium and high, different physical parameters also tended to increase (Table 1). There existed significant variation between classes for BC score, BW, HG, AG, TTH, STTH, STP, STR and STB. There were significant coefficients of correlation between BC score and all the other physical parameters. The values of the correlations were high (0.71 and 0.67) in STTH and STP measurements, respectively, and were followed by STB (0.48), TTH (0.48), STR (0.38), AG (0.30) and BW (0.29). Regression analysis indicated that every one point change in the BC score was associated with corresponding change of 32.44 kg in BW. The corresponding change in other physical parameters was 4.84, 2.16, 0.98, 0.60, 0.41, 0.13 and 0.21 cm for HG, AG, TTH, STTH, STP, STR and STB respectively. The accuracy of prediction, however, was low and ranged from only 4% in case of BW to 50% in case of STTH measurement. Similar results were reported by Wildman *et al.* (1982) and Nicholson and Little (1989) in *Bos taurus* and African zebu cattle respectively.

The comparison of body condition class-wise least square means of different production parameters revealed that the current level of milk production was more, albeit nonsignificantly, in the animals having low and medium body conditions than those having high body condition (Table 1). Yields of fat and SNF also followed the pattern observed in FCM. However, the animals having low body condition were at a disadvantage in the long run *vis a vis* their medium and high yielding counterparts as indicated by their lower 90d and 180d milk yields and the difference again was not significant.

The BC score was negatively correlated (r= -0.20 to - 0.38) with the traits of current milk production (FCM, fat, SNF and protein yields), whereas Cal/kg, PKY, 90d MY and 180d MY had positive association with BC score. The magnitude of the relationship, however, was low (r= 0.20-0.24). Body condition of the animals had nonsignificant regression on the production parameters. Every 1 point increase in BC score was associated with a corresponding decrease in 1.16 kg of FCM/ day. Near similar trend was also observed in case of yields of milk fat, SNF and protein also. On the contrary, 1 point increase in BC score was associated with 72.88 and 144.26 kg increase in first 90-day milk yield and first 180-day milk yields respectively. The accuracy of prediction was, however, very small signifying that the BC score alone may not be a satisfactory measure for predicting the production performance of the crossbred dairy cows. Near similar relationship between body condition and milk yield of dairy cows was reported by Frood and Croxton (1978).

The results of the study indicate towards the superiority of animals having medium body condition over those having low or high body conditions. It may be concluded that the yield of milk and milk constituents improve with the increase in body condition up to BC score of 4.5 beyond which there may not be any added advantage in feeding extra to further increase the body condition.

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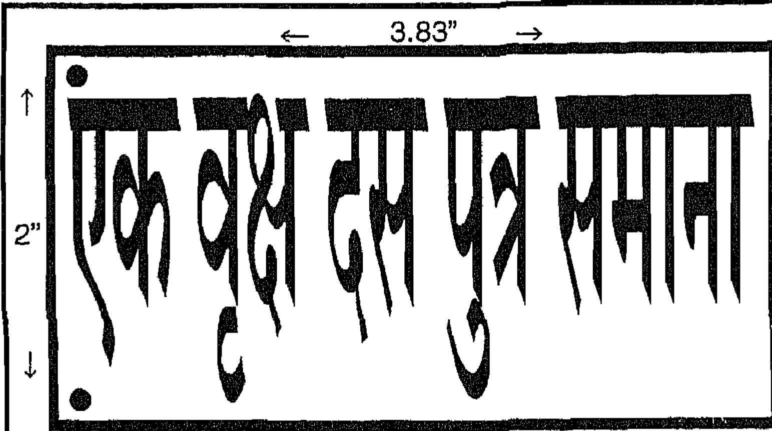

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