ICAR PUBLICATIONS

D. K. M. A.

636 (05) CODEN : IJLAA4 **70** (4) : 335-440 (2000) II ISSN 0367-8318

The Indian Journal of Animal Sciences

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THE INDIAN JOURNAL OF
ANIMAL SCIENCES
Previous Issue : Vol. 70, No. 3, pp. 223-334

Vol. 70, No. 4 April 2000

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Indian Journal of Animal Sciences **70** (4): 337-339, April 2000

Humoral and cell-mediated immune responses of lambs against bluetongue virus

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Received: 17 February 1999; Accepted: 7 October 1999

ABSTRACT

The bluetongue virus (BLU-1) was isolated from field outbreaks in Maharashtra. The virus was adopted on BHK-21 cell lines. Humoral immune responses of lambs were evaluated by using competitive enzyme linked immunosorbent assay (cELISA) and complement fixation test (CFT). The cell-mediated immune response (CMI) was evaluated by using leucocyte migration inhibition test (LMIT). The cell culture adopted BLU-1 virus was a strong immunogen. The antibody to BLU-1 was produced on seventh day post-inoculation which persisted at high level after challenge infection. The CMI was developed from seventh day post-inoculation and persisted at high level after challenge infection.

Key words: BLU-1, Bluetongue virus type 1, Competitive ELISA, Complement fixation test, Cell-mediated immunity, Leucocytes migration inhibition test

While bluetongue (BLU) viruses can infect most ruminants, BLU disease primarily affects sheep. Attenuated BLU virus vaccines are being used but the efficacy and protective ability of the vaccine needs to be properly evaluated. The development of humoral immunity in BLU virus infected sheep was reported earlier (Jochim 1985, Stott and Osburn 1990). The possible role of cell-mediated immune (CMI) response in protecting the sheep from BLU virus has been suggested by Stott *et al.* (1985) following immunization with an inactivated virus preparation.

Present study was planned to evaluate the development of humoral and CMI responses against cell culture adopted attenuated BLU-1 virus, and to find out the efficacy of the experimental cell culture adopted bluetongue vaccine in protecting the lambs against challenge infection.

MATERIALS AND METHODS

Lambs of Osmanabadi breed 8-10 month age were used in present experiment. The bluetongue virus was isolated from the field outbreaks in sheep from the Maharashtra. The virus was isolated, adopted, and maintained on BHK-21 cell lines (10th passage). The virus was serotyped as BLU-1 from the Australian Animal Health Laboratory (AAHL), Geelong, Australia.

The bluetongue antigen was prepared by using BLU-1 serotype on the lines of Jochim and Chow (1969). The antigen was confirmed by using reference antibody supplied by Dr Jochim, USA. The method of Mehrotra (1991) was used for raising BLU antibodies in sheep. The antiserum was tested against reference antigen supplied by Dr Jochim, USA.

Competitive enzyme-linked immunosorbent assay (cELISA) was standardized and performed on the lines of Afshar *et al.* (1989) as prescribed by Veterinary Diagnostic Technology, Inc., USA. The reagents of cELISA supplied by Veterinary Diagnostic Technology, Inc., USA, were used in the experiment. The per cent inhibition (PI) of BLU monoclonal antibodies were calculated by using following formula:

$$PI\ (\%) = \frac{\text{Adjusted average OD test serum sample}}{\text{Adjusted average OD negative serum}} \times 100$$

Complement fixation test (CFT) was performed as per the method of Boulanger *et al.* (1967) with slight modifications. The test was performed as micro CFT.

Leucocyte migration inhibition test (LMIT) (McCoy *et al.* 1967) was used to measure *in vitro* migration of leucocytes. The migration indices (MI) of the leucocytes were calculated as per following formula:

$$MI = \frac{\text{Average area of spread of leucocytes in presence of antigen}}{\text{Average area of spread of leucocytes in absence of antigen}}$$

An experiment was planned with 16 lambs (Table 1) of either sex, aged 8-10 months. All these lambs were sero-negative for BLU antibodies and apparently healthy. The lambs were housed separately group-wise with separate feeding and maintenance management. The lambs were housed in insect proof building. The heparinized blood samples were collected for the LMIT whereas the serum

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Table 1. Details of lambs used in the experiment

Group	No. of lambs	Details of infection	Dose and route	Days of observation
Lamb used for BLU-1 passage	02	Infected with BLU-1 (blood) on 0 day*	5 ml whole blood intravenously**	0 – 15 days
Healthy control lambs (HC group)	06	Kept as healthy throughout the experiment	–	0, 7, 14, 21, 28, 35, 42 and 49th day
Lambs inoculated with cell culture adopted BLU-1 (TV group)	06	(a) Inoculated on '0' day with cell culture adopted BLU-1	5 ml (each containing $10^4 CCID_{50}$ BLU-1 subcutaneously)	0, 7, 14 and 21st day
		(b) Challenged on 21st day with blood containing virulent BLU-1 of lambs used for passage	5ml whole blood intravenously	28, 35, 42 and 49th day
Infection control lambs	02	Infected with BLU-1 (blood) on 21st day	5 ml whole blood intravenously	0-20 days (kept healthy), from 21st day kept with TV group

*'0' day, day of starting the experiment; **the blood was collected at the height of thermal reaction from lambs infected with virulent BLU virus.

samples were collected for the cELISA and CFT at weekly intervals of study.

The BLU virus was passage in 2 lambs by inoculating 5 ml whole blood with BLU-1 virulent virus on the '0' day of the experiment. The thermal reaction of the lambs was recorded in addition to clinical observations. The blood of these lambs was collected at the height of temperature. Presence of BLU virus was confirmed by isolation and identification. The blood drawn from these lambs was used for challenge infection to TV group of lambs on 21st day post-inoculation and infection of 2 control lambs.

The mean PI of BLU monoclonal antibodies were recorded by employing cELISA. Mean CF antibody titers were recorded by CFT whereas mean migration indices of leucocytes were recorded by LMIT. The observations were recorded at weekly intervals. The data were analyzed for determining levels of significance by student - t test.

RESULTS AND DISCUSSION

Humoral immune response

The humoral immune response of lambs against BLU-1 was evaluated by using cELISA and micro CFT.

The test serum sample was considered positive for BLU antibodies in cELISA, if the PI was equal to or greater than 50%. The serum samples with PI less than 50% were considered negative. The PI values of TV group lambs were above 50% from 7th to 49th day post-inoculation. The specific antibody was detected from seventh day post-inoculation. The PI values reached (Table 2) to 91.23% on seventh day post-challenge with cell-culture adopted attenuated BLU-1 virus (tenth passage). The increased PI persisted till the end of the experiment. The results of cELISA clearly pointed out that the lambs inoculated with attenuated BLU-1 virus developed specific humoral immune response against BLU-1 virus. The level of immunity was sufficiently high to protect the lambs from virulent virus which was evident from the results of challenge infection of these lambs. The results are in agreement with Neitz (1948) who also observed the protective role of antibody in BLU-1 challenged lambs.

The CF antibodies were detected from seventh day post-inoculation. The antibody titers increased subsequently reaching peak on 21st day post-inoculation. The lambs were challenged with BLU-1 virus on 21st day. The CF antibody titers declined to 106.00±16.93 on seventh day post-challenge. The titers again increased up to 226.00±32.13 till the end of the experiment. Osburn (1991) could detect the BLU antibodies by cELISA and CFT within first or second week following infection. The CF antibody titers decreased might be due to the neutralization of the challenge virus by existing antibodies whereas rise in titers one week post-challenge indicated strong immunogenicity of the virus. Clinically the TV group of lambs were healthy due to the protective immunity of lambs acquired from exposure to cell culture adopted attenuated BLU-1 virus. All the lambs previously inoculated with attenuated BLU virus and later challenged with virulent BLU virus did not suffer the BLU disease and also showed very high levels of BLU antibodies. Infection control lambs showed the typical clinical picture of BLU, the symptoms were hyperaemia, cyanosis of buccal mucous, erosions of mucous membranes of buccal cavity and nasal cavity were noticed with thermal reaction. These lambs were sacrificed for postmortem examination.

Cell-mediated immune response

The CMI response against BLU-1 virus was evaluated *in vitro* by LMIT. The HC group of lambs showed average MI in the range of 1.00 to 1.20. Comparison of MI of TV group with that of HC group indicated that the mean MI started decreasing significantly from seventh day (0.83±0.03) post-inoculation. In TV group of lambs, the MI further decreased

Table 2. Details of results of humoral and cell-mediated immune responses

Day	PI values (%)		CF titers ± SE		MMI ± SE	
	HC group	TV group	HC group	TV group	HC group	TV group
0*	25.00	25.52	0.00 ± 0.00	0.00 ± 0.00	1.20 ± 0.10	1.12[a] ± 0.00
7	20.25	88.54	0.00 ± 0.00	20.00[a] ± 4.48	1.12 ± 0.05	0.83[a] ± 0.02
14	18.00	85.84	0.00 ± 0.00	30.00[a] ± 4.48	1.04 ± 0.06	0.74[a] ± 0.05
21**	17.75	87.22	0.00 ± 0.00	120.00[a] ± 17.95	1.00 ± 0.10	0.69[a] ± 0.03
28	22.45	91.23	0.00 ± 0.00	106.00[a] ± 16.93	1.04 ± 0.04	0.31[a] ± 0.14
35	30.25	91.10	0.00 ± 0.00	120.00[a] ± 23.25	1.04 ± 0.04	0.13[a] ± 0.08
42	35.25	79.35	0.00 ± 0.00	213.30[a] ± 23.79	1.20 ± 0.14	0.47[a] ± 0.12
49	31.75	83.57	0.00 ± 0.00	226.00[a] ± 32.13	1.08 ± 0.05	0.50[a] ± 0.03

*Day of inoculation of BLU-1 group, ** day of challenge in TV group, nonsignificant; a, $P < 0.01$.

after challenge infection for 1 week. The inhibition of leucocyte migration in TV group was maximum (0.13±0.08) on 14th day post-challenge. The inhibition of migration of leucocytes was observed till the end of the experiment. It is evident from these observations that CMI developed in lambs following cell culture adopted attenuated BLU-1 virus inoculation. The CMI response was observed from 7th to 14th day post-challenge suggesting probable role of CMI in protecting the lambs against BLU. The role of CMI in protective immunity was demonstrated by Stott *et al.* (1985) in sheep following immunization with an inactivated virus preparation. The development of blastogenic response to BLU virus antigen in a lymphocyte-stimulation assay was associated with protection.

ACKNOWLEDGEMENTS

The authors thank the Indian Council of Agricultural Research (ICAR), New Delhi, for providing financial assistance to the ad-hoc project. Thanks are also due to Dr Bryan Eaton, AAHL, CSIRO Geelong, Australia, for serotyping of BLU virus. The authors are grateful to Dr Jochim, USA for providing reference BLU antigen and antibodies.

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Indian Journal of Animal Sciences **70** (4): 340-342, April 2000

Humoral immune response in broilers against inclusion body hepatitis virus*

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Received: 17 February 1999; Accepted: 7 October 1999

ABSTRACT

Humoral immune response was studied in broilers against inclusion body hepatitis (IBH) inactivated oil adjuvanted vaccine, and virulent PDRC-1 strain of the IBH virus by using agar gel immunodiffusion (AGID) and enzyme linked immunosorbent assay (ELISA). IBH antibody was detected from seventh onwards in IBH vaccinated and infected groups of chickens by both the tests. Infected and challenged groups exhibited strong immune response when compared with the vaccinated group. The immunosuppressed group of chickens infected with IBH virus showed poor development of IBH antibody.

Key words: Agar gel immunodiffusion, Enzyme-linked immunosorbent assay, Inclusion body hepatitis, Virus neutralization

The neutralising antibodies developed in IBH infected chickens have protective value (Saifudding and Wilks 1991). An ELISA is as sensitive as virus neutralization (VN) test to detect protective antibody (Dawson *et al.* 1980).

The present study was therefore planned to evaluate development of humoral immune response of chickens against IBH vaccination, infection and also to find out the effect of immuno-suppression on immune responses of chickens against IBH virus.

MATERIALS AND METHODS

Day-old broiler chicks were used. The reference IBH antigen and antiserum was obtained from Professor Colin R Wilks, Massey University, New Zealand. The PDRC-1 strain of IBH virus and experimental inactivated oil adjuvant IBH vaccine were obtained from Poultry Diagnostic and Research Center, Pune. The cyclophosphamide IP was used for immuno-suppression. The anti-chicken IgG HRPO conjugate was used for ELISA. The IBH antigen was prepared as McFerran (1980).

Antiserum to IBH virus was raised in rabbits. The rabbit was given 1ml of IBH protein antigen (1 000 µg/ml) mixed and emulsified with 1 ml of Freund's incomplete adjuvant in interdigital space. The second injection of the 1 ml protein antigen (1 000 µg/ml) with 1 ml Freund's complete adjuvant was administered on 14th day after injection intramuscularly. Fourteen days after second injection 1 ml (100 µg/ml) IBH protein antigen was injected intravenously. The test bleeding was done 7 days after last injection to confirm production of IBH antiserum. The AGID test was standardized as described by Cruickshank *et al.* (1975). An indirect ELISA was used on the lines of Piela and Yates (1983). The experiment was planned as detailed in Table 1. The evaluation of results was done on the basis of statistical analysis (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

Precipitating antibodies to IBH antigen were detected in all serum samples from seventh day post-vaccination or infection (Table 2). The antibodies persisted for longer duration in all the sera of IBH infected group (I group) when compared with IBH vaccinated group (V group), suggesting that the immunogenicity of virulent virus was superior to vaccine virus.

Immunosuppresive effect of cyclophosphamide was distinctly noticed. The development of precipitating antibody was transient in Cy/I group. The findings confirm the importance of immuno-suppression stress in the pathogenesis of IBH virus infection (Rosenberger *et al.* 1975, Fadly *et al.* 1980).

ELISA was more sensitive to determine the IBH antibody titers in the test sera (Table 3). The antibodies were noticed from seventh day post-vaccination or infection and reached peak by 21st day. These observations corroborate the earlier findings of Singh and Oberoi (1994).

Part of Ph.D. Thesis submitted to the Marathwada Agricultural University,Parbhani.

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Table 1. Details of the groups of chicks used in the experiment

Group	Total No. of chicks	Details of inoculation post-inoculation (DPI)	Day of inoculation/ (days)	Duration of study samples collected	No.of serum samples collected	No. of heparinized blood
HC	20	NIL	Uninoculated control	0 – 63	200	200
V	20	IBH killed vaccine, 0.5ml, s/c per chick	0	0 – 63	200	200
VI	20	(i) IBH killed vaccine, 0.5 ml, s/c per chick	0	0 – 63	200	200
		(ii) IBH virulent virus, 100 ELD 50 orally per chick	42 DPI			
		(iii) IBH virulent virus, 100 ELD 50 orally per chick	42 DPI			
V2	20	(i) IBH killed vaccine, 0.5ml, s/c per chick	42 DPI	0–63	200	200
		(ii) IBH virulent virus, 100 ELD 50 orally per chick	42 DPI			
Cy/I	20	(i) Cyclophosphamide, 4mg per chick, i/m (ii) 3 days prior to infection (iii) IBH virulent virus, 100 ELD 50 orally per chick				
I	20	IBH Virulent virus, 100 ELD 50 orally per chick	0	0–63	200	200
Total					1200	1200

0 day means seventh day of age.

Table 2. Detection of IBH precipitating antibodies in different groups

Days post vaccination/ infection	Groups					
	No. of positive samples/No. of samples tested					
	HC	V	V1	V2	Cy/I	I
0*	0/20 (0.00)	0/20 (0.00)	0/20 (0.00)	0/20 (0.00)	0/20 (0.00)	0/20 (0.00)
7	0/20 (0.00)	20/20 (100.00)	20/20 (100.00)	20/20 (100.00)	09/20 (45.00)	20/20 (100.00)
14	0/20 (0.00)	20/20 (100.00)	20/20 (100.00)	20/20 (100.00)	10/20 (50.00)	20/20 (100.00)
21**	0/20 (0.00)	20/20 (100.00)	20/20 (100.00)	20/20 (100.00)	10/20 (100.00)	20/20 (100.00)
28	0/20 (0.00)	17/20 (85.00)	20/20 (100.00)	17/20 (85.00)	10/20 (50.00)	20/20 (100.00)
35	0/20 (0.00)	17/20 (85.00)	20/20 (100.00)	17/20 (85.00)	10/20 (50.00)	20/20 (100.00)
42***	0/20 (0.00)	15/20 (75.00)	20/20 (100.00)	16/20 (80.00)	10/20 (50.00)	17/20 (85.00)
49	0/20 (0.00)	13/20 (65.00)	20/20 (100.00)	20/20 (100.00)	10/20 (50.00)	14/20 (70.00)
56	0/20 (0.00)	10/20 (50.00)	20/20 (100.00)	20/20 (100.00)	10/20 (50.00)	14/20 (70.00)
63	0/20 (0.00)	10/20 (50.00)	20/20 (100.00)	20/20 (100.00)	7/20 (35.00)	14/20 (70.00)

0 day means seventh day of age; * day of vaccination/infection to all groups except HC group; ** day of challenge to V1 group with virulent IBH virus;
*** day of challenge to V1 and V2 groups with virulent IBH virus; figures in parenthesis indicate percentage of positive serum samples.

The challenge to vaccinated chickens caused remarkable drop of ELISA titers for seventh day post-challenge followed by a sharp rise in antibody till the end of experiment. A drop in titres clearly indicate involvement of vaccinal antibodies in interaction with virulent virus. An anamnestic response to precipitating antibodies was also similar. Fadly and Winterfield (1975) demonstrated the specific lines of precipitation with sera from chickens previously vaccinated and challenged with IBH virus.

The observations of this study revealed that in spite of comparatively lower titres of antibodies in vaccinated group, the vaccine virus was effective and potential immunogen to stimulate antibody response so as to protect chickens from clinical disease of IBH. All vaccinated and challenged groups of chickens were healthy. Pathogenic effects of IBH virus were observed in I group of chicks in addition to immunogenicity of the virus. Usually, immune system recognizes the immunogenicity but never the pathogenicity.

Antibody response estimated by ELISA revealed that the chicks inoculated with cyclophosphamide and infected with IBH virus had very low titers as compared to IBH infected group at each interval of study from third week post-infection. These observations clearly pointed out the reduced immunological competence of chicks due to immunosuppression caused by cyclophosphamide. The immunosuppressive effect of cyclophosphamide on both humoral and cell-mediated immunity is well known (Rouse and Szenberg 1974). The importance of immunosuppression stress is well documented (Fadly *et al.* 1976). The low IBH antibody titers in immunosuppressed chickens provide an

Table 3. Mean ELISA titers of different groups

Days post-vaccination infection	Mean titers ± SE					
	Groups					
	HC	V	V1	V2	Cy/I	I
	(N=20)	(N=20)	(N = 20)	(N=20)	(N =20)	(N=20)
0*	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
7	0.00 ± 0.00	20.50 ± 02.11	16.00 ± 01.68	15.00 ± 01.14	20.00 ± 01.77	22.00 ± 01.86
14	0.00 ± 0.00	41.00 ± 04.22	36.00 ± 04.00	40.00 ± 04.35	64.50 ± 05.05	60.00 ± 04.58
21**	0.00 ± 0.00	288.00 ± 37.80	230.00 ± 31.25	200.00 ± 21.33	180.50 ± 20.61	324.00 ± 35.06
28	0.00 ± 0.00	136.00 ± 13.11	184.00 ± 27.90	164.00 ± 16.90	127.00 ± 17.79	192.00 ± 17.80
35	0.00 ± 0.00	110.00 ± 10.81	248.00 ± 18.26	104.00 ± 14.91	94.00 ± 10.57	140.00 ± 16.29
42***	0.00 ± 0.00	66.00 ± 04.37	344.00 ± 37.22	76.00 ± 09.13	43.50 ± 05.29	88.00 ± 10.30
49	0.00 ± 0.00	36.00 ± 03.11	160.00 ± 17.41	62.00 ± 10.75	25.50 ± 02.23	56.00 ± 04.49
56	0.00 ± 0.00	21.00 ± 02.39	236.00 ± 28.13	152.00 ± 21.45	14.50 ± 01.14	37.00 ± 03.91
63	0.00 ± 0.00	14.00 ± 01.12	248.00 ± 30.66	196.00 ± 22.09	12.50 ± 00.99	25.00 ± 02.35

0 day means seventh day of age, * day of vaccination/infection to all groups except HC group, ** day of challenge to V1 group with virulent IBH virus, *** day of challenge to V1 and V2 group with virulent IBH virus N indicates No. of chickens used in a group.

evidence to the fact that the susceptibility of chickens to IBH infection was greatly enhanced.

ACKNOWLEDGEMENTS

The authors are grateful to the management of the Venkateshwara Hatcheries Ltd, Pune, for giving materialistic support to the study, and Associate Dean and Principal of the institute for providing necessary facilities for the present study.

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Indian Journal of Animal Sciences 70 (4): 343-345, April 2000

Pathogenicity of chicken and pigeon isolates of Newcastle disease virus

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Received: 28 December 1998; Accepted: 22 October 1999

ABSTRACT

Pathogenicity of chicken and pigeon isolates of Newcastle disease virus was studied in 3-week-old chickens. Clinical signs following inoculation of chicken isolate by oronasal route were observed after an incubation period of 4-5 days that included dullness, depression, dyspnoea, diarrhoea and leg paralysis with mortality of 60%. Pigeon isolate infected group exhibited very mild clinical signs with mortality of 6%. Postmortem examination of birds infected with chicken isolate revealed haemorrhages at the tip of the glands of proventriculus and caecal tonsil, while in pigeon isolate group, no gross changes were observed. However, histopathological changes were seen invariably in bursa of fabricius, spleen, caecal tonsil and trachea of both the groups. Virus was also isolated from various organs of both the groups from 5-10 days post infection.

Key words: Chicken, Newcastle disease virus, Pathogenicity, Pigeon

Newcastle disease virus (NDV), the prototype of the paramyxovirus (PMV), is the most important avian pathogen throughout the world.The virus is known to infect not only chicken but also free flying birds like pigeon which because of close contact with poultry offer ample opportunities for disease transmission. The virus isolated from pigeon in various parts of world has been characterized as PMV-1 (Alexander *et al.* 1984, 1986) and these isolates were found antigenically similar but not identical to NDV.

The pathogenicity of NDV strains vary greatly with the host. NDV strains isolated from pigeons were highly specific for pigeons and were non-pathogenic to chickens (Biancifiori and Fioroni 1983, Viane *et al.* 1983). On the other hand, there were reports of disease and death in chickens experimentally infected with pigeon isolate (Alexander and Parsons 1984, Sulochana and Mathew 1991). Therefore, the present experiment was undertaken to study the pathogenicity of chicken and pigeon isolates of NDV in 3-week-old non-immune chickens.

MATERIALS AND METHODS

The virus isolates used in this study were procured from Division of Avian Diseases of the Institute. The chicken and pigeon isolates were named as CNDV and PNDV throughout the study. The CNDV was isolated from an outbreak of Newcastle disease (ND) in chicken. The PNDV was recovered from the pigeon showing torticollis of neck and paralysis of legs and wings. The virus isolates were serially passaged in embryonated chicken eggs (ECE) by terminal dilution of positive allantoic fluid and finally the virus at fifth passage level was preserved in small aliquots at –20°C for further use.

Embryonated chicken eggs (ECE)

Embryonated chicken eggs were obtained from Hatchery Unit of Central Avian Research Institute (CARI), Izatnagar, and incubated (till 11th day) until use in the experiment.

Chicks

Day-old chicks were procured from the CARI and maintained in the Division of Avian Diseases, until use for experimental purpose.

Experimental design

White Leghorn chickens (80) negative for ND-HI antibodies were divided into 3 groups keeping 30 birds each in 2 groups and 20 birds in third group as control. Chickens of group 1 were inoculated with CNDV (0.1 ml, 10^6EID_{50}) and group 2 with PNDV (0.1 ml, 10^6EID_{50}) isolates oronasally. Birds of each group were kept in isolation units and observed for development of clinical signs. From each group 3 birds were sacrificed at 3, 5, 10, 15, 21 and 28 days post-infection (DPI) and were examined for gross lesions. Materials were collected for histopathological examination and virus isolation

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from brain, trachea, lung, liver, kidney, bursa of fabricius, caecal tonsil, proventriculus and spleen.

Virus isolation

A 20% suspension of the tissue was clarified at 3 000 rpm for 10 min and the supernatant was treated with penicillin (1 000 IU/ml) and streptomycin (2 mg/ml) and tested for sterility on blood agar. For each sample, three 11-day-old ECE were inoculated and 3 embryos inoculated with NSS were kept as control. Embryos dying after 24 hr and those surviving 5 days post inoculation were chilled at 4°C for 4 hr and then AAF was tested for presence of NDV by spot HA test.

Histopathological examination

The formalin fixed tissues were washed in running tap water and dehydrated in ascending grades of alcohol. The processed tissues were embedded in paraffin and were sectioned at 4-5 µm thickness and stained with H & E stain as routine.

RESULTS AND DISCUSSION

Pathogenicity of 2 Newcastle disease virus isolates from chicken and pigeon was studied in 3-week-old chickens. Following inoculation of CNDV, clinical signs like dullness, depression, off-feed and severe dyspnoea were observed 3 DPI. Some birds developed leg paralysis 4-5 DPI followed by greenish white diarrhoea in all the birds by 6 DPI. Mortality started on 5 DPI (Table 1) and maximum mortality occurred at 8 DPI. After that 4 birds survived and they were apparently normal without showing any clinical signs. Overall mortality became 60% in this group. Similar findings have also been observed in chickens infected with NDV (Allan *et al.* 1978). Hanson (1978) reported that velogenic viruses caused an illness of short duration and mortality of 90% or higher.

Chickens infected with PNDV did not exhibit any pronounced clinical symptoms except dullness, depression and anorexia with 6% mortality (Table 1). Lancaster (1981) reported that mesogenic viruses spread rapidly within a flock producing respiratory and nervous symptoms and mortality up to 50%. Strains of NDV highly specific to pigeons have also been reported by Viane *et al.* (1983) and Pearson *et al.* (1987). However, some workers had described disease and death in experimentally infected chickens (Sulochana and Mathew 1991).

Macroscopic examination of dead and sacrificed birds of CNDV group revealed haemorrhages at the tip of the glands of proventriculus and caecal tonsil. The involvement of gastrointestinal tract by this virus confirmed its viscerotropism. No significant macroscopic lesion was observed in PNDV group. Alexander (1991) reported that gross lesions of the disease is directly related to the intensity of clinical signs.

In the birds of group 1, microscopic changes in bursa of fabricius observed from 3 DPI included depletion and degeneration of lymphocytes in intrafollicular area and hyperplasia of lining epithelial cells in the trachea. Spleen revealed reticuloendothelial cell hyperplasia, depletion and degeneration of lymphoid cells. The changes in the brain noticed from 7 DPI comprised neuronophagia, endotheliosis, mild perivascular cuffing and focal glial cell accumulation in cerebrum. Degeneration and necrosis of lymphocytes were the main changes in the lamina propria of caecal tonsils noticed from 7 DPI.

Similar type of histopathological changes in various organs of chickens infected with NDV have been reported by various workers (Beard and Easterday 1967, Cheville *et al.* 1972). Hanson (1978) reported that abnormalities in CNS were the most significant feature of ND and he found neuronal degeneration, glial foci, perivascular lymphocytic infiltration and hypertrophy of endothelial cells in the brain.

In PNDV group, the changes in bursa of fabricius, caecal tonsil and spleen were detected from 3 DPI and mainly consisted of depletion, degeneration and necrosis of lymphoid tissues. In trachea, the changes were noticed only at 10 DPI that included hyperplasia of epithelial cells. Histopathological changes in all the organs were comparable to CNDV group.

In CNDV group, at 3 DPI, virus was recovered from all the organs except brain and proventriculus, whereas from 5-10 DPI, virus was isolated from all the organs tested. It is in

Table 1. Morbidity and mortality pattern of 3-week-old chickens following inoculation with CNDV and PNDV isolates

Birds	No. of total birds	Route	State of chickens after inoculation	Days post infection										
				1	3	5	6	7	8	9	10	19	20	24
3-week-old chicken	30 (10^6EID_{50}/0.1 ml CNDV)	Oronasal	Sick	–	–	3	18	6	7	6	2	N.A.		
			Paralysed	–	–	–	3	8	–	–	–	N.A.		
			Dead	–	–	1	–	7	7	1	2	N.A.		
3-week-old chicken	30 (10^6EID_{50}/0.1 ml PNDV)	Oronasal	Sick	–	–	–	2	–	–	–	–	1	–	–
			Paralysed	–	–	–	–	–	–	–	–	1	–	–
			Dead											

Total mortality in CNDV group, 18; mortality percentage, 60, total mortality in PNDV group, 2; mortality percentage, 6.

agreement with the findings of Parede and Young (1990). In PNDV group, the frequency of virus isolation, was less. However, virus was detected in some of the organs viz. brain, spleen and bursa of fabricius from 3-15 DPI; and virus was also detected in lungs, caecal tonsils and trachea. From spleen, virus was recovered even at 21 DPI.

In conclusion, chicken isolate caused higher mortality with marked involvement of gastrointestinal and respiratory tract, whereas pigeon isolate produced very low mortality with mild clinical signs. It was also reported that passage through chickens was necessary for pigeon isolate to regain their potential virulence for chickens. Along with that the virulence of pigeon isolate for pigeon also get exalted (Alexander and Parsons 1984). There is every possibility that the mesogenic pigeon isolate may cause outbreaks in chicken, therefore it is advisable to vaccinate pigeon as well as chicken wherever needed.

ACKNOWLEDGEMENT

The authors are grateful to the Director, IVRI, for providing necessary facilities to carry out the research works.

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Indian Journal of Animal Sciences **70** (4): 346-348, April 2000

Strain differentiation of Newcastle disease viruses by laboratory tests

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Received: 11 November 1998; Accepted: 22 October 1999

ABSTRACT

Studies on characterization and strain differentiation of 3 Newcastle disease virus isolates originated from the chicken, guinea fowl and pigeon were undertaken by pathogenicity tests and by some unrelated laboratory tests. Chicken and guinea fowl isolates were viscerotropic velogenic in nature, whereas pigeon isolate was mesogenic in nature. All the 3 isolates have similar type of haemagglutinating properties indicating the presence of same terminal carbohydrate residues. Among the 3 isolates, pigeon isolate was inactivated very soon at 56°C, whereas guinea fowl isolate resisted heat for longer time. As far as elution pattern is concerned, chicken isolate was a rapid eluter while guinea fowl and pigeon isolates were slow eluters. However, rate of elution was very slow for pigeon isolate than guinea fowl isolate. No relationship could be established between the pathogenicity indices and other laboratory tests.

Key words: Chicken, Guinea fowl, Newcastle disease virus, Pigeon, Strain differentiation

Newcastle disease virus (NDV) isolates differ in their ability to induce disease and respond to laboratory tests. Based upon the pathogenicity for chicken and chick embryo, NDV strains are divided into lentogenic, mesogenic and velogenic groups.

The importance and impact of a particular NDV isolate is directly related to its virulence. Various laboratory tests which have been used for assessing the pathogenicity of the virus are mean death time (MDT) in embryonated chicken eggs (ECE), intracerebral pathogenicity index (ICPI) in day-old chicks, intravenous pathogenicity index (IVPI) in 6-week-old susceptible chickens and cloacal and conjunctival mean death time (CCMDT) in 8-week-old chickens (Alexander and Allan 1974, Allan *et al.* 1978, Alexandr and Parsons 1984). The strain differentiation of NDV isolates is based on haemagglutination (HA) of mammalian erythrocytes, thermostability of haemagglutinin, and their HA elution pattern. In the present investigation the above all laboratory tests were used to group the isolates and then to differentiate the NDV strains from one to another.

MATERIALS AND METHODS

Virus

All the NDV isolates of chicken, guinea fowl and pigeon origin, procured from Division of Avian Diseases of the institute, were serially passaged in embryonated chicken eggs by terminal dilution of positive allantoic fluid and finally fifth passaged material was preserved in small aliquotes at –20°C for future use. Chicken, guinea fowl and pigeon isolates were named as CNDV, GNDV and PNDV, respectively, throughout the study.

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Table 1. Pathogenicity indices of NDV isolates

Pathogenicity index	Virus		
	CNDV	GNDV	PNDV
MDT	56 hr	48 hr	84 hr
ICPI	1.75	1.50	1.16
IVPI	2.51	2.57	1.01
CCMDT	4 days	6 days	–

No mortality.

Embryonated chicken eggs (ECE)

Embryonated chicken eggs were obtained from the Hatchery Unit of Central Avian Research Institute (CARI), Izatnagar, for the experimental purpose.

Chicks

Day-old chicks were raised under same environmental condition till used in different experiments. Chicks negative for ND-HI antibodies were used for experimental purpose.

MDT, ICPI and IVPI

The tests were performed as per Allan *et al.* (1978).

Table 2. Agglutination of RBCs of different species with NDV isolates

Virus	HA titre (log_2) of viruses with 1% RBCs				
	Cattle	*Rabbit*	*Horse*	*Chicken*	*Sheep*
CNDV	4	*	6	6	6
GNDV	4	*	6	7	4
PNDV	4	*	6	6	6

*Not agglutinated.

CCMDT

The test was performed as described by Hanson (1980). Eight- week-old ND seronegative chickens (4 birds per each virus) were inoculated by swabbing 0.1 ml of undiluted virus infected amnioallantoic fluid (AAF) onto conjunctiva and into cloaca. Birds of each group were kept separately and observed daily until death or for 10 days.

Agglutination of mammalian erythrocytes (1%)

A standard micro HA test was performed for all the 3 NDV isolates with chicken, cattle, rabbit, sheep and horse RBCs (Allan *et al.* 1978).

Thermostability of haemagglutinin and infectivity

Stability of HA to temperature was carried out following the procedure of Hanson and Spalatin (1978).

Haemagglutination (HA) elution

To assess the HA elution pattern of viruses, the test was conducted by using the protocol of Spalatin *et al.* (1970).

RESULTS AND DISCUSSION

Pathogenicity indices of 3 NDV isolates are presented in Table 1. These values fall within the range given for velogenic strains, as reported by Singh and Singh (1970) and Allan *et al.* (1978). The MDT, ICPI and IVPI of PNDV fall within the range prescribed for mesogenic strains. Similar reports were given by Allan *et al.* (1978) and Alexander and Parsons (1984).

Cloacal and conjunctival mean death time of 3 NDV isolates was conducted. The GNDV and CNDV upon inoculation onto conjunctiva and into cloaca of 8-week-old chickens caused diarrhoea and death of all the birds within 4-6 days post infection (DPI) and postmortem examination revealed haemorrhages in proventriculus and caecal tonsils in both the groups. Therefore, both the isolates were classified as viscerotropic velogenic virus. No mortality was recorded in the chickens following PNDV inoculation, however, mild clinical signs were observed in all the 4 birds that confirmed the isolate to be a mesogenic strain. Alexander and Allan (1974) reported CCMDT (4-8 days) to be a valuable method to differentiate between the viscerotropic and neurotropic strains of NDV.

Based upon MDT, ICPI, IVPI and CCMDT of 3 NDV isolates, CNDV and GNDV were identified as viscerotropic velogenic strains and PNDV a mesogenic strain.

Haemagglutination pattern of 3 NDV isolates revealed that all of them agglutinated horse, chicken and sheep erythrocytes more or less in similar manner, whereas with cattle RBCs, titre was low and with rabbit RBCs, there was no agglutination (Table 2). Various workers reported that ability to agglutinate cattle, goat, sheep, swine and horse cells varied with the strains of NDV within pathotypes (Winslow *et al.* 1950, Vijayaram *et al.* 1992). Similar haemagglutination pattern of the 3 virus isolates with RBCs of various species indicated the presence of same carbohydrate moieties as the terminal residues of their haemagglutinin, however, they varied in virulence. It is in accordance to the finding of Winslow *et al.* (1950), who reported the ability of some NDV strains to haemagglutinate horse or sheep erythrocytes, while other could not, apparently indicated that the viruses had different carbohydrate moieties present as the terminal residues of their haemagglutinin.

Thermostability of haemagglutinin and infectivity of all the 3 NDV isolates were estimated by exposing viruses to 56°C for 5-120 min. The HA activity of PNDV and CNDV was lost after 30 and 45 min, respectively, whereas HA activity of GNDV was completely lost only after 120 min of exposure. However, infectivity of all the 3 isolates has taken longer time to get inactivated than their haemagglutinin. Infectivity of PNDV and CNDV were completely inactivated after 45 and 60 min of exposure, respectively, while GNDV was not inactivated completely even after 60 min of exposure. This may be due to the involvement of different viral proteins (HN for HA activity and HN and F for infectivity) for both the activities. On the other hand, Allan *et al.* (1978) reported that most of the strains of chicken origin got inactivated within 30 min at 56°C. Tolba and Eskarous (1962) reported that the

Table 3. Haemagglutination elution pattern of NDV isolates

Events of elutions	Viruses		
	CNDV	GNDV	PNDV
Elution time	2 hr	120 hr	Not eluted within 120 hr
Elution pattern after 24 hr	Eluted	Not eluted	Not eluted
Elution pattern after resuspension	Eluted	Not eluted	Not eluted
Original HA titre*	10	11	10
HA titre* before resuspension	<1	7	7
HA titre* after resuspension	<1	7	7

*HA titre expressed in log_2

HA activity was not necessarily inactivated at the same rate as was the infectivity of virus. In our study, no correlation between the rate of inactivation of infectivity and haemagglutinin with the virulence of viruses was observed. These findings are in accordance with those of Hanson and Spalatin (1978).

Haemagglutination elution pattern of all the 3 NDV isolates revealed that CNDV was a rapid eluter, whereas GNDV and PNDV strains were slow eluters. While GNDV strains was eluted within 120 hr, the pigeon isolate did not elute even after 120 hr. The test was also conducted by an alternate method where after 24 hr the contents of the wells were resuspended and the reading was taken after 2 hr. The findings of both the tests were similar. Spalatin *et al.* (1970) also reported that the second method gave comparable results to the first method. Elution pattern was different even among the velogenic viruses (CNDV and GNDV), as CNDV was a rapid eluter and GNDV was slow eluter. Spalatin *et al.*(1970) also reported that both slow and rapid eluters were present equally in 3 NDV pathotypes. In this present study no relationship was found between HA elution pattern and other properties of the same strain, viz. pathogenicity index, HA activity and thermostability of haemagglutinin, as reported previously by Spalatin *et al.* (1970).

From the above all studies, it is concluded that chicken and guinea fowl isolates were viscerotropic velogenic in nature and pigeon isolate was a mesogenic strain. However, all the strains differ from one to another in the laboratory tests. Even among velogenic isolates, strain variation was there. Haemagglutination pattern was almost similar for all the strains, whereas the elution pattern was different for all of them. Thermostability of haemagglutinin and infectivity was also different. No relationship could be established between the pathogenicity tests with other laboratory tests. It seems that origin of all the 3 isolates may be different.

ACKNOWLEDGEMENT

The authors are thankful to Director, IVRI, for providing facilities to carry out the research works.

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Indian Journal of Animal Sciences **70** (4): 349-352, April 2000

Role of *Lymnaea luteola* in spreading *Schistosoma incognitum* in an endemic area

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Received: 27 July 1998; Accepted: 11 October 1999

ABSTRACT

The potential transmission role of *Lymnaea luteola*, the snail intermediate host of *Schistosoma incognitum* in an endemic area, was studied. Out of 3 991 *L. luteola* collected from Marhai, Suhagi and Gorakhpur areas of Jabalpur, 134 (3.35%) were positive for *S. incognitum* cercariae with marked seasonal variation. *L. luteola* were experimentally infected in an artificially prepared tank with pig faeces (60 or 160 g) harbouring *S. incognitum* miracidia. The snails started shedding *S. incognitum* cercariae on day 28 or 38 day post-infection (DPI). Cercarial shedding was the highest on 28 to 43 DPI with the highest infectivity of 26 to 35%. Subsequently, the snails discharging cercariae diminished to 5-10% with no shedding of cercariae was seen from 58th to 65th DPI reflecting heavy mortality of positive snails within a short period. The field collected snails (39) were found to discharge *S. incognitum* cercariae for 1-15 days (average 6 days) till their death. The daily cercarial output per snail varied from 6.6 to 326.6 (minimum) and 106.6-1 006.6 (maximum). The total number of *S. incognitum* cercariae shed by a single snail during its life time ranged from 73 to 4 929 depending on days of survival of the snail.

Key words: Freshwater snails, Laboratory infection, *Lymnaea luteola*, Prevalence rate, *Schistosoma incognitum*

The blood-fluke, *Schistosoma incognitum*, was first detected in human stools (Chandler 1926) and later in pigs (Rao and Ayyar 1933). Since then, the parasite is reported from South East Asia infecting primarily pigs but sheep, goats and other animals are also incriminated (Agrawal and Shah 1989). By experimental infection, its intermediate host was confirmed to be *Lymnaea luteola* as other snail species failed to develop cercariae of *S. incognitum* (Sinha and Srivastava 1960). A whole year survey revealed 2.18% (Sinha and Srivastava 1960) or 3.5% (Kohli 1991) *L. luteola* to be positive for *S. incognitum* cercariae with seasonal differences. *L. luteola* has been used for supply of *S. incognitum* cercariae for experimental schistosomosis (Ahluwalia 1968, Borkakoty 1975, Bhatia *et al.* 1976, Agrawal 1978) but without studying details of the snail or emergence of the cercariae. Therefore, no detailed studies appeared to have been made on the role of *L. luteola* in spreading *S. incognitum* to understand the epizootiology of the infection. Recently, we have reported the role of *Indoplanorbis exustus* in spreading schistosomosis in the endemic areas (Mishra and Agrawal 1998). The present studies have been undertaken on *L. luteola* infected with *S. incognitum* and reported here under.

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MATERIALS AND METHODS

Studies were undertaken to observe seasonal variation if any in the shedding of schistosome cercariae by *L. luteola* snails in the natural habitat and the cercarial emergence pattern in experimentally induced infections.

Screening of wild L. luteola

Freshwater snails including *L. luteola* were collected from Suhagi, Marhai and Gorakhpur areas of Jabalpur which are known endemic foci for *S. incognitum*. *L. luteola* were separated and exposed to artificial light, first in groups of 10 and later individually from positive groups either in a glass beaker or in specimen tubes. This beaker or glass-tube was checked for presence or absence of *S incognitum* cercariae by placing an eye piece (6 ×) in an inverted position on the glass wall which facilitated observation of gross morphological features of the emerging cercariae. Mammalian schistosome cercariae which concentrated on upper water surface were considered as those of *S. incognitum*. In a few cases, the cercariae were differentiated on the basis of their furcal tips (Kohli and Agrawal 1995).

Cercarial emergence

Screening of *L. luteola* by the above method facilitated separation of the snails shedding *S. incognitum* cercariae.

Table 1. Screening of field *Lymnaea luteola* for *Schistosoma incognitum* cercariae

Month of collection	Area of collection	No. of *L. luteola* positive/ collected	Percentage positive
Jan. 97	Marhai	13/238	5.46
Feb. 97	Marhai	4/126	3.17
Mar. 97	Gorakhpur	5/201	2.48
Apr. 97	Marhai	2/54	3.7
Aug. 97	Marhai	0/74	0
Aug. 97	Marhai	13/100	13
Sep. 97	Suhagi	3/69	4.34
Sep. 97	Marhai	4/560	0.71
Sep. 97	Marhai	13/383	3.39
Oct. 97	Marhai	0/50	0
Oct. 97	Marhai	7/386	1.84
Oct. 97	Suhagi	1/100	1
Nov. 97	Marhai	2/150	1.33
Jan. 98	Marhai	4/300	1.33
Jan. 98	Marhai	13/200	6.5
Feb.98	Marhai	50/1000	5
Jan 1997 to Feb 1998		134/3991	3.35

Table 2. Pattern of *Schistosoma incognitum* cercarial shedding by the field *Lymnaea luteola* snails

No. of positive snails	Total cercarial discharge period (in days)	No. of cercariae discharged snails per day		Total cercaria - shed by per individual snail in its life span		Average total cercariae shed by the snail in its life
		Min	Max	Min	Max	
2	1	73.3	106.6	73.3	106.6	89.9
1	2	326.6	360	686.6	686.6	686.6
2	3	13.3	433.3	746.5	766.6	756.5
3	4	80	653.3	506.5	1619.9	944.3
7	5	26.6	879.9	933.1	2709.6	1814.4
5	6	13.3	699.9	1033.1	3279.8	1787.7
5	7	6.6	1006.6	1326.5	4093.1	2451
8	8	6.6	626.6	1499.7	2797.5	2102.7
3	10	6.6	673.3	1193	4279.3	2839.5
1	12	199.9	630	3749.5	3749.5	3749.5
2	15	189.9	889.9	3289.1	4929.3	4109.2
39	1 to 15	6.6	1006.6	73.3	4929.3	89.9-4109.2

These positive snails were transferred individually in test-tubes each containing 15ml of stored water and piece of mulberry leaf as its feed. These snails were kept in diffused light without their exposure to artificial light; water from the tubes was removed for cercarial count every day at 10.00 AM and tubes were refilled with stored water along with addition of mulberry leaf. The procedure was repeated till death of the snail. For counting of the cercariae, 3 aliquots, each of 0.5ml, were removed from the test-tube after thorough mixing of the water. A drop of aqueous iodine was then added to stain the cercariae before counting under a stereoscopic microscope. The average of 3 aliquots was multiplied with total quantity of water (15ml) to know total number of cercariae shed by a snail in a day. Thus, the cercarial output per day per snail, their minimum and maximum number, duration of their discharge and total cercarial output during their life time were estimated from 39 positive *L. luteola* snails.

Laboratory infection

The previous workers (Sinha and Srivastava 1960, Borkakoty 1975, Avsatthi 1976, Kaur 1985) infected laboratory bred *L. luteola* with miracidia of *S. incognitum* individually in the test-tubes. This method was not identical to field conditions, led to heavy snail mortality, and required daily personal attention. In present work *L. luteola* were maintained and bred in the artificial tank to simulate field conditions as per the method of Agrawal (1999). A small tank of 2.50 m diameter with 0.325 m depth in centre and 0.30 m on the periphery containing sand, pond mud, *Hyacinth, Vallisnaria, Nymphaea* spp. was used for present study. To this pond, pig faeces, checked and found highly positive for *S. incognitum* miracidia, were added. In all, 3 experiments were conducted; the first was in September 1995 with addition of about 500 miracidia, second in October 96 with 160 g pig faeces and third in October 97 with addition of 60 g pig faeces. In the first experiment, 60 snails were removed from the pond on 28th day post infection (DPI) and were checked for *S. incognitum* cercariae. The positive snails were separated while negative snails were re-exposed on subsequent days and this procedure was continued till no snail turned positive. This was to know the incubation period in different snails which were exposed to pig faeces simultaneously at one time. In second and third experiments, 20 or more snails were checked individually at frequent intervals by exposing them to artificial light till they were confirmed negative. The positive snails were not added back into the tank but used for other work. A total of 520 snails were checked in second experiment while 635 snails were checked in third experiment (Table 3).

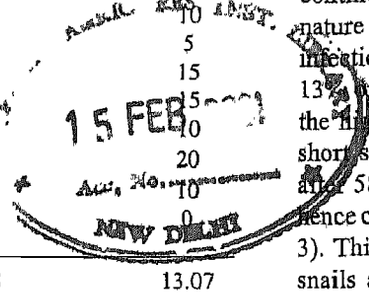
RESULTS AND DISCUSSION

We have checked the wild snails directly in the glass beaker/specimen tube with the help of an eye piece (6×) where-as previous workers (Borkakoty 1975, Avsatthi 1976, Shrivastava 1977, Agrawal 1978) checked the water either with naked eye or a hand lens or part of the water under a stereoscopic microscope. Our present method detected even 2-3 cercariae present in the water with little efforts. Likewise, we have used artificial tank for infecting *L. luteola* with *S. incognitum* whereas Sinha and Srivastava (1960) infected laboratory bred *L. luteola* with miracidia of *S. incognitum* individually in the test-tubes. The present method was more close to field conditions and enabled observations in large number of snails under simulating field conditions.

The field survey revealed that 3.35% *L. luteola* were positive for *S. incognitum* cercariae whereas Sinha and

Table 3. Number of laboratory infected positive snails at different time intervals

Days after infection	No.of snails examined	No.of positive snails	Percentage
	Experiment No. 2		
28	50	13	26
30	50	11	22
33	20	5	25
34	20	4	20
36	20	4	20
38	20	1	5
41	20	3	15
42	20	4	20
43	20	2	10
44	20	3	15
47	20	1	5
48	20	2	10
50	20	1	5
51	20	3	15
54	20	3	15
55	20	2	10
56	20	4	20
57	20	2	10
58-65	100	0	0
Total	520	68	13.07
	Experiment No. 3		
38	50	10	20
40	60	9	15
41	40	4	10
42	22	4	20
43	20	7	35
45	20	2	10
46	20	0	0
47	50	3	6
49	20	0	0
50	20	2	10
54	20	2	10
55	20	0	0
56	20	0	0
57	20	0	0
58	97	3	3
59-64	138	0	0
Total	635	46	7.24



Srivastava (1960) and Kohli (1991) reported 2.18% and 3.5% to be naturally infected from Barielly and Jabalpur respectively. The results also suggested seasonal variation in prevalence rates (Table 1). However, more important was the negativeness of all the 74 snails of Marhai collected on 20.8.97 but positiveness of 13% snails when collected from same pond only 2 days later. Likewise, marked variations in positiveness was observed during September and October (Table 1). These observations suggested that though prevalence of schistosome cercariae in *L. luteola* was seasonally affected, there are other factors also which are determining positiveness of the snails. These might be size and genetics of snail population, spot, and time and number of snails collected. It will be justifiable to study the factors other than the season which are influencing prevalence of schistosome cercariae in freshwater snails.

The overall prevalence rate in wild *L. luteola* did not exceed 13% but when these snails were exposed to *S. incognitum* miracidia in the artificial tank, as high as 35% of the snails were found to discharge cercariae. Thus, there was a marked variation in number of snails discharging cercariae under 2 circumstances. The higher prevalence rate in artificial infection became more important since snails were exposed only once with pig faeces whereas in nature they are continuously being exposed to the miracidia. Perhaps, in nature too such a higher proportion of the snails pick up the infection but heavy mortality reduces the prevalence rate to 13% or so. This appeared so as in present experiment also, the higher positiveness of 35% reduced to 5-10% within a short span of 20 days with detection of no positive snails after 58 DPI. Perhaps, positive snails were further reduced hence could not be detected in 20 or 50 snails examined (Table 3). This could happen only by heavy mortality of infected snails and coming up of new uninfected snail population during the ensuing period. A heavy infection of *S.spindale* in *I.exustus* was also responsible for early death of *I exustus* (Mishra and Agrawal 1998). This pathology might be related to degree of inherited resistance of the snails towards trematode or schistosome infections. Additionally one interesting observation was about difference in incubation period and negativeness as revealed in first experiment of laboratory infection of the snails. The group started shedding the cercariae on 28th DPI. On 34th DPI out of 53 snails, 24 were negative. Twenty-three of these negative snails were re-exposed on 35th DPI when 4 snails shed the cercariae. Likewise, out of these 16 (3 died) negative snails, 5 turned positive on 37th DPI. However, no negative snails out of 11 turned positive when examined on 38th and 39th DPI. Neither their teasing revealed any schistosome cercariae. These results not only reflected differences in the incubation period in the snails, exposed at the same time, but also identified 11 snails which were capable to resist the schistosome infection. Similarly, about half of the population of *L. luteola* in other experiments (Table 3) did not develop *S. incognitum* infection despite presence of *S. incognitum* miracidia in the environment. These events appear genetically related. Therefore, it is essential to study population dynamics of the snails to understand the relative susceptibility and / or refractory nature and threshold levels leading to mortality of freshwater snails due to schistosome infections.

The wild positive *L. luteola* survived only for 1-10 days

except for 1 snail which survived 12 days and 2 snails which survived 15 days (Table 2). This is in contrast to the reports of survival of positive *I.exustus* for 11-35 days (Mishra and Agrawal 1998). Beside duration of the survival, the 2 snails differed greatly in minimum, maximum and total number of cercariae shed by these snails. Thus, as high as 77 890 *S.spindale* cercariae were shed by a single *I.exustus* during its life span (Mishra and Agrawal 1998), whereas only 4 929 *S. incognitum* cercariae were discharged by a single *L. luteola* (Table 2). The maximum number of cercariae shed by *L. luteola* in a day was also lower (1 006) in comparison to that of *I.exustus* (13 000) (Mishra and Agrawal 1998). Nevertheless, *L. luteola* also continued to shed the cercariae of *S. incognitum* till its survival much like *I.exustus*. These observations suggested that *L. luteola* has lower potential for spreading schistosomosis in comparison to that of *I.exustus*.

ACKNOWLEDGEMENT

This work was carried out under National Fellow Project financed by Indian Council of Agricultural Research, New Delhi.

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Indian Journal of Animal Sciences 70 (4): 353-355, April 2000

Haematological changes in experimental haemonchosis in Barbari goats

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Received: 26 February 1999, Accepted: 7 October 1999

ABSTRACT

The information on caprine haemonchosis is meagre. Therefore, present study was made to study the haematological changes in experimental *Haemonchus contortus* infection in Barbari goats. The 12 male goats of 6-9 months age were divided in 2 groups A and B having 8 infected and 4 control animals respectively. Selected animals were first screened and made free from any endo- and ecto-parasites. These were kept under strict hygienic conditions and provided with clean water and tree tops without any grazing. The animals in group A, were administered with 5 000 L3 *(H. contortus)* orally, while those in group B were kept as uninfected control. Both the groups were bled every week and blood samples were examined for haematological parameters, viz. PCV, Hb and TEC and erythrocytic indexes of MCV, MCH and MCHC were worked out. Statistical analysis of the data revealed significant fall of PCV, Hb, and TEC in infected goats when compared with control indicating anaemia marked by no appreciable change in MCV and thus absence of reticulocytosis. Pathogenesis of anaemia was attributed to haemorrhage and haemodilution after infection.

Key words: Goats, Haematology, Haemonchosis

The information on caprine haemonchosis is meagre. The present study was undertaken to examine the haematological changes which occurred in Barbari goats experimentally infected with *H. contortus*.

MATERIALS AND METHODS

Male Barbari goats (12) of 9-12 months age having approximately same weight and health status were used for the present study in the Department of Parasitology, Veterinary College, Mathura. The goats were screened for haemoprotozoans along with other endo- and ecto-parasites. All the animals, irrespective of presence of infection, were treated with broad spectrum anthelmintic orally twice at 3 weeks interval for internal parasites and 0.8% malathion acaricide through dipping. After 1 month, post-treatment, interval the animals were divided into 2 groups A and B, consisting of 8 and 4 animals and designated as infected and control groups respectively. Both the groups were maintained in strictly hygienic conditions without grazing and provided with clean water, tree toppings and concentrate to avoid unwanted infection. The third stage larvae (L3) used for experimental infection were obtained through mass larvae culture from a laboratory reared donor sheep with pure *H. contortus* infection. The animals of group A were only given about 5 000 L3 of *Haemonchus contortus* orally after overnight starvation. The animals of both the groups were bled weekly and blood samples were examined for packed-cell volume (PCV), haemoglobin (Hb) and total erythrocyte count (TEC). These haematological observations were used to calculate the erythrocytes indexes i e MCV, MCH and MCHC.

The 'mean' herein refers to least-squares means as the data available was analyzed using Harvey (1975) analysis for comparison of groups, infections, and weeks. The significance of the difference between means was determined by using critical difference tests.

RESULTS AND DISCUSSION

Haematological observations recorded under the present study in *H. contortus* infection in Barbari goats, showed decline in infected group than control animals (Tables 1, 2).

The mean PCV in infected group dropped by 33.98% than control over 6 weeks of study. The sharpest decline in PCV was recorded in first week when it fell to 19.62 ± 0.77 cm in infected animals in contrast to mean PCV value of 26.75 ± 1.09 cm observed in control. The changes were not significant after 2 weeks of infection.

Haemoglobin concentration (g/dl) changed significantly

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Table 1. Analysis for variance for haematological parameters in *H. contortus* infection

Source	df	Mean sum of squares					
		PCV	Hb	RBC	MCV	MCH	MCHC
Infection	1	1413.93**	76.73**	326.73*	23.38	16.29**	127.30**
Weeks	5	4.12	0.39	5.89**	35.02**	4.07*	3.13
Infection weeks	5	7.90	0.36	6.35**	24.64**	2.71**	1.85
Remainder	54	4.77	0.46	0.40	7.00	0.73	6.06

(P>0.01) against control. Mean Hb concentration in infected animals dropped by 27.37% over 6 weeks. The lowest recorded Hb concentration in this group was 5.70 ± 0.28g/dl (Table 2) showing a drop of 29.80% in sixth week. The weekly mean Hb concentration in infected animals, however, showed variability and remained lower than control.

Mean erythrocyte count (TEC) in group A, was observed to be 8.32 ± 0.09 million / mm³) which was significantly (P<0.05) lower than 12.95 ± 0.12 million / mm³) recorded in controls. Thus, there was a drop of 35.75% over 6 weeks. Weekly mean value also showed significant (P<0.05) variations (Table 1) which were higher in first 3 weeks than in following weeks.

There was no significant change in mean MCV index. But weekly mean MCV increased significantly in group A for first 4 weeks before it declined during next 2 weeks. MCH value in the animals of group A was significantly higher than control. Weekly mean corpuscular haemoglobin also varied significantly with respect to time interval and a steady increase was noted by fourth week. Thereafter, the index reduced gradually. The MCHC calculated in infected animals was significantly higher than in control animals.

Pradhan and Johnstone (1992) reported apparent drop in PCV, Hb concentration and RBC count at 7 day in sheep and described the anaemia as macrocytic with increased MCV and MCH but without appreciable change in MCHC. Dargie and Allonby (1975) described anaemia due to haemonchosis developing in 3 stages. Anosa (1977) described anaemia as normocytic normochromic with low PCV, RBC and Hb concentration. Ahmad and Ansari (1989) reported depletion in RBC, WBC, PCV and Hb concentration. Chartier and Benoit (1992) described haematological changes in goats and reported moderate but continuous decline trend of about 25-30% in Hb, RBC and PCV. Significant drop of PCV, RBC and Hb in our study in goats was generally comparable to earlier reports. Marked drop in PCV in first 3 weeks associated with significant decline in TEC encountered, can be attributed primarily to massive abomasal haemorrhage by active feeding habit of developing stages and the adult worms, and secondarily delayed erythropoietic system activation was also considered to be a contributory factor (Dargie and Allonby

Table 2. Factor-wise least squares means of various haematological parameters in *H. contortus* infection in goats

Factor	Observation	PCV(%)	Hb (g/dl)	RBC (million/mm³)	MCV (fl)	MCH (pcg)	MCHC (g/dl)
Overall	66	23.59±0.28	7.10±0.08	10.63±0.08	22.56±0.33	6.86±0.10	30.44±.31
Groups							
A	42	18.78±0.33	5.97±0.10	8.32±0.09	23.18±0.40	7.38±0.13	31.89±0.38
B	24	28.41±0.44	8.22±0.14	12.95±0.12	21.94±0.54	6.35±0.17	29.00±0.50
Weeks							
I	12	23.18±0.66	7.03±0.21	11.98±0.19	19.29±0.81	5.85±0.26	30.75±0.75
II	11	24.37±0.86	7.19±0.2	11.07±0.19	21.85±0.82	6.49±0.06	30.04±0.77
III	11	24.39±0.68	7.25±0.21	10.11±.19	24.39±0.82	40±0.26	30.32±077
IV	11	23.57±0.68	7.34±0.31	10.21±0.19	23.89±0.82	7.54±0.26	31.420.77
V	11	23.07±0.68	6.98±0.21	10.14±0.19	23.15±0.82	7.08±0.26	30.52±0.77
VI	10	23.00±0.70	6.80±0.22	10.28±0.20	22.81±0.85	6.81±0.27	29.80±0.79
Groups × weeks							
A× I	8	19.62±0.77	6.23±0.24	11.10±0.22	17.97±0.93	5.64±0.30	31.90±0.87
B×I	4	26.75±1.09	7.28±0.34	12.86±0.31	20.79±1.32	6.07±0.42	29.25±1.23
A×II	7	19.00±0.82	5.91±0.26	9.12±0.24	20.87±1.00	6.49±0.33	31.54±0.94
B×II	4	29.75±1.09	8.47±0.34	13.03±0.31	22.82±1.32	6.50±0.42	28.54±1.23
A×III	7	18.28±0.82	5.95±0.26	7.32±0.24	25.16±1.00	8.19±0.32	32.57±0.93
B×III	4	30.50±1.09	8.55±0.34	12.91±0.31	23.62±1.32	6.62±0.42	28.07±1.23
A×IV	7	19.14±0.82	6.28±0.26	7.35±0.24	26.35±1.00	8.19±0.32	32.82±0.93
B×IV	4	28.00±1.09	8.40±0.34	13.08±0.31	21.43±1.32	6.62±0.42	30.02±1.23
A×V	7	18.14±0.82	5.77±0.26	7.37±0.24	24.62±1.00	8.65±0.32	31.66±0.93
B×V	4	28.00±1.09	8.20±0.34	12.92±0.31	21.67±1.32	6.43±0.42	29.38±1.23
A×VI	6	18.50±0.82	5.70±0.28	7.66±0.22	24.30±1.08	7.50±0.34	30.85±1.00
B×VI	4	27.50±1.09	7.90±0.34	12.90±0.31	21.33±1.32	6.12±0.42	28.74±1.23

1975). Mean MCV values, in the present study, showing no significant variation in 2 groups but with significant decline in Hb and TEC (Table 2) are suggestive of selective depression of erythropoiesis with no evidence of reticulocytosis to compensate for the resulting anaemia. However, significant increase in mean weekly MCV in first 3 weeks clearly indicate delayed erythropoietic response which is very much clear from increase of RBC count and MCH after third week.

Though significantly higher MCH value of infected animals, in the present study, was within normal range of 5-8 pg (Schalm *et al.* 1975) reported in non-anaemic goats, but looked higher than the values reported by Somvanshi *et al.* (1987) and Rastogi and Singh (1990). The higher MCH level seems to be a compensatory device to maintain the haemoglobin level. Mean MCHC value of infected animals, though in normal range (Rastogi and Singh 1990), was significantly more than value observed in control. As MCHC is influenced by both erythrocytic size and haemoglobin concentration the finding may be attributed to more severe changes in PCV through haemodilution and delayed or depressed erythropoiesis. MCHC values in our study differed from Somvanshi *et al.* (1987) who reported these in Indian Pashmina goats. The overall changes in haematological values in *Haemonchus contortus* infection seems to lead to anaemia which is normocytic and normochromic in nature.

ACKNOWLEDGEMENT

Authors thank the Dean, College of Veterinary Science and Animal Husbandry, Mathura, for his kind co-operation and facilities provided.

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Indian Journal of Animal Sciences **70** (4): 356-361, April 2000

Clinical, haematological and biochemical observations following plasma and glycerin stored tendon allografting in equids

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Received: 7 June 1999; Accepted: 4 October 1999

ABSTRACT

There is need of better tendon preservation techniques for feasibility and healing of tendon after plasma stored and glycerin preserved tendon allografting in donkeys. Therefore, study was performed after tendon allografting of superficial digital flexor (SDF) tendon and surgically traumatizing deep digital flexor (DDF) tendon in 16 donkeys. In group 1 (n=8) plasma stored, and in group 2 (n=8) glycerin preserved tendon grafts were used. The grafted SDF and traumatized DDF tendons were encased in amniotic membrane. External coaptation applied for 8 weeks. Donkeys were observed at various stages up to 120 days. Animals of both the groups showed a significant rise of temperature, respiration and heart rates on first 2 operative days. Swelling reduced significantly on the fifth day and disappeared on the 42nd day in majority of the animals of group 1, whereas it persisted in 2 animals of group 2, which is also evident from the girth measurements of the operated limb. Girth measurements remained significantly more than normal up to 10 days ($P< 0.01$) and were normal by day 35 and showed no significant variation thereafter till the end of study period in the plasma stored tendon allografted animals (group 1). However, the girth measurements remained more and substantially higher in glycerin stored tendon allografted animals (group 2) till the end of study period. Contralateral limbs showed appreciable increase in the girth measurements up to 28 days in both the groups. Majority of the plasma stored allografted animals showed normal tendon gliding movements whereas glycerin stored tendon allografted animals (group 1) did not exhibit tendon gliding movements. Weight bearing on walk became normal in 33.6±1.8 days and 36.4±3.4 days in groups 1 and 2 respectively. However, haematological and biochemical parameters showed no clinically significant change. It is concluded that plasma stored tendon allografts were clinically better than glycerin stored tendon allografts in equids.

Key words: Equids, Preserved allografts, Superficial digital flexor, Tendon

Better tendon preservation techniques are required for feasibility, and healing of tendon after plasma stored and glycerin preserved tendon allografting in donkeys. Therefore the present work was done.

MATERIALS AND METHODS

The study was conducted on 16 clinically healthy male donkeys aged 3 to 8 year and weighing 70 to 90 kg (mean±SE = 79.4±2.18). Superficial digital flexor (SDF) and deep digital flexor (DDF) tendons in the mid metacarpal region of the fore-limbs were chosen for the purpose of study. The animals were divided randomly into 2 groups comprising 8 animals each.

The tendon tissues (grafts) were collected aseptically, washed in sterile normal saline repeatedly and preserved by any of the 2 methods until their use *ie* stored in plasma at 4°C for use in group 1 and stored in 20% glycerine saline at 4°C for use in group 2 animals. The amniotic membrane was collected from normal delivery in women. Detailed procedure for collection of tendon and amniotic membrane is mentioned elsewhere (Saini *et al.* 1997a, Saini *et al.* 1998). Grafts were preserved for 30 to 56 days. Stored tendon grafts and amniotic membrane were slowly brought to room temperature (25°C) before grafting.

For tendon grafting chloral-hydrate-thiopentone anaesthesia was given and left mid-metacarpal region was prepared for aseptic surgery. The SDF and DDF tendons were exposed surgically. Then SDF tendon was separated bluntly from DDF tendon. About 3 cm long piece of SDF tendon was transected. The DDF tendon was surgically traumatized. After preparation of tendon grafts, these were sutured at the graft bed by single-locking loop tendon suture technique (Pennington 1979) through graft (Fig. 1) using No.2 polyamide. In group 1, plasma stored tendon allografting of SDF tendon was done whereas in group 2, glycerin stored tendon grafts were used. Thereafter, a piece of human amniotic membrane prepared for this purpose was wrapped

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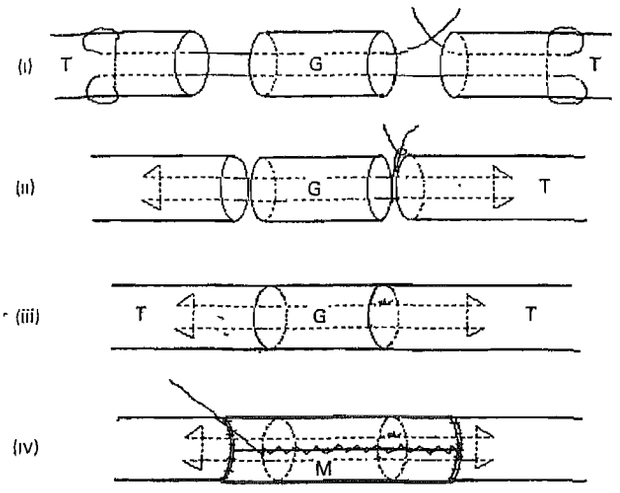

Fig. 1. Diagramatic representation of single locking loop tendon suturing technique used for tendon grafting where T=tendon stumps, G=tendon graft, and M=amniotic membrane encasement.

around the grafted tendon and sutured with 6-0 coated polygalactin 910 violet to form a casing. Similarly encasement was formed and sutured around traumatized DDF tendon. Wound was closed in routine manner and painted with povidone-iodine solution and covered with sterile gauze piece and dressed for 10 days. Operated limb in all the animals was immobilized by application of plaster of Paris splints for 8 weeks. The trimethoprim-sulfadiazine combination was given intravenously @ 1 ml/15 kg body weight for 7 days. Intramuscular injection of 7-8 ml of analgin was administered twice daily for 3 days. The skin sutures were removed after 10th day. All the donkeys were observed from 15 to 120 days after grafting for general condition, wound condition (swelling, exudation warmth, pain), girth measurements, tendon gliding movements, weight bearing (at rest, at walk), haematological monitoring and biochemical analysis.

General condition

All the animals were observed for their general health, behaviour and alertness throughout the period of study. Rectal temperature, heart rate and respiration rate were monitored in all the animals before and after tendon surgery.

Wound condition

Operated limb was observed for gross appearance, gaping and status of healing. Swelling was graded as: 0-no swelling (comparable to contralateral limb), 1-mild swelling (circumference of limb increased up to 0.5 cm), 2-moderate swelling (circumference of limb increased between 0.5 and 1.0 cm), and 3-severe swelling (circumference of limb increased >1 cm). Exudation at the wound site was quantitatively graded as: 0-no exudate, 1-very mild exudate (small exudate sticking to gauge piece only), 2-mild exudate {few drops (2-3) flowed on palpation} 3-moderate exudate {many drops (8-10) flowed on palpation} and 4-severe exudate (free flowing exudate streak). The warmth at the operated site was graded as: 0- normal, 1-warm and 2-moderately warm. Severity of pain on gently pressing at the operated site was graded as: 0-no pain, 1-mild pain (animal reacted to pain but did not withdraw its limb), 2-moderate pain (animal showed reaction to palpation and some times withdrawn and sometimes did not withdraw its limb) and 3-severe pain (animal always has withdrawn its limb on palpation).

Girth measurements and tendon gliding movements

Girth measurements of both operated and contralateral limb were recorded with a measuring tape at the mid-metacarpal region at the graft level. The presence or absence of tendon gliding movements were observed before and after surgery.

Weight bearing and locomotion

The animals were observed for weight bearing in standing posture when at rest and was graded as: 1-toe touching the ground, 2-moderate weight bearing and 3-full weight bearing. Weight bearing and lameness at walk were assessed as: 1-support the limb on ground and less weight bearing, 2-moderate weight bearing and apparently lame, 3-full weight bearing but apparently lame, and 4-full weight bearing and apparently not lame.

Haematological monitoring

Blood samples from jugular vein were collected a day before surgery and postoperatively. Haematological monitoring included: Haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC) and differential leukocyte count (DLC).

Biochemical analysis

Biochemical analysis included total plasma protein (g/dl of plasma) estimation by Biuret reagent (modified) on spectrophotometer at 550 nm, plasma albumin (g/dl plasma) was estimated by buffered dye reagent on spectrophotometer at 600 nm; plasma globulin (g/dl of plasma) was calculated as: plasma globulin (g/dl) = total plasma protein(g/dl) -plasma albumin(g/dl), A/G ratio was calculated by dividing plasma albumin (g/dl) with plasma globulin (g/dl), and lactic dehydrogenase (LDH): LDH (n mol pyruvate reduced/min/ml) was estimated (Wootton 1964).

The difference's between the 2 means for parametric observations and medians for non-parameteric observations based on individual recordings were determined by student's t test and Mann-Whitney test (Campbell 1981) for parametric and non-parameteric observations respectively.

RESULTS AND DISCUSSION

All the animals remained alert, active and took feed and water throughout the post-operative period. A significant rise in rectal temperature, respiration and heart rates for first 2 days in both the groups was observed after tendon surgery

which did not vary much during rest of the post-operative period. These changes may be the result of acute trauma and surgical stress. Variations in the rectal temperature, heart and respiration rates after tendon repair have been reported in horses (Bertone *et al.* 1990) and buffalo calves (Verma *et al* 1983).

Surgical wounds appeared apparently healthy throughout in all the animals. None of the wounds exhibited the gaping or infection and healed by first intention, thereby, indicating the effectiveness of the aseptic procedures and chemotherapeutic measures adopted during the experiment. A similar healing of surgical wounds after tendon repair has been reported in equine (Brown and Pool 1983, Bertone *et al.* 1990).

Table 1. Girth measurments (cm) [mean ± SE] of operated and contralateral limbs after tendon allografting.

Days	Groups			
	I		II	
	Operated	Contralateral	Operated	Contralateral
0	10.76±0.29	10.76±0.11	11.23±0.11	11.23±0.72
1	11.97±0.26**	10.87±0.30	12.70±0.18**	11.33±0.11
2	11.97±0.37**	10.92±0.28	12.67±0.19**	11.41±0.17
3	11.78±0.28**	10.92±0.24	12.55±0.21**	11.35±0.08
5	11.76±0.31**	11.0±0.28	12.35±0.26**	11.48±0.06*
7	11.63±0.27**	11.12±0.36	12.21±0.27**	11.50±0.11*
10	11.56±0.24**	10.92±0.26	12.05±0.26**	11.40±0.12
14	11.32±0.23	10.92±0.27	11.90±0.23**	11.46±0.13
21	11.14±0.19	10.91±0.33	11.85±0.21**	11.51±0.16*
28	11.07±0.19	10.91±0.33	11.75±0.15**	11.48±0.16
35	11.02±0.19	11.11±0.33	11.63±0.23	11.46±0.23
42	11.08±0.23	11.06±0.33	11.60±0.26	11.48±0.22
49	11.10±0.41	11.04±0.33	11.62±0.27	11.48±0.22
56	11.12±0.39	11.06±0.35	11.64±0.26	11.48±0.22
70	11.06±0.52	11.00±0.43	11.54±0.14	11.30±0.16
84	11.06±0.52	11.00±0.43	11.54±0.14	11.36±0.14
90	11.06±0.52	11.00±0.43	11.54±0.14	11.36±0.14

*Siginificant at P<0.05 level, ** significant at P<0.01 level.

Moderate to severe swelling (score range 2-3) present during first 2 days in both the groups. It started reducing significantly (P< 0.05) on day 3. Swelling disappeared by the day 42 in majority of the animals of groups 1 and 2, except in 2 animals of group 2 that showed persistence of mild swelling (score 1). Presence of severe to moderate swelling and pain observed after tendon surgery/tendinitis is due to acute inflammatory change (Stromberg and Tufvesson 1977, Foland *et al.* 1992). The persistence of swelling for longer period glycerine stored grafted animals could be due to inflammatory and fibrotic reaction. Pain on palpation decreased significantly (P< 0.02) on day 3 in both the groups. Pain disappeared in 13.0±0.93 and 15.75±1.02 days in groups 1 and 2 respectively. Pain on application of pressure to the tendon was even noticed 2 months after transplantation and slight palpable thickening of tendon and subcutaneous tissue remained up to 6 months (Stromberg and Tufvesson 1977). These findings were further substantiated by air-tendographic observation (Saini *et al.* 1999) which revealed moderate thickening in the animals where glycerin stored tendon grafts has been used. Moderate to severe wound exudation (score range 3 to 4) decreased significantly in groups 1 (P< 0.01) and 2 (P< 0.002) on day 2. Warmth at the site also decreased significantly in group 1 (P< 0.02) and 2 (P< 0.05) on second day. The wound exudation and local increase in warmth for first few post-operative days was the result of the acute inflammation after surgical trauma in all donkeys.

Girth measurements of the operated (left) limb in the mid-metacarpal region remained significantly (P< 0.01) higher until day 10, in group 1 (Table 1). Thereafter, the girth measurement started decreasing and reached near normal on the day 35, and no significant change was observed till the end of experiment (Fig. 2). Contralateral (right) limb showed no significant variation in girth measurements (Table 1). In group 2 a significant increase (P< 0.01) in the girth measurements of the operated limb in mid-metacarpal region was observed until day 28 (Fig 2). Thereafter girth measurements remained slightly on higher side than

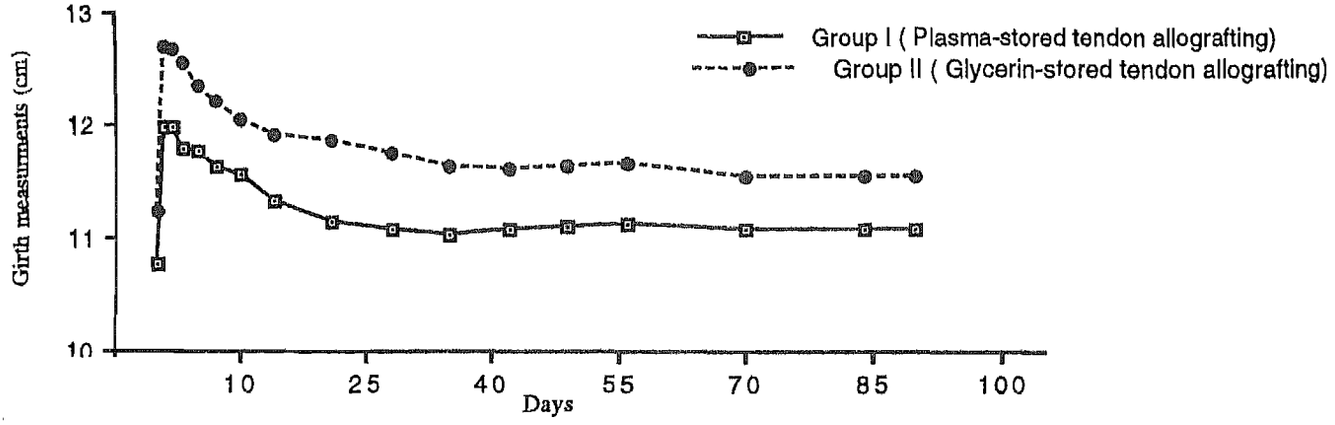

Fig. 2. Girth measurement after tandon allografting in groups 1 and 2.

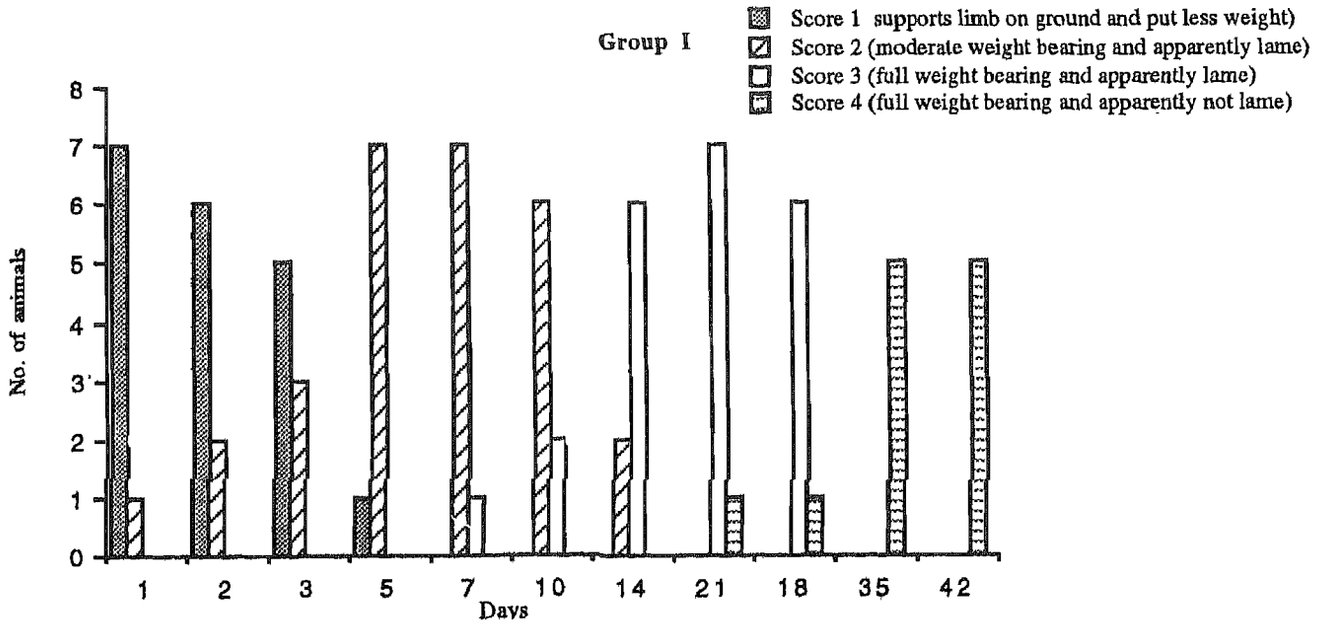

Fig. 3. Scores of weight bearing at walk after plasma stored tendon allografting.

contralateral limb. Contralateral (right) limb showed a significant ($P< 0.05$) increase in girth measurements in the 2mid-metacarpal region on days 5, 7 and 21 (Table 1). The increase in the girth measurements for first few days following tendon surgery was associated with the signs of inflammation. While Gift *et al.* (1992) found that the limb circumference returned within 7 mm of their original circumference in 6 weeks after collagen induced DDF tendinitis in equine in control and sodium hyaluronate treated limbs. Intermittent increase in the girth measurements of the contralateral limb in both the groups was due to excessive fluid accumulation around tendons/ligaments by induced stress tendinitis/desmitis due to compensative and excessive weight bearing. The excessive fluid accumulation has been evidenced by ultrasonographic observation on the contralateral limb (Saini *et al.* 1997b).

The extension and flexion at the fetlock revealed gliding movements of the tendons in the grafted region in all, except 1 animal of group 1, in which it could not be felt. Gliding movements of SDF tendon could not be felt in 5 animals in group 2, whereas in the remaining 3 animals the gliding movement of SDF tendon could be palpated. In majority of

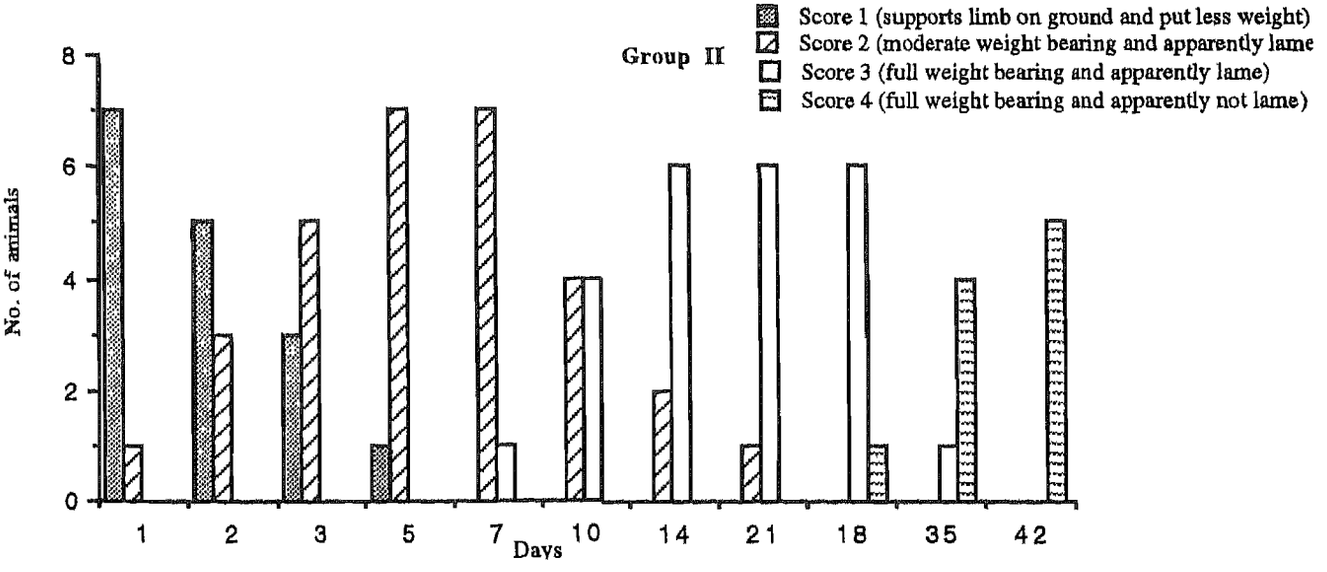

Fig. 4. Scores of weight bearing at walk after glycerin stored tendon allografting.

glycerin stored tendon grafted animals the absence of gliding movements was indicative of formation of peritendinous adhesions. Successful restoration of gliding requires that scar produced by healing of coapted tendons be separated from that produced by healing of surrounding soft tissue so that one scar can move independently of the other (Peacock 1964). This objective was achieved to large extent in plasma stored grafted SDF and traumatized DDF tendons. Amniotic membrane helps to maintain free gliding surface of SDF and DDF tendons in equine (Saini *et al.* 1996, Saini *et al.* 1998). The immobilization also contributes mild to moderate degree of adhesion formation in the sutured tendons. The adhesions thus formed are observed when splintage is discarded (Matthews and Richards 1976). In the present study, immobilization has played at least some role in restricting gliding movements of flexor tendons temporarily in some of the animals.

Weight bearing improved significantly ($P< 0.03$) on day 3 (score range 2-3) in standing posture in both the groups. It became normal (score 3) in 4.5±0.71 and 4.8±0.84 days in groups 1 and 2, respectively (Fig. 3). At walk weight bearing increased significantly ($P< 0.03$) on day 5, in both the groups. It became normal (score=4) in 33.6±1.83 and 36.4 ±3.43 days after grafting in groups 1 and 2, respectively (Fig. 4). This indicated that weight bearing increased significantly during first couple of weeks after grafting and gradually returned to normal between 4 and 6 weeks in all the animals. A marked lameness was reported by Stromberg and Tufvesson (1977) for 1-2 weeks which decreased gradually and persisted for 2-4 months after tendon transplantation in horses. Gift *et al.* (1992) reported that lameness disappeared in 6 weeks in control and sodium hyaluronate treated tendinitis in horses.

In general there was no appreciable change in the mean haematological values of all the animals in both the groups. But TLC showed a significant ($P< 0.05$) rise on days 1, 3 and 5, in group 1 and on days 1, 3, 5 and 10, in group 2. Neutrophil count showed a significant rise on days 1 and 3, in both the groups. Lymphocyte count decreased significantly ($P< 0.01$) on first and third day in both the groups. Significant increase in the TLC and neutrophils for first 2-3 days after surgery could be the reaction to postoperative surgical trauma, that lead to temporary increase in these parameters, though, not of a clinical significance.

The mean values of total protein contents in plasma at 0 day before surgery were 7.5±0.57 g/dl in groups 1 and 7.60±0.48 g/dl in group 2, respectively. The mean values of albumin contents in plasma on 0 day before surgery were 3.42±0.25 g/dl in group 1 and 3.5±0.32 g/dl in group 2 respectively. While globulin contents in plasma on 0 day before surgery were 4.08±0.36 g/dl in group 1 and 4.07±0.16 g/dl in group 2, respectively. The albumin/globulin A/G ratio on 0 day before surgery was 0.84±0.04 in group 1 and 0.84±0.05 in group 2, respectively. However, these values also did not show any appreciable variation during the postoperative period. The LDH levels on 0 day before surgery were 156.5±3.0 and 155.4±6.39 n mol pyruvate reduced/min/ml in groups 1 and 2, respectively. In group 1, it showed significant rise on 1 ($P< 0.01$) and 3 ($P < 0.05$) days. Thereafter it showed no significant change up to day 90. In group 2, LDH exhibited a significant change only on first ($P < 0.005$) day after grafting. The significant variations observed in the LDH levels at few instances after grafting may be due to tissue trauma or stress during and after surgery. These changes, in LDH, however, have no clinical significance.

It is concluded that plasma stored tendon allografts proved clinically better than glycerin preserved tendon allografts in equids for providing normal function and locomotion.

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Indian Journal of Animal Sciences 70 (4): 362-365, April 2000

Postoperative pain management in dogs: Efficacy of pre-emptive analgesia with lignocaine and ketamine

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Received: 12 February 1999; Accepted: 4 October 1999

ABSTRACT

The study was designed to evaluate the efficacy of epidural lignocaine and ketamine for post-operative pain management in experimental tibial fracture repair in 12 adult dogs divided in 3 equal groups. After atropine sulphate and triflupromazine groups premedication, normal saline (2 ml), lignocaine hydrochloride (2%) @ 4 mg/kg and ketamine hydrochloride (5%) @ 3 mg/kg were given epidurally at lumbosacral space in animals of groups 1, 2 and 3, respectively, to assess their efficacy in pain management. Anaesthesia was induced and maintained with thiopental sodium in all the groups. Left tibia fracture created at proximal metaphysis was repaired with double pinning and interfragmentory wiring.

Wound hyperalgesia and swelling in animals of group 1 (normal saline) were significantly ($P<0.05$) higher than the group 3 (epidural ketamine). In group 2, wound hyperalgesia and swelling were lesser as compared to group 1 but higher than group 3. Movement associated pain scores showed early weight bearing on affected limb in animals of group 3 as compared to groups 1 and 2. The results of this study suggest that epidural ketamine, if given preoperatively can pre-empt the post-operative hyperalgesia, swelling and pain. Lignocaine had only little beneficial effects.

Key words: Dog, Ketamine hydrochloride, Lignocaine hydrochloride, Pain, Pre-emptive analgesia

Conventional therapy for pain i e intermittent use of analgesics has failed to provide freedom from pain in animals and human beings. Hence, present study was designed to evaluate the efficacy of NMDA antagonist, ketamine in the management of post-operative pain in dogs operated for experimental tibial fracture repair and to compare it with that of lignocaine hydrochloride, a popular local anaesthetic.

MATERIALS AND METHODS

Healthy adult mongrel dogs (12) of either sex weighing between 13 and 22 kg (av. 16.31 kg) were divided into 3 groups (1, 2 and 3) of 4 animals each. All the animals were kept off feed for 18 hr and water was withheld for 4 hr before the start of surgery. Preanaesthetic medication included atropine sulphate injected i/m @ 0.65 mg/animal in all the groups. It was followed, 10 min later, by triflupromazine hydrochloride @ 1 mg/kg i/m. After a gap of 10 min, lignocaine hydrochloride (2%) @ 4 mg/kg body weight and ketamine hydrochloride (5%) @ 3 mg/kg body weight were administered epidurally in animals of groups 2 and 3, respectively, as pre-emptive analgesic agents. In animals of group 1 (control), 2 ml of normal saline was given epidurally.

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Ten min later, anaesthesia was induced with 2.5% solution of thiopental sodium given to effect in all the groups. Fracture of proximal tibial metaphysis was created in all the animals by heck saw after approaching tibia from medial aspect. It was repaired using cross pinning and interfragmentary wiring. The efficacy of epidural lignocaine and ketamine to prevent the post-operative morbidity, swelling and pain was assessed using different parameters and compared with control group. Anaesthesia during the surgical procedure was maintained by thiopental sodium till effect. Surgical anaesthesia was achieved by constant monitoring of palpebral reflexes and repeated administration of small quantities of thiopental as needed. Post-operatively, a common protocol of diclofenac sodium @ 1 mg/kg daily for 3 days and streptopenicillin @ 10 mg/kg bid for 5 days was used in all the animals.

Animals were assessed for post-operative wound hyperalgesia, swelling at site of operation and pain (movement associated) for 7 consecutive days. Post-operative wound hyperalgesia (increased sensitivity of nociceptors at the site of operation) was noted by recording pain response (crying, groaning, avoiding, lifting of leg) to manual pressure applied at the operated site using thumb and 2 fingers. The response was recorded on 0-3 score scale as 0- no pain (animal did not show signs of pain to moderate pressure); 1- mild pain (animal allowed touch and occasionally showed pain to moderate

pressure); 2- moderate pain (animal allowed touch but frequently showed pain to moderate pressure); and 3- severe pain (animal either did not allow to touch, (tried to bite/ protected limb or showed pain on just touching).

Post-operative swelling was recorded in cm for first 7 days after the operation by measuring the circumference of the operated limb at 3 different sites, viz. mid of tibia, at stifle and at a centre point between these 2 sites as incision was made at medial aspect of tibia between stifle joint and mid of tibia in all the animals. The average girth for each day was obtained by calculating the mean of these 3 readings. It was compared with preoperative mean girth in each animal. The gain over the initial girth reflected the extent of postoperative swelling. The percentage increase in swelling was also calculated in all the animals.

The pain on movement was recorded daily for 7 days using a score scale and was graded as 0- no pain (animal was able to move without limping); 1- animal supported weight but occasionally limping while moving; 2- frequent limping at each step; 3- keeping full paw on ground while standing but lifting limb while walking; 4- Just touching the ground with toe even while standing; 5- carrying the limb; and 6 -animal reluctant to move.

Mann Whitney test was used to compare mean scores for wound hyperalgesia and movement associated pain, between the groups at corresponding intervals and paired 't' test was used to compare post-operative swelling with base values (Campbell 1981).

RESULTS AND DISCUSSION

Maximum wound hyperalgesia was recorded in animals of group 1 as compared to animals of groups 2 and 3 (Fig. 1) from days 1-7 post-operatively. In group 2, it was relatively lesser, than group 1 but nonsignificantly more than group 3. Wound hyperalgesia was minimum and significantly less in group 3 as compared to control animals (group 1), from days 1-7 post-operatively. After surgery antidromic impulses in axon, promote the release of substance P(sp) from the nerve endings and in combination with other algogenic substances like prostaglandins (PG), leukotrienes, bradykinin, serotonin and histamine, sp promotes inflammation and sensitization of afferent nociceptors (Yaksh 1987, Levine *et al* 1988). The afferent nociceptive barrage during and after surgery may also induce hyperexcitability of central neurons (Woolf 1983) and elicit spinal cord reflexes that increase the activity of post-ganglionic sympathetic effereuts which in turn amplify inflammation (Levine *et al.* 1988). Thus both somatic and sympathetic nervous system may play roles as mediators of inflammation and hyperalgesia.

Maximum wound hyperalgesia in group 1 as compared to treatment groups suggests maximum peripheral or/and central sensitization in this group, probably due to more nociceptive impulses and supports the findings of Tverskoy *et al.* (1990) who reported that general anaesthesia alone in contrast to spinal anaesthesia, can not prevent the transmission of all impulses from operation site to spinal cord.Reduced hyperalgesia in group 2 could be attributed to local anaesthetic action of lignocaine. Local anaesthetics are known to inhibit transmission of impulses from operated sites to spinal cord and also to reduce neurogenic inflammation by blockade of axon reflex (Barnes *et al.* 1990). Therefore, lignocane could reduce wound hyperalgesia either by preventing the establishment of spinal or reducing peripheral sensitization or both. These findings are in accordance with the observations of Tverskoy *et al.* (1990) who recorded reduced

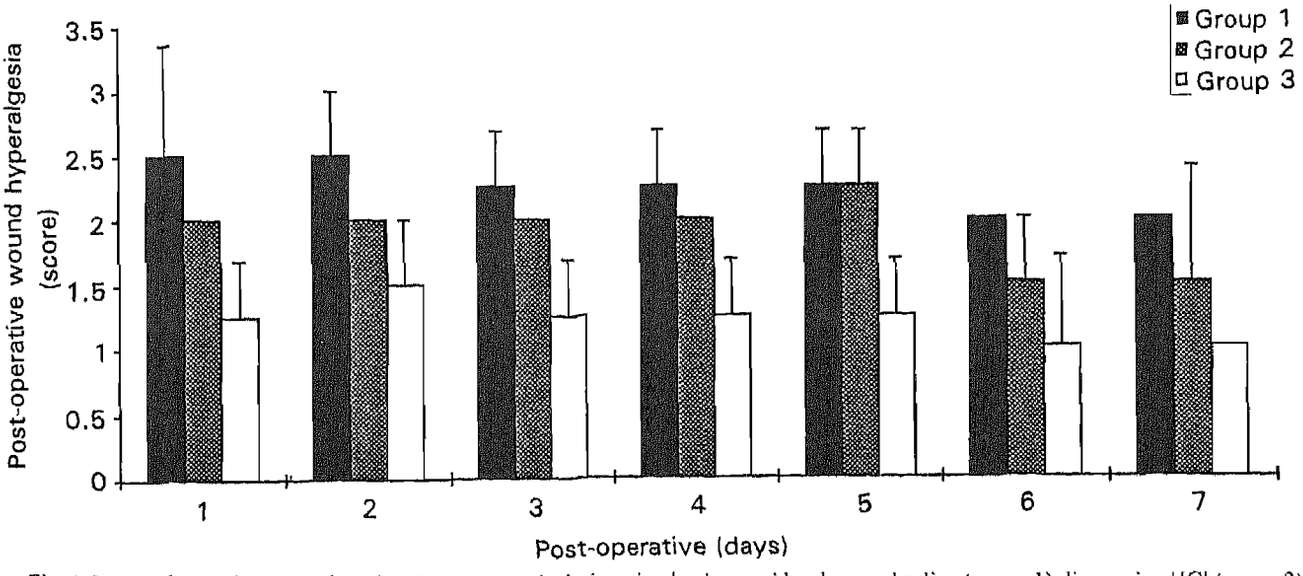

Fig. 1. Wound hyperalgesia on days 1 to 7 post-operatively in animals given epidural normal saline (group 1), lignocaine HCl (group 2) and ketamine HCl (3).

wound hyperalgesia for 10 days in patients operated under spinal block with bupivaccaine as compared to general anaestheria group. Minimal and significantly ($P < 0.05$) lesser wound hyperalgesia recorded in animals of ketamine group 3 as compare to control animals was attributed to direct antagonistic action of ketamine on NMDA receptors in spinal cord. NMDA receptors are involved in establishment of hyperexcitability state of spinal cord (Thompson 1990) and NMDA receptor antagonists have been found effective in preventing this state in experimental models (Woolf and Thompson 1991). Ketamine is a specific and noncompetitive antagonist of NMDA receptors and therefore might have reduced the wound hyperalgesia in the present study as earlier reported by Amarpal *et al.* (1995).

Girth of the operated limb increased significantly and maximum increase was recorded on days 3-4 post-operatively in all the groups. Increase was, however, more significant in groups 1 and 2 ($P<0.01$) as compared to animals of group 3 ($P<0.05$). A reduction in swelling was recorded after 3-4 days in all the groups and by day 7, post-operative swelling had returned to near pre-operative values in animals of groups 2 and 3 but it was still significantly higher than preadministration value in group 1. Percentage increase in swelling was also maximum in animals of group 1 and minimum in group 3. At the site of surgery traumatized tissues release a number of inflammatory mediators like histamine, bradykinin, norepinephrine, prostaglandins, cytokines, 5- hydroxytryptamin, leucotriencs, neuropeptides, phospholipase A_2 and superoxide etc (Treeds *et al.* 1992). These agents are directly responsible for post-operative inflammation and oedema and thus swelling. On the other hand, sustained nociceptive impulses from the site of surgery may also trigger spinal hyperexcitability and initiation of spinal cord reflexes which can further increase the swelling at the site by induction of neurogenic inflammation. Reduced swelling recorded in animals of treatment groups as compared to animals of group 1 suggests that thiopental sodium general anaesthesia is unable to completely check the transmission of noxious impulses at spinal cord. In animals of group 2 where lignocaine was administered epidurally reduced swelling could be attributed to the ability of lignocaine to block the axon reflexes through its local anaesthetic action at axon level as reported by Barnes *et al* (1990). Dhal *et al.* (1994), however, have reported direct anti-inflammatory action of local anaesthetics through inhibition of granulocyte and lymphocyte function, platelet aggregation, production and release of phosphlolipase A_2, superoxide and histamine. Thus antiinflammatory effect of lignocaine might have acted synergistically with diclofenac given post-operatively and therefore reduced the post-operative swelling. Minimum swelling in animals of group 3 where ketamine was administered could be explained on the basis of multiple action of ketamine. Ketamine not only abolishes the spinal hyperexcitability which plays an important role in post-operative inflammation (Woolf 1989) but also has anti-inflammatory action by inhibition of histamine release (Gatean *et al.* 1989). In addition Hirota *et al.* (1995) reported that ketamine decreases vascular permeability and thus it can reduce the post-operative oedema, which also contributes to the increase in girth of operated limb.

Movement associated pain represents the deep tissue pain and indicate functional disability of the organ. It is manifestation of neuropathic pain which is caused by mechanical hypersensitivity resulting from central sensitization evoked by A- high threshold mechanoreceptors which normally do not produce painful sensations (Woolf and Chong 1993). Mean + SE scores for pain are shown in Fig. 2. On first post-operative day, pain was significantly

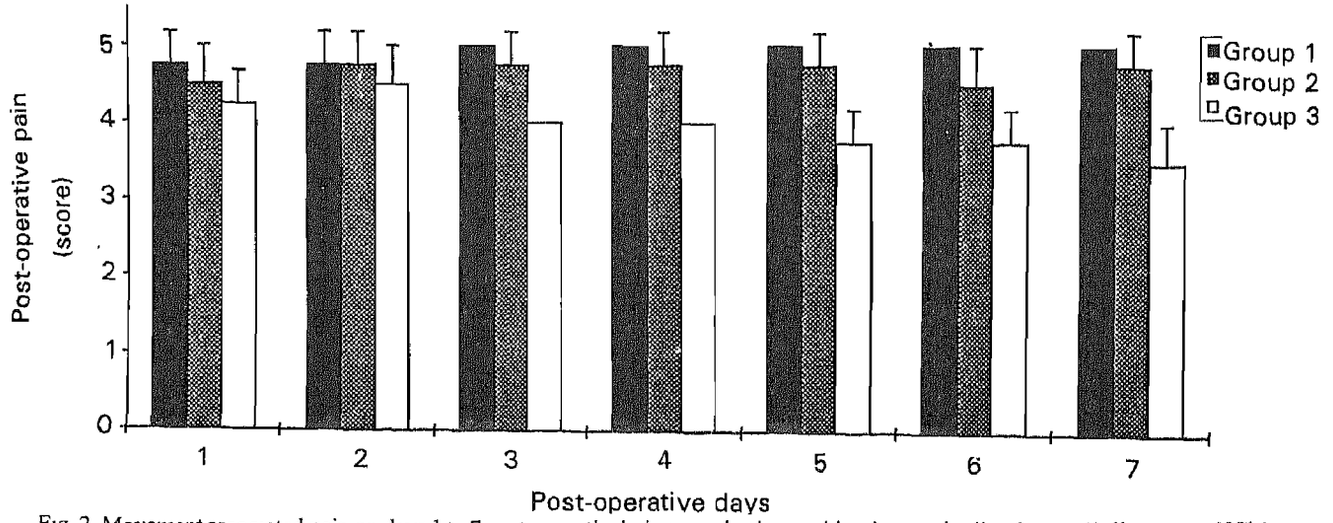

Fig. 2. Movement associated pain on days 1 to 7 post-operatively in animals given epidural normal saline (group 1), lignocaine HCl (group 2) and ketamine HCl (group 3).

($P<0.05$) lesser in groups 2 and 3 as compared to group 1. Form day 2 onwards, though animals of group 2 exhibited lesser pain as compared to group 1, the difference was not significant. On the other hand, animals of group 3 showed significantly ($P<0.05$)lesser pain than the animals of group 1 throughout the observation.

In the present study lignocaine was effective to prevent the post-operative pain significantly for first post-operative day probably by blocking the noxious signals at nerve root level during surgery and thereby preventing central sensitization. The results are in accordance with the finding of Sinclair *et al.* (1993) who reported that lignocaine was effective in reducing the post-operative requirement of meperidine only on first operative day in human beings. The lignocaine was, therefore, effective only for shorter duration. Minimum pain was recorded in animals of group 3 up to last observation day (7th day). Similar to local anaesthetics, ketamine has local anaesthetic action (Durrani *et al.* 1989) and antiinflammatory activity (Hirota 1995) but more pronounced and longer lasting pre-emptive effect of ketamine on pain might be due to specific antagonism of NMDA receptors which are responsible for establishment of spinal excitability. NMDA receptor antagonists, in addition to preventing the induction of spinal hyperexcitability can reduce it, once it is established (Woolf and Thompson 1991).

The results of the present study indicated that pre-operative administration of epidural lignocaine and ketamine in addition to general anaesthesia are useful in minimizing the post-operative would hyperalgisia, swelling and pain. Lignocaine, though had only short lived advantage over general anaesthesia alone, the ketamine produced more consistent and longer lasting effect. It seems that preoperative epidural ketamine may reduce post-operative, inflammation, swelling and disability and therefore, may be useful in reducing post-operative care and medication.

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Indian Journal of Animal Sciences **70** (4): 366-368, April 2000

Status of reproductive performance in rural buffaloes artificially inseminated using deep frozen semen

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Received : 17 February 1999; Accepted : 4 October 1999

ABSTRACT

Data on 6 762 artificial inseminations on 5 793 village buffaloes belonging to 2 650 farmers spread over 17 villages in Maharashtra during June 1991 to September 1996 were analysed for studying reproductive performance in rural buffaloes artificially inseminated using deep frozen semen. The average farmer herd size was of 2.19. The mean conception rate was observed to be 51.84 ± 0.61%. The local buffalo breeds (Pandharpuri and Nagpuri) exhibited higher conception rate than Murrah or Mehsana breeds. Generally conception rate improved from first lactation (39.36±1.61%) to third lactation (56.25±1.67%) and then declined. Parity, season of insemination, uterine horn tone and AI sequence had significant effect on conception rate. Presence of mature follicle on either right or left ovary, condition of cervix, type of vaginal discharge and even the thawing temperature of water did not affect the extent of conception rate. It was recommended that among other factors, farmer observation on vaginal discharge, vulval swelling, bellowing can be relied upon for improving conception rate.

Key words: Artificial insemination, Conception rate, Deep frozen semen, Rural buffalo

Variability of response to AI by the farmers, is because of the use of different breeds of buffaloes, ignorance about the heat symptoms, silent heat, variation in expression of heat, seasonal fluctuations in heat expression, variation in feeding and management practices. Scanty literature available on response of village buffalo to AI does not provide sufficient information for suitable recommendations for improvement in conception rate under village condition. An attempt, therefore, was made to study different factors affecting AI in rural buffaloes.

MATERIALS AND METHODS

Artificial insemination data, generated from June 1991 to September 1996 of 5 793 buffaloes belonging to 2 650 farmers spread over to 17 villages in Kolhapur district of Maharashtra. The breed and parity-wise distribution of buffaloes was made (Table 1).

The breed-wise classification was made on the general appearance of the animal to the respective breed. The graded animals are also thus included in the respective breed class. The calls for inseminations were received at the centre headquarters and the animals were inseminated with deep frozen semen at the door of the farmers. The heat symptoms like bellowing, tumefaction of valvular lips, vaginal discharge,

Table 1. Breed and parity-wise distribution of buffaloes

Breed	Parity						Total
	1	2	3	4	5	6	
Non-descript	467	272	653	927	543	79	2941
Pandharpuri	14	21	62	78	30	5	210
Mehsana	25	26	51	82	18	1	203
Nagpuri	9	43	208	199	113	24	596
Surti	225	350	621	396	128	8	1728
Murrah	24	171	14	38	20	2	115
Total	764	729	1609	1720	852	119	5793

cervical and uterine tonicity, extent of cervical opening were recorded. The thawing temperature of water was recorded to study the effect of water temperature on conception. The semen was deposited inside the body of uterus. The animal, if not repeated after AI, was examined after 90 days for pregnancy. In the area of study, 0.43% of buffaloes were found sold or transferred. Generally the sale or transfer is effected after the animals are confirmed pregnant. Thus if the inseminated animals were not reported back for AI and were found sold before they were confirmed pregnant, they were treated as pregnant. Three seasons (rainy - July to October, winter - November to February, and summer - March to June) were considered for studying the effect of season of insemination on conception. Standard statistical procedures

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Table 2. Breed-and parity-wise conception rate (%) of rural buffaloes

Breed	Parity																		Overall		
	1			2			3			4			5			6					
	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE
ND	554	36.89	2.09	303	56.48	2.85	733	53.12	1.84	1089	54.22	1.51	618	52.64	2.08	91	47.31	5.25	3388	50.88	0.85
Pandharpuri	20	50.05	11.46	25	44.06	0.12	66	68.21	5.77	92	63.08	5.05	34	52.99	8.68	6	16.75	6.65	243	58.89	3.16
Mehsana	31	38.77	8.88	31	48.44	9.11	59	47.51	6.55	104	35.64	4.71	32	37.56	8.68	1	0.10	0.00	258	40.37	3.05
Nagpuri	9	44.50	17.55	47	63.87	7.08	228	56.62	3.28	251	53.83	3.15	136	50.78	4.30	32	50.05	8.97	703	54.53	1.87
Surti	276	43.90	2.98	409	52.37	2.47	719	59.15	1.83	467	52.94	2.31	149	54.41	4.09	12	41.73	14.85	2032	53.84	1.10
Murrah	26	34.66	9.50	19	47.42	11.45	17	47.11	12.47	47	40.49	7.22	26	42.37	9.87	3	33.40	33.0	138	41.36	4.20
Overall	916	39.36	1.61	834	54.00	1.73	1822	56.25	1.16	2050	53.02	1.10	995	51.91	1.58	145	45.57	4.15	6762	51 84	0.61

were used to analyse the data (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

The average buffalo herd size was 2.19 at village level. The overall mean conception rate in buffaloes at village level was recorded as 51.84 ± 0.61% Hassan and Zia-ur-Rahman (1994),Bhosrekar *et al.* (1994) under field conditions and Baruselli *et al.* (1994) on farm conditions recorded higher, whereas Jain and Tailor (1995) have reported lower conception rate when compared with present study. Breed, and parity-wise conception rates are presented in Table 2. The animals of local area (Pandharpuri and Nagpuri breed) exhibited higher conception rate (58.89 ± 3.16 and 54.53 ± 1.87% respectively) compared to Mehsana and Murrah type of animals (40.37 ± 3.05 and 41.36 ± 4.20% respectively). The conception rate observed in Surti type buffaloes was in similar range (53.84 ± 1.10%) to that observed in Nagpuri buffaloes. The large class of animals was non-descript and showed slightly lower conception rate of 50.88 ± 0.85%. The higher conception rates in local buffaloes compared to graded Murrah also been noted by Singh and Singh (1988). Generally the conception rate increased from first lactation (39.36 ± 1.61%) to third lactation (56.25 ± 1.16%) and declined thereafter. Heifers as well as animals beyond fifth lactation conceived less than those in second to fifth lactation. Agarwal and Purbey (1983) on a small sample size observed higher conception rate in heifers compared to buffalo cows.

Analysis of variance technique was used to study the effect of village, breed, parity, season of AI, thawing temperature of water, tumefaction of valvular lips, cervical tonicity and partial or total openness of Os, uterine tonicity, type of vaginal discharge, bellowing etc. and the results are presented in Table 3. Effect of both breed and parity were significant on conception rate of field buffaloes. The variation due to villages was not important and did not affect conception rate in buffaloes. The conception rate among villages ranged from 38.95 ± 11.81 to 62.54 ± 10.08%. High standard errors indicated large variation in conception rate amongst the villages.This could have caused because of between and within village variation in buffalo management practices adopted by the farmers.

Variation was observed in conception of buffaloes inseminated in different years and seasons. Animals inseminated during winter showed significantly higher conception rate (55.58 ± 0.92%) than inseminated during summer (46.95 ± 1.18%). Jain and Tailor (1995) also observed higher conception rate in Surti buffaloes during period of moderate temperature and humidity. The year-wise conception rate ranged from 49.16 ± 1.31 to 55.53 ± 1.30% from 1991 to 1996 and the differences were important.

Thawing water temperature is known to affect the conception of animal. In the present study, thawing water temperature ranged from 101 to 107 °F (38.3 to 41.6 °C). The variation in the conception rate was not statistically important in the consiffered range of temperature owing to thawing water temperature. Although thawing temperature of 105 °F (40.5 °C) and above resulted in more number of conceptions 59.13 ± 4.30%.

Majority of buffaloes (85.67%) were inseminated only

Table 3. Analysis of variance of conception rate in rural buffaloes

Source of variation	Degree of freedom	Mean square
Year	5	18124.93**
Village	16	1836.39
Breed	5	1068.81
Parity	5	23866.04**
Season	2	25919.21**
AI sequence	2	7540.86 *
Thawing temperature	3	2145.03
Horn tone	3	123713.41 **
Vaginal discharge type	4	3031.21
Ovary activity	4	2728.80
Bellowing	1	9900.02 **
Vulval swelling	1	79382.53 **
Cervix status	1	5.67
Os opening	1	7938.64
Micturation	1	680.31
Breed bellowing	5	897.19
Breed vulval swelling	5	1208.17
Breed Os status	5	629.98
Residual	6692	2275.35

P <00.05 *, P 00<.01 **

once.Amongst those inseminated 12.79% reported for second heat while only 1.5% animals reported for third or more heats. The mean conception rates for these 3 classes were 51.13 ± 0.65, 56.92 ± 1.68 and 49.09 ± 4.92% respectively. The higher conception rate during second AI could be because of suitable treatment to buffaloes prior to AI and more heifers getting conceived during second insemination rather than first.

Farmers consider vaginal discharge as one of the important symptoms of heat. The nature and manner of vaginal discharge of animals in heat was recorded and was divided in 5 groups for assessing its effect on conception, viz. transparent and clear, pussy, clear and roapy, clear and watery and absence of discharge at the AI but buffalo owner claiming his noting the discharge. Although animals showing clear and roapy vaginal discharge did exhibit higher conception rate (58.21 ± 5.00%),and those with pussy vaginal discharge exhibited low conception rate (31.73 ± 3.30%). There was no significant difference in conception rates among different groups. The buffalo owners information about presence of vaginal discharge during heat can be relied up on as the conception rate in this group was observed to be 51.62 ± 2.21%.

The tonicity of uterine horn was different for different animals inseminated. The uterine horns of less than one-tenth (9.33%) animals were flaccid, the conception rate in these animals was low (18.31 ±1.53%). Majority of animals (77.6%) exhibited good uterine horn tone and more than 54% of these animals conceived. Animals (6%) showed excellent horn tone and 60% of these animals conceived on insemination. Nearly 7% of animals showed coiled uterine horns.

The incidence of conception in relation to presence of graafian follicle on ovary, at the insemination was examined by grouping the data in 4 groups (presence of mature follicle on, left ovary, right ovary, both the ovaries and data not available.) The presence of mature follicle on either right or left ovary did not significantly affect the extent of conception, the conception rate in this group was 53% . The pregnancy rates in inseminated animals showing mature follicles on both the ovaries were less frequent 43.13 ± 6.18% .

The 0.5% inseminated animals exhibited hard cervix, 0.74% kinked-cervix, while 0.21% animals showed abnormally small-sized cervix. In view of small numbers in each of these subgroups, they were grouped as one. This group recorded significantly low conception rate (25.05 ± 4.43%) compared to rest of the group of animals (52.22 ± 0.61%). Amongst the total inseminations carried out, the cervix was partially closed in 3.85% inseminations, resulting in significantly low conception rate (16.62 ± 2.31%) as compared to rest of the group showing conception rate of (53.25 ± 0.62%). The 2.29% of buffaloes were recorded micturating when presented for insemination. Although more of these animals conceived (57.46 ± 3.98%) compared to others (51.71 ± 0.61 5) the difference between the group was not important.

There is need to be considered for improvement in the conception rate in rural buffaloes, the factors like breed and season. Farmer observations on vaginal discharge, vulval swelling, bellowing can be relied up on. Amongst other symptoms of heat uterine tonicity, Os condition, type of vaginal discharge and thawing temperature are equally important.

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Indian Journal of Animal Sciences **70** (4): 369-385, April 2000

Biology of ovarian follicles in the goat: A review

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Received: 29 September 1998; Accepted: 30 September 1999

Key words: Goat, Ovarian follicle biology

The goat, a multipurpose animal, is used since long for the production of milk, meat, hide, wool and even for transport in remote hilly areas (Sawhney 1992). It is a hardy species and survives well on hill sides, deserts and in plains as well. The goat population in India (102 million) is number one in the world (FAO Yearbook 1989) and contribute about Rs 350 crores annually to the Gross Net Production (GNP) of the country despite minimum yield/production on world basis. The goat is a prolific breeder with a litter size varying from 1 to 4. During the last decade, a number of research papers have appeared on the morphology, histochemistry, biochemistry and physiology of various aspects of folliculogenesis and ovum maturation both *in vivo* and *in vitro* including the involvement of various extragonadal, intragonadal, paracrine and autocrine factors. There is an urgent and timely need to integrate this information and then to point out the gaps in our knowledge for future investigation, especially in the light of recent advances in the biology of ovarian follicles in other ruminants including cattle, sheep, swine, etc.

Various reports on the improvement of genetic potential of goats through artificial insemination or through embryo transfer technology had been used (Chauhan and Anand 1991, Pawshe *et al.* 1994a, b, Chauhan *et al.* 1997a). *In vitro* fertilization has recently been implicated to the successful development of blastocysts for studying their genetic superiority, polyembryony, production of transgenic embryos and subsequent embryo transfer (Barnes *et al.* 1993, Madan *et al* 1994, Chauhan *et al.* 1999).

Therefore, an attempt was made to review the available information on different aspects of biology of ovarian follicles in the goat ovary as well as to identify the areas for future investigations to enhance and efficate productivity of this small ruminant.

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Ovarian structure

The caprine ovaries are oval in shape 0.7 to 1.9 cm long, 0.5 to 0.9 cm in width and 0.2 to 0.4 cm in thickness and weigh 0.8 to 1.2 g (Roberts 1971, Gretty 1975, Singh and Prakash 1988). The bisection of the ovary has revealed 2 demarcated zones, the peripheral zone or cortex or zona parenchymatosa (375 to 490 mm in thickness) and central zone or medulla or zona vasulosa (215 to 305 mm in thickness) (Singh and Prakash 1988). The broad cortex of the goat ovary contains largely the primordial follicles and follicles in various stages of growth and atresia, interstitial gland tubules and some epithelial cords (Sharma *et al.* 1992). Cortical stroma is intricate and a number of blood vessels and nerves are seen in it. The cortex is externally surrounded by a surface epithelium earlier known as germinal epithelium. It is simple and comprised squamous, cuboidal or columnar cells (5 to 20 µm in height). It is cuboidal to columnar in ovaries of young goats and transforms to squamous with the advancement in age (Singh and Prakash 1988). Beneath this lies a thick layer of tunica albuginea composed of coarse aveolar connective tissue with fibroblast cells and collagen fibres oriented parallel to the ovarian surface (Singh and Prakash 1988). The term albuginea was assigned to it because of its white colour (Ham and Cormack 1979). The cortical stroma consists of a large number of fibroblast-like cells and collagen fibres oriented in various directions. These cells are spindle-shaped and contain large oval and darkly stained nuclei. The continuity of stroma is interrupted by follicules at various stages development or regression, and corpora lutea. Of the 5 ovarian compartments, viz. the normal developing follicles, atretic follicles, corpora lutea, interstitial gland tissue and the stroma, we shall restrict our focus only on the normal developing follicles.

The primordial follicles lie just close to the surface epithelium. They consist of large centrally placed oocyte surrounded by a single layer of flattened follicular or granulosa cells separated from the surrounding stromal elements by a non-cellular basal lamina. The size of the granulosa cell nuclei is large in proportion to their cytoplasm. As follicle grows the granulosa cells multiply and form a multi-layered follicle

wall around the developing oocyte (Sharma *et al.* 1992). The normal developing follicles can be studied under 7 stages (Kaur and Guraya 1983):

Stage I: Preantral follicles with 2 to 3 layers of granulosa cells surrounding the oocyte

Stage II: Preantral follicles with 4 to 5 layers of granulosa cells encircling the developing oocyte

Stage III: Preantral follicles with 6 to 7 layers of granulosa cells

Stage IV: Largest preantral follicle with 8 or more layers of granulosa cells around the oocyte

Stage V. Follicle with early signs of antrum formation

Stage VI: Follicle with a single medium-sized cavity or two small cavities on either side of the oocyte

Stage VII. Graafian follicle with large well formed antrum.

In a fully differentiated follicle, the oocyte is surrounded by the zona pellucida, membrana limitans is present between the theca and granulosa. The cellular stratum of granulosa appeared heterogenous. Theca interna is glandular while theca externa is fibrous. Antrum formation starts at 4 to 5 layered stage. Cumulus oophorus is distinct in large follicles. Zona formation and theca differentiation start in 1 to 2 layered follicle but, thereafter, they increase only in size (Sharma *et al.* 1992).

Primordial follicle

Like other mammalian species primordial oocytes in the goat ovary remain arrested at diplotene stage of meiotic prophase for a long duration in the ovarian cortex and resume maturation (meiosis) only after the goat has attained sexual maturity (6 months) then the hormonal changes induce active follicular recruitment growth and maturation or atresia (Pineda 1989). The primordial follicles are separated by thick stromal components like connective tissue, fibroblasts, collagen fibres, vascular bed, and nerve fibres. Primordial follicle is a simple structure consisting of primordial oocyte surrounded by a single layer of flattened granulosa cells demarcated from the stroma by the non-cellular basal lamina. These follicles are lodged just beneath the surface epithelium on the dorsal facet of the ovary. These oocytes are characterized by large lightly stained ooplasm and a large spherical nucleus and a large darkly stained eccentrically placed nucleolus. Nucleoplasm shows the presence of darkly stained small dot-like chromatin structures, some of such structures are seen adhering the nuclear envelope and a few lying on or by the side of nucleolus. Ooplasm (16μm × 32μm to 80μm × 96μm in diameter, Singh and Prakash 1988) showed the presence of coarse granules of various sizes, shapes and staining reactivity (Sharma and Sawhney, unpublished observation). In ultrastructure, the primordial oocytes showed the presence of a large crescent juxtanuclear congregation of different cell organelles called as cytocentrum or archeoplasm or Balbiani's vitelline body or yolk nucleus. It comprised stacks of Golgi complex, spherical mitochondria, ribosomes meshwork of granular endoplasmic reticulum and pleiomorphic bodies (Sharma *et al.* 1994). Numerous cisternae tubules and large vesicular bodies of Golgi are amongst the prominent structures of the yolk nucleus aggregation. Particles of medium to high electron density are also seen in the congregation. A magnified version of the saccules of Golgi complex with secretory material of electron-dense and homogenous nature was clearly visualized. A number of microtubules and a few stacks of microfilaments are also seen in this complex. Similar fine structural details were reported in oocytes of cattle, buffaloes and other mammalian species (Guraya 1968, 1979b, Dovrak and Tesarik 1980, Guraya 1997, 1998). This structural similarity opines that the paranuclear complex is structurally equipped for synthesis of various RNAs and proteins. Electron microscopic studies on unilamellar follicles of rat, guinea pig, rabbit and hamster revealed rather a simple aggregates of vesicles or saccules as large Golgi complex in mouse (Guraya 1985, 1997, 1998, Wassarman 1998).

Some of the Golgi complexes are scattered throughout the ooplasm generally interspersed with mitochondria and are also seen in the peripheral region of this paranuclear complex. The Golgi complexes are mostly associated with elements of endoplasma reticulum near the oolemma. Their membranes appear as continuous structure with the endoplasmic reticulum. Masses of ribosomal fibrils units and heterogenous spherical bodies are seen in the vicinity of the nuclear membrane in some oocytes, in others they lie at some distance away from the nucleus (Sharma *et al.* 1994). Oocytes in which polyribosomal fibrils and microfilaments are attached or are lying adjacent to the nucleus, the membranes of endoplasmic reticulum are structurally linked to the outer leaflet of the nuclear envelope at intervals around its entire circumference. These fibrils are also associated with Golgi complexes scattered throughout the cytoplasm. The rough-surfaced paired membranes of polyribosomal fibrils and microfilaments are continuous at their periphery with granular peripheral endoplasmic reticulum. Vesicles of endoplasmic reticulum in the peripheral cytoplasm are frequently filled with an electron dense amorphous material. No vesicular smooth endoplasmic reticulum was observed. Individual mitochondria associated with flattened elongated endoplasmic are also visualized at some places. The basic organization and cell organelle associated with this complex or Balbiani's vitelline body in goats (Sharma *et al.* 1994) are identical to that of other mammalian species in general and bovine in particular (Guraya 1968, 1979b, Dovarak and Tesarik 1980, Guraya 1997, 1998). However, particulate paranuclear complex commonly observed in bandicoot (Guraya 1985) oocyte is not seen in goat thereby indicating either species specific differences or different paces of follicular development.

The paranuclear Golgi complex in goat primordial oocytes (Sharma *et al.* 1994) is similar to the large Golgi complex associated cell organelle organization reported in human

primordial oocytes and has been designated as cytocentrum (Devorak and Tesarik 1980, Guraya 1985, 1997). The presence of pleiomorphic bodies and endoplasmic reticulum associated with this Golgi complex is a modification of endoplasmic reticulum only (Hertig and Barton 1973, Guraya 1985, 1997). Basic organization and cell organelles associated with this Golgi complex or Balbiani's vitelline body in goat are identical to that of other mammalian species (Sharma *et al.* 1994). The ooplasm of primordial oocyte in the goat resembles that of dog in the sense that both contain large rounded mitochondria, vesicular elements of SER associated with Golgi (Tesoriero 1981). The sparsely distributed mitochondria are usually spheriodial or slightly elongated in shape. Mitochondria possess a few prominent peripheral arched cirstae and their matrix contain a few granules (Sharma *et al.* 1994). Elongated or filamentous mitochondria are few in number and rarely seen. The pleiomorphic variations in mitochondrial form, organization and structure in goat oocytes (Sharma *et al.* 1994) possibly represent mitochondrial division by fission and their role in metabolism of the developing oocyte (Dvorak and Tesarik 1980). Individual mitochondria closely associated with flattened or elongated cisternae of RER in goat oocyte, seems to be a characteristic association of bovine species. All these ultrastructural characteristics clearly indicate that primordial follicles acquire all cytological organelleles required for the biosynthesis of RNAs, proteins, etc. (Sharma *et al.* 1980).

Scanning electron microscopic studies have revealed that primordial oocytes in the goat ovary are enclosed in smooth textured amorphous material. A number of small and even undulations were commonly observed on the oocyte surface. This layer is comprised fine granules and appeared slightly fibrous in nature (Sawhney 1992). Similar structural organizations have already been reported in mice, rat and rabbit oocytes (Motta and VanBlerkome 1974).

Histochemically, the primordial oocytes of goat share general mammalian oocyte characteristics (Guraya 1985, 1997, 1998) as evidenced by their positive reactions with Feulgen, methyl green pyranin-Y, periodic acid Schiff's, Sudan black B, mercury bromophenol blue, toludine blue and alkaline phosphatase (Guraya Unpublished observations).

The structural organization of follicle wall of primordial follicles of goat revealed that oocyte is only in partial isolation from extra-follicular ovarian compartments (Sharma *et al.* 1992, Sawhney 1992). Substances from the surrounding cortical regions have an assess to diffuse into and influence oocyte. Anderson (1979) demonstrated transfer of horse radish peroxidase grains from adjacent lacuonae between the follicle cells into the oocyte. Zamboni (1974) further elaborated that oocyte surface is exposed to various components of stroma that all includes blood capillaries.

Follicular growth

Oocyte

The growth and differentiation of goat oocytes during folliculogenesis are governed by the activity of granulosa cells whose activity is regulated by various extra ovarian and intra ovarian hormones and growth factors (Guraya 1998). The oocyte and follicle structure, composition and organelle distribution show considerable variability during the course of follicular growth and maturation (Sharma and Chowdhury 1998).

Ultrastructurally, the growing oocyte shows the presence of scattered mitochondria, elements of endoplasmic reticulum, vacuoles, glycogen and lipid droplets throughout the follicular growth (Sharma and Chowdhury 1998). The oolemma is thrown into numerous slender cytoplasmic processes, micropapillae and microvilli. Their number and size increased with growth of the oocyte. These thick processes interact with cumulus cells traversing through the zona pellucida. Golgi complexes are mostly localized in the vicinity of oolemma (Sharma and Chowdhury 1998). The mitochondria appear round to oval and no marked change in their shape and size is observed during oocyte growth. However, they are randomly distributed in the oocytes of medium-sized follicles, whereas, mitochondria get concentrated in the peripheral ooplasm of large oocytes. Proliferation of mitochondria by fission forms a characteristic feature of large goat oocytes (Sharma and Chowdhury 1998). Lipid droplets are oval to oblong in oocytes from medium-sized and are slightly elongated in large-sized follicles. Their distribution is uneven, however, their number is much higher in fully grown oocytes. Presence of lipid bodies in the cumulus cells suggest the strong possibility of their transport into the growing oocytes as in other mammals (Guraya 1985, 1997). Most of the cell organelles like Golgi complexes, mitochondria, glycogen bodies and phospholipid droplets lie scattered in the cortical ooplasm of oocytes in the large antral follicles, imparting central ooplasm a less populated homogeneous core (Sharma and Chowdhury 1998).

The increase in the number and dimensions of microvilli on the oocyte surface from medium to large oocytes are indicative of uptake of nutritional components from the surrounding somatic cells. In this regard, goat oocyte resembles cattle oocyte (Guraya 1997) and endorse the finding of Moor and Smith (1979). The intimate association between the mitochondria, endoplasmic reticulum and Golgi complex in goat oocytes advocates further the findings of Weakley (1966), who demonstrated temporarily dilation of endoplasmic reticulum indicative of transitory phases of storage of products acquired from the surrounding granulosa cells (Norrevang 1968).

The aggregation of mitochondria just beneath the ooplasmic membrane in goat oocytes is similar to that in other mammalian species (Guraya 1985, 1997). The presence of hooded mitochondria in large oocytes, strongly supports observations of Fleming and Saake (1972) who suggested that this hooding is indicative of an increase in the surface area for creation of specific micro-environment to efficate

exchange of metabolites with endoplasmic reticulum. This may be the possible reason for enhanced mitochondrial number and activity during later phases of oocyte development. The peripheral congregation of endoplasmic reticulum, mitochondria, Golgi complex and glycogen and lipid droplets in goat oocyte strongly endorse the earlier findings in other bovine in specific oocytes (Wassarman and Albertini 1994, Guraya 1998).

The localization of cortical granules beneath the plasma membrane in large oocytes is similar to that of bovine species (Szollosi *et al.* 1978, Guraya 1985, 1997). The presence of large lipid and glycogen droplets in the oocytes of the goat is also similar to that observed in bovine species in general (Fleming and Saake 1972, Guraya 1997). The increase in the number of these lipid droplets during oocyte growth are indicative of increased pace of lipid yolk deposition in large oocytes required to sustain early development after fertilization. A similar phenomenon of lipid yolk deposition was reported by Guraya (1985, 1997).

Two distinct genetically determined biochemical (metabolic) programmes function: (i) for differentiation of immature oocyte, and (ii) maturation of oocyte before ovulation (Guraya 1985, Moor and Gandalfi 1987, Leibfried-Rutledge *et al.* 1987 1989, Wassarman and Albertini 1994, Guraya 1997). The oocyte growth is initiated by (i) increase in size and (ii) extension of G2 (growth phase-2) of its meiotic cycle. The augmentation of oocyte growth is at very fast pace and the oocyte diameter increases from 15 to 125 μm, whereas, volume increase is more than 100-fold (Sharma, Unpublished observations).These changes are associated with increased accumulation of RNAs, proteins, lipids and other macromolecules and intra-oocytic organelle *de novo* synthesis and their reorientation (Sharma and Chowdhury 1998a, b). Quantitative analysis of DNA in cumulus-oophorus complex depicts a decline from 3.28 ± 0.01 to 2.93 ± 0.36 μg/mg wet weight as the complex advances from medium (2-5 mm in diameter) to large (> 5 mm) follicle (Sharma and Chowdhury 1998b). A similar pattern of changes is depicted by granulosa cells and oocyte. The decline in DNA content of cumulus-oophorus complex is attributable to the comparatively large volume and weight of the oocyte. In mouse, the oocyte volume is approximately 64-times higher than an average normal somatic cell (Wassarman 1983). As the volume increases the DNA/mg wet weight falls as also evidenced by increased incorporation of proteins and other macromolecules (Thiabault *et al.* 1987, Rabahi *et al.* 1993, Lorenzo *et al.* 1994, Sharma and Grewal 1996, Guraya 1997).

The RNA per μg of DNA content is increased from 1.41±0.03 to 1.43 ± 0,13 in oocyte. A similar trend is observed in cumulus-oophorus complex and the granulosa cells as they advance from medium to large follicle (Sharma and Chowdhury 1998b). The elevated quantity of RNA in granulosa cells of large follicles clearly is indicative of their active involvement in transcriptional activity and imparting these macromolecules onto the developing oocyte wherein this effect is not shown because the rate of incorporation of storage macromolecules was much higher than these RNAs (Wassarman 1983, Madan *et al.* 1994, Modesit *et al.* 1994, Saccharum and Bovister 1995).

Quantity of protein/μg of DNA increased significantly from 15.31 ± 1.13 to 21.31 ± 2.25 in cumulus-oophorus complex. This endorses the concept that oocyte continues to grow and acquire nutritional milieu and finally maturation inducing macromolecules from the surrounding granulosa cells (Buccione *et al.* 1990). Experiments proved that approximately 50% of the proteins are provided by granulosa cells to the growing oocyte, but the nature and functions of these proteins are still to be investigated (Bachvarova 1985, Guraya 1985, VanBlerkom 1985, Moor and Gandolfi 1987, Buccione *et al.* 1990, Wasserman and Albertini 1994, Guraya 1997).

Relatively very little information is available on the changes in transcription and translation during oocyte growth (Sharma and Chowdhury 1998a, b). The quantity of RNA/DNA ratio increases from 1.40 ± 0.03 to 1.43 ± 0.13, whereas protein/DNA increase is from 18.00 ± 4.40 to 18.71 + 1.50 as oocyte advances from medium antral follicle to large antral follicle (Sharma and Chowdhury 1998b). The pattern of changes in quality of proteins in a stage specific faction is being studied in our laboratory, however, such information is available on other mammals (Bachvorova 1985, Guraya 1985, VanBlerkom 1985, Leibfried-Rutledge *et al.* 1989, Wassarman and Albertini 1994). The contribution of exogenous protein from granulosa to the growing oocyte is yet to be precisely characterized.

Besides the accumulation of specific proteins and various types of RNAs, goat oocyte store deeply sudanophilic lipid droplets which are designated as lipid yolk bodies. The lipoidal aggregates are largely due to the coalescence of transported precursors from the surrounding granulosa cells by pinocytosis and phagocytosis (Guraya 1985, 1997, 1998). The physiological significance of the stage-specific accumulation of regulatory protein (proteins) and other macromolecules is a very challenging area of research.

Cytochemistry of developing oocytes shows considerable variations. Glucose-6-phosphate dehydrogenase (G-6-PDH) and lactate dehydrogenase (LDH) are reported in growing oocytes of mammals (Brinster 1966, Mangia and Epstein 1975, Sharma and Guraya 1998). The activity of these enzymes is directly proportional to the oocyte size up to a diameter of 85 mm and, thereafter, no further increase occurs in larger oocytes. Succinct dehydrogenase and acid phosphatase show similar trend in mice and guinea pig oocytes (Sharma and Guraya 1998). Such studies are lacking on goat oocyte. Little or no uptake of glucose lactate and succinate was demonstrated in mouse oocytes. Pyruvate is most efficiently utilized. Carbon dioxide release from pyruvate increased logarithmically with oocyte growth and reaches a

plateau when oocyte acquires a diameter of 50 μm (Eppig 1985, Sharma and Guraya 1998). Western-blotting analysis revealed that immuno-reactive-proteins to anti-ovarian aldose reductase antibodies are present in oocytes at all stages of folliculogenesis in low amount. Investigations on the enzymatic changes and selective utilization of substrates would be of immense value in determining the specific requirements of the developing oocytes in goat *in vitro*.

Cortical granules in the goat oocytes are observed at the primordial oocyte stage. They appear as small spherical, slightly ovoid, membrane limited organelles (Sawhney 1992). The glycoproteinaceous nature of these granules was demonstrated using PAS and bromophenol blue stains (Sharma Unpublished observations). As the oocyte grow in size, they get randomly distributed and finally come to lie just beneath the plasma membrane. For their structure, biochemistry and physiological role, they are similar to those of other mammals. The analysis of the cortical granules for establishing the maturational competence *in vivo* and *in vitro* would be rewarding to determine the cytoplasmic maturation of the oocytes required in *in vitro* fertilization and embryo transfer technology programme. Till date, use of lectins to study the different glycoconjugate binding sites and their variations during oocyte growth has not been undertaken for goat oocytes. The research in this area will open new vistas revealing the dynamic changes in the membrane-bound and free-macromolecular assemblies associated with molecular biology of developing oocyte.

The growing oocyte possess a large, spherical germinal vesicle (nucleus) with prominent nucleolus with one or more nucleoli which show conspicuous changes in their number and size during oocyte growth (Monga 1990). Extranuclear bodies mainly appear in the oocytes of growing antral follicles. They are of more common occurrence in the goat oocytes than those of sheep. These bodies could be the granulosa cells which have passed into the oocyte apparently by phagocytosis as observed in the growing oocytes of the Indian mole rat (Kaur and Guraya 1986) and lizards (Guraya 1989, Sharma and Grewal 1996). The another school of thought lead by Antonine *et al.* (1989) postulated that these extranuclear bodies originate from nucleolar mass, suggesting extrusion of nucleolar material essentially ribonuclear proteins into the ooplasm. The origin and functions of extranuclear bodies need to be investigated more precisely. However, their function seems to supply informational macromolecules (RNAs and proteins) to the growing oocytes required for early developmental stages (Guraya 1998). Small RNA containing vesicles were reported in forest door mouse *Dryomys nitedule* (Zybina *et al.* 1980). A huge quantity of ribonuclear proteins (RNPs) is produced by nucleoli and lampbrush chromosomes of germinal vesicle, and are transported into the ooplasm (Guraya 1979a, 1985, 1997, 1998).

The nucleoli of the growing oocytes from stage I to IV follicles appear granular and stain intensely for RNA. The fibrillogranular and vacuolated nucleoli present at the onset of antrum formation may represent metabolic status indicative of active RNA synthesis (Wassarman and Josefowicz 1978, Crozet *et al.* 1981). The germinal vesicle develops a dense Feulgen-positive body from the primordial follicle to Graafian follicle stage (Kiknadze *et al.* 1980). Nucleolar organizer of the nucleolus also reacts positively (Monga 1990).

The increase in oocyte dimensions both qualitative and quantitative depend not only on the synthetic activities of the oocyte itself, but also on surrounding follicle cells (Guraya 1997, 1998). Enhancement in oocytic biomembranes (cell organelles + plasma membrane folds) are crucial in this regard. The spatial redistribution of cell organelles endorses the changed (enhanced) metabolic attributes of the plasma membrane of the oocyte. The zona pellucida, an acellular barrier between corona cells and oocytes also adds to its thickness during follicle growth. The growth and further differentiation of the oocytes is maintained by numerous, precise heterologous contact zones which have a number of microvilli and micro-papillae on the oocyte surface that extend across the zona pellucida, interact with granulosa cells, and serve as the lifeline for the growing oocyte (Eppig 1985, Thibault *et al.* 1987, Wassarman 1994, Sharma and Sawhney 1999). The corona cells have one or more than one foot processes which join or terminate as bulbous extension (Thiboult *et al.* 1987, Sharma and Guraya 1990, Guraya 1997). Along the such apposing membranes junctional complexes are formed. These juxtaposed membrane specializations form the communication channel between the granulosa cells and the oocyte.

In the small antral follicles, the apposed cell membrane areas of contact associate by means of desmosomes (Sawhney 1992, Guraya 1997). As follicle advances, the number and dimensions of individual junction increase (Amsterdam *et al.* 1979, Sawhney 1992). Nexus (gap junctions) are highly specialized portions of plasma membrane between the 2 interacting cells (granulosa cells, granulosa-oocyte cells) and appear as minute attachments. In rabbit, abutment and annular nexus were reported (Bjersing 1978). Sharma and Sawhney (1998) identified such interacting surfaces in goat follicles, but their mechanism of operation and the permeability-permissible limits need to be investigated further. There does not exist any reduction in gap junction mediated communication between oocyte and cumulus cell up to germinal vesicle breakdown (Epigg 1985). The persistence of these anatomical structures, therefore, endorse the hypothesis that gonadotrophin-induced maturation is influenced by the loss of gap junctional complex leading to a decrease in meiosis arresting substances (Buccioni *et al.* 1990, Amsterdam 1979). Gap junction channels are made up of connexin family of proteins (Sutovsky *et al.* 1993). Sutovsky *et al.* (1993) demonstrated that connexin-43 positive gap junction was present between corona radiata cells and oolemma disappear after 6 hr of culture and synthesis of

connexin-32 starts after 6 hr thereby suggesting dynamic changes in channel structure and function during near the germinal vesicle breakdown stage. Such studies are still lacking in goat.

Follicle wall

As the oocyte from the quiescent pool of primordial oocytes enters into growth phase, the cellular components encircling the oocyte undergo dynamic changes in their shape, structure and functions. The components of the follicle wall facing the stroma get differentiated into theca, and the layer of cells immediately in contact with oocyte transforms into the granulosa. This demarcation ooccurs right when oocyte is surrounded by a single layer of granulosa cells (Sharma *et al.* 1992).

Thecal components: Theca of the developing ovarian follicles of goat is differentiated into theca externa and theca interna. Theca externa acts largely a cellular barrier, while theca interna as steroidogenic entity (Sharma *et al.* 1996). The light microscopic analysis of theca externa reveals that it comprises fibrous cells, collagenous filaments and a number of blood vessels, and nerve transverse this structure (Guraya *et al.* 1991, Sharma *et al.* 1996).

The ultrastructural details of goat theca externa cells revealed the presence of profiles of rough endoplasmic reticulum and elongated mitochondria possessing tubular cristae (Sharma *et al.*1996). Similar structural details of fusiform cells in theca externa of cattle are on record (Espey and Lipner 1994). The frequent occurrence of lipid droplets, glycogen granules and ramifying rough endoplasmic reticulum further endorse the non-steroidogenic function of these cells as already demonstrated in other mammalian species (Mestwerdt *et al.*1977, Dvorak and Tesarik 1980, Guraya 1985, 1997).

Theca interna consists of elongated to polyhedral cells and a thick bed of capillaries and lipoidal tissue lines this structure (Sharma *et al.* 1996). Ultrastructurally, the theca interna cells of the goat shows numerous profiles of smooth endoplasmic reticulum, ovoid mitochondria with tubular cristae, a number of Golgi complexes and lipid droplets (Sharma *et al.* 1996) indicating basic similarity in organizational plan in Bovidae as evidenced by the observations on proestrous cattle theca interna (Priedkalns and Weber 1968a, b).

The changes in mitochondrial shape and appearance of smooth endoplasmic reticulum in theca interna cells recorded from proestrous to estrous phase in cattle (Priedkalns and Weber 1968a, b) were not observed in the goat. The appearance of similar battery of cell organelles in theca interna cells of members of Bovidae is associated with development of steroidogenic enzymes (Label and Levy 1968) indicating acquisition of steroidogenic potential (Guraya 1997). An identical ultrastructural organization was reported in mammal in general (Guraya 1985, 1997). The presence of Golgi complexes in the goat theca interna cells is indicative of its involvement in the exchange of materials from the extra follicular reserves (Sharma *et al.* 1996) as also supported by the increasing acid and alkaline phosphatases activities related to the enhancement of membrane permeability (Sangha and Guraya 1989). Desmosomes, hemidesmosomes and other types of cellular junctions were also observed (Sharma *et al.* 1996).

Basal lamina: The general structural organization of basal lamina in follicles of goat resembles largely with that of other mammals (Anderson *et al.* 1978, Hirshfield 1991) and further strengths the concept that this layer acts as substratum upon which peripheral layer of membrana granulosa is laid. Biochemically, this layer contains hexose containing mucopolysaccharides, acid mucopolysaccharides, sialomucins, chondroitin sulphate, fibronectin, hyaluronic acid and fibrin sulphate (Anderson *et al.* 1978, Axelsson and Heinegard 1980, Bagavandoss *et al.* 1983, Polotie *et al.* 1984, Guraya 1985, 1997, 1998, Sharma 1987, Sharma *et al.* 1995a). The presence of various junctions like structures advocates further that this layer functions as sieve and selected molecules are transported into the developing follicle (Anderson *et al.* 1978, Hirshfield 1991).

The confluency of the materials of basal lamina origin with granulosa cells further indicates that this membrane is permeable to some molecules such as glucose and lactose (Guraya 1985) and selectively permeable to macromolecules (Hirshfield 1991). The localization of some membrane bound material (particulate) is indicative of the transport of enclosed material onto the developing follicle, whereas, its membrane is involved in the formation of basal lamina as suggested by Hirshfield (1991) that basal lamina increases 500-fold as from small-sized follicle to antral follicle. Lectin staining studies support this concept (Sharma 1987).

Most of the ovarian and thecal endocrine and non-endocrine (including nutritional milieu) components are transported onto the membrana granulosa through a non-cellular basal lamina which acts as sieve and allows only selected macromolecular size fraction of blood transudate to be passed onto the intrafollicular environment (Sharma *et al.* 1995a). Ultrastructural studies on basal lamina revealed several undulations and uneven thickness of this non-cellular layer bipartite structure comprised heterogenous stratum of electron dense material. This forms the stratum upon which the peripheral layer of granulosa cells is laid (Sharma *et al.* 1995a). It comprised of collagenous fibres, fibrin, laminin and other finely granulated material. A number of free and membrane bound granules of varied electron opacity are also frequent. At some places, a large number of such granules are congregated. This structure reveals a number of membrane modifications and at some places, the material of some cells is confluenced with the material of basal lamina, still at other sites desmosomes, hemidesmosomes and annulate type of modifications are common (Sharma *et al.* 1995a).

Membrana granulosa: With the oocyte growth, the layer

of cells immediately enclosing the oocyte undergoes rapid multiplication forming single layer of flattened granulosa cells of the primary follicles. The size of granulosa cell nuclei is larger in proportion to their cytoplasm (Sharma *et al.* 1992). As the follicle advances in size, the granulosa cells multiply and form a multi-layered structure which subsequently acquires small intercellular gaps leading to the formation of antrum that contains exudates from the surrounding granulosa cells (Sharma *et al.* 1992). Morphologically, histochemically and functionally membrana-granulosa is a heterogenous entity. The mural granulosa cells are more or less columnar in their general outline while cumulus granulosa cells are more or less spherical in shape. The corona radiata cells are highly differentiated group of granulosa cells (Sharma and Guraya 1990, Sharma and Sawhney 1999). Under scanning electron microscope, the mural cells appear more or less columnar in shape and are arranged in continuous matrix. The fine amorphous material is commonly found coagulated on the surface of these cells under such conditions, the cells appeared to be arranged in rows and are approximately of the same size, imparting a crater-like morphology to the whole structure. Small ruffles and intermittent membranous blebs are frequently observed on their surfaces (Sharma and Sawhney 1999). The cumulus cells are more or less irregular in shape and long cytoplasmic extensions (mostly unipolar) are observed in corona radiata cells. However, small granulosa to granulosa cell interacting extensions are also observed (Sharma and Sawhney 1999). Finely granulated material similar to that present in follicular fluid adheres to the surfaces of cumulus cells. A few granulosa cells with uneven outline showed short stumpy extensions on their surfaces. The corona radiata cells are attached to the zona pellucida by typical elongated cellular projections. The granulosa to granulosa extensions are fragile and few in number (Sharma and Sawhney 1999). The mural goat granulosa cell morphology resembles largely that of rabbits, mouse and rat (Narimoto *et al.*1986, Sharma and Guraya 1990). The heterogeneity in membrana granulosa observed in goat is similar to that described in rat (Sharma and Guraya 1990, Vrobeva and Tsitologiya 1990, Rao *et al.* 1991).

Under transmission electron microscope the granulosa cell cytoplasm showed the presence of Golgi complex, rough endoplasmic reticulum (RER), smooth endoplasmic reticulum (SER), and eliptical to round mitochondria with simple cristae (Sharma and Sawhney 1999). Multiple large clumps of fine granular material adhere to the nuclear envelope. The ultrastructural details of caprine granulosa cells resemble largely those of the rat, mouse, sheep, and bovine granulosa cells (Guraya 1985, 1997, 1998). The ultrastructural details indicate that granulosa cells are largely protein synthesizing in nature as in cattle and buffaloes (Guraya 1997).

The relative abundance between smooth and rough endoplasmic reticulum changes with the alteration in position within the follicle and with the advancement in size of the follicle (Sharma and Chowdhury 1998a). The number of mitochondria, glycogen and lipid droplets, and pleomorphic bodies also shows a change closely related to the metabolic obligations of the stage at specific site with the membrana granulosa (Sharma, Unpublished observation). Ax *et al.*(1984) have suggested that these changes are largely attributable to the variations in the oestrogen nidre.

The peripheral granulosa cells show tightly opposed membranes in the basal granulosa cells and those facing the follicular fluid (antrum) show an intercommunicated complex cellular meshwork through the invaginations and evaginations of the cell membranous structure. The junctional complexes with adjacent cells are a specialized area of plasma lemma which extends over a considerable length involving plasma membrane or cell process which invades into the cytoplasm. SEM studies have revealed such extensive processes. True intracellular inclusions, derived from a pinched off portion of the micro-extensions were reported in a number of animals (Merk *et al.*, 1973, Zamboni 1974, McGughey *et al.* 1990, Phillips and Dekel 1991, Sharma and Sawhney 1999).

The ultrastructural analysis of cumulus oophorous complex has revealed the structure of interacting sites between the oocytes and the granulosa cells. The presence of such interacting areas are suggestive of the metabolic cooperativity between the oocyte and the enclosing granulosa cells (McGughey *et al.* 1990). A number of investigations confirmed the nutritional and regulatory role of somatic cells. Sharma and Sawhney (1999) revealed that gap-junctions are the sites implicated in cell to cell communication and metabolic interaction between the granulosa cells and the oocyte. They further revealed that an elaborate gap junctional meshwork is present in membrana granulosa of goat. This is in agreement with the reports on other mammalian species (Guraya 1985, 1997). The ultrastructural details of caprine ovarian follicle are suggestive of the fact that follicle as a whole encloses membrana granulosa and oocyte in a common syncytium, and it provides structural basis for the transfer of nutritional and regulatory molecules from the somatic cells into the oocyte.

The membrana granulosa of goat antral follicles reveals a high degree of pleiomorphism in morphology and junctional complexes in different follicles (Sharma and Sawhney 1999). Similar variations in gap junctions were reported in rat, hamster and rabbit (Larsen *et al.* 1981, 1987, Recowsky *et al.* 1989). Different hypothesis were postulated to explain and specifiy the role of these interacting areas. It has recently been suggested that, these are involved in (i) the maintenance of gap junction integrity and prolonging the meiotic arrest through the transfer of meiotic arrest proteins from the granulosa cells into the oocyte, and (ii) physical disruption of granulosa syncytium network by pre-ovulatory LH range interrupts the transfer of this protein thereby permitting the resumption of meiosis (Sawhney 1999).

Follicular fluid: The follicular fluid appears between the

spaces in membrana granulosa of preantral follicles. It is more viscous and consistent in early phases. With the advancement in size of the follicle these small fluid filled spaces coalesce leading to the formation of antrum. In higher mammals especially sheep, goat, cattle and buffalo, the antrum acquires the huge dimension and in mature preovulatory follicles, it becomes slightly less viscous. Following types of follicular fluid were described in mammals (Guraya 1985, 1997):

(i) primary follicular fluid of membrana granulosa origin,
(ii) secondary follicular fluid of blood plasma origin, and
(iii) follicular fluid having leucocytes, cell debris that plugs ruptured follicle.

The straw coloured follicular fluid viscosity varies with the follicular growth and there appears a complex network of fibrous mucopolysaccharides. The pH of follicular fluid is normally 7.0, whereas pCO_2 varies with the stage of follicular growth and species (Guraya 1985). Histochemically, methyl green pyronin Y, periodic acid Schiffs and alcian blue (pH 1.0) reactivity increases with follicular growth and strong to intense reaction were reported in pre-ovulatory, antral follicular fluid of goat (Monga 1990). However, Feulgen test for DNA was negative in the follicular fluid. Chemically, follicular fluid contains proteins-albumins, globulins, fibrinogens, lipoproteins, galactosamine, brodykinin, heparin, IgA, IgM (Guraya 1985), amino acids-Asp, Thr, Glu, Glu-NH2, Gly, Ala, Met, carbohydrates-glucose, fructose, mannose, galactose, fucose, glycosamine, galactosamine, hyaluronic acid (Guraya 1985), enzymes-aminopeptidase, alkaline phosphatase, adenosine triphosphatase, acid phosphatase, aspartate amino transferase, alanine amino transferase, endopeptidase, collagenase, fructose bisphosphate aldolase, hyaluronoglycosidase, proteinase, plasmin, dipeptidase, lactate dehydrogenase, pyrophosphatase (Guraya 1985), non-nitrogenous metabolites-citric acid, lactic acid and sialic acid, steroids-androgens, cholesterol, oestrogens and progestagens, gonadotropins-FSH, LH, prolactin and their subunits (Guraya 1985), inhibitors and stimulators-FSH receptor binding inhibitor, inhibin or FSH-suppressing substance, luteinization inhibitor, luteinization stimulator, oocyte maturation inhibitor (Guraya 1985), elements and salts of copper, calcium, chlorine ions, sodium, potassium, inorganic phosphate, magnesium, zinc, phosphorus, sulphur (Sharma *et al.* 1995b, Sharma and Sharma 1997, Sharma and Vats 1998).

The follicular fluid biochemistry in the goat has largely been ignored (Guraya 1997). The follicular level of FSH in medium-sized antral follicles (2-5 mm in diameter) increased from 0.6 to 1.15 mIU/ml in large antral follicles (>5 mm in diameter). However, variations in follicular LH titre was from 5.8 to 7.8 mIU/ml in both the categories of the follicles, respectively (Sharma and Chowdhury, Unpublished observations). The intra-follicular progesterone level increased from 44 to 70 ng/ml from medium to large follicles. The variations in estradiol titre was from 2 300 to 5 800 pg/ml (Sharma and Chowdhury, Unpublished observations). These variations in follicular fluid hormonal titre corresponds to the circulatory gonadotropins.

The production of progesterone and oestradiol by ovarian follicles was high in PMSG-induced superovulation, in goats (Kumar *et al.* 1992). These fluctuations in follicular steroidogenesis are not related to the enhanced incidence of premature chromatin condensation in oocytes of PMSG-treated animals. PMSG reduced fertilization rates through elevated steroid production in the periovulatory period leading to the disturbances in gamete transport in the reproductive tract (Robinson 1973, Trounsn and Moore 1974, Crisman *et al.*1980, Evans and Armstrong 1984, Kumar *et al.* 1992). It is also suggested that cytoplasmic maturation of the oocyte is influenced by the changing level of hormones leading to the reduced fertilization rate (Kumar *et al.* 1992).

The quantity of sodium (Na) declined significantly ($P<0.05$) from 5281.63 to 4476.09 μg/g, as follicle advanced in size from early antral (<2 mm in diameter) to ovulatory (>6 mm in diameter), whereas, the concentration of potassium (K) increased from 141.54 to 211.87 μg/g (Sharma *et al.* 1995b). The variations observed are in agreement with earlier findings (David *et al.*1973, Chang *et al.* 1976, Knudson *et al.*1979, Guraya 1985). The elevated quantity of Na and K is in agreement with the findings of Knudson *et al.*(1979) who attributed it to the follicular fluid maturation from medium to large antral follicles. Investigations of Schuetz and Anisowicz (1974) and Chang *et al.*(1976) reported higher K values in the follicular fluid and ovaries obtained from slaughterhouse. The anoxia and intra-follicular acidosis because of ischaemia prior to collection may induce postmortal degeneration of granulosa cells leading to the leaching out of intra-cellular K into follicular fluid (Sharma *et al.* 1995b). Sharma and Vats (1998) analysed Fe, Mn, and Cu levels in 6 categories of normal developing follicles of goat. The Fe content increased as follicle advanced from early antral to preovulatory stage. These variations corresponds to the changes in steroid hormones (progestrone and oestrogen) that induces haemodynamic pulses in the vascular shunt of the developing follicles (Guraya *et al.* 1991). Development of smooth musculture arround the follicle also attributes to this variation. The decline recorded in the ovulatory follicles of goat may be due to ischemia leading to the rupture of follicle wall at stigma (a vascular deficient portion) (Guraya *et al.* 1991). The Mn level varied irregularly possibly because of changes in its utilazation and uptake (Sharma and Vats 1998). The variations in the Cu levels in different categories of follicles showed a positive relationship with oestrogen concentration that increases the ceruloplasmin synthetase (Sharma and Sharma 1997, Sharma and Vats 1998). The minute variations in different categories of follicles are possibly due to synergistic effect of progesterone and oestrgen (Sharma and Sharma 1997), as well as mobilization of Cu to erythrocuprein, heptocuprein, cytochrome-C oxidase, tyrosine monoamine

oxidase, ascorbic acid oxidase and a number of coenzymes in dehydrogenases required in fatty acid metabolism (Sharma *and Sharma 1997, Sharma and Vats 1998).*

The concentration of oestradiol in the follicular fluid after sponge withdrawal is nonsignificantly ($P<0.05$) different. However, in PMSG-primed goats, it is significantly higher and a significant increase in the onset of estrous is observed (Kumar *et al.* 1992). At 18 hr after hCG administration, the oestradiol decreased markedly in follicular fluid (Kumar *et al.* 1992). The quantity of progesterone is 0.2 n mol-1 ml and a significant increase is recorded after 18 hr of hCG treatment (Kumar *et al.* 1992). The mechanism by which PMSG alters follicular steroidogenesis *in vivo* in goat (Kumar *et al.* 1992) and sheep (Moor *et al.* 1985) is unknown. It may be due to predominant LH-like activity of PMSG (Farmer and Papkoff 1979, Henderson *et al.* 1990) that initially activates the theca interna in follicles recruited for growth to synthesize elevated quantity of androgen precursor that are acted upon by aromatase enzyme of granulosa cells leading to an increase in oestradiol accumulation after 48 hr of treatment (Kumar *et al.*1992). Moreover, the prolonged half-life of PMSG (McIntosh *et al.*1975, Schams *et al.* 1978), coupled with the ability of oestradiol to increase the responsiveness of granulosa cells to FSH (Richards 1980) further stimulates the follicles to continue producing increased amounts of oestradiol (Kumar *et al.* 1992). The follicle needs to be primed with oestradiol before FSH can induce increased synthesis of LH receptors (Richards 1980) thus responsible for the higher intra-follicular progesterone concentration (Kumar *et al.* 1992).

Regulation of follicle growth

Follicular development, differentiation and growth are controlled by intra-ovarian, intra-follicular and extra-ovarian endocrine and growth factors (Guraya 1997). Follicular growth is regulated under the dynamic interaction between FSH and LH produced by the pituitary (Greenwald and Terranova 1988, Greenwald and Roy 1994). Follicular growth is mediated by cAMP and is modulated by a number of factors including steroid hormones (progesterone, androgens, oestrogens), and non-steroidal factors-growth factors and neuro-regulatory peptides (Hirshfield 1989a, b, Tsafriri and *Adasha 1994, Guraya 1997). The pituitary gonadotropins,* ovarian steroids and non-steroidal factors interact to regulate the initiation of meiosis in foetal ovary, recruitment of primordial follicles and selection of ovulatory or atretic follicles (Tsafriri and Adashi 1994). *In vitro* studies demonstrated that local factors are of extreme importance as they react with the reproductive hormones to control specific ovarian functions. The isolation, chemical characterization, synthesis, functions and mechanisms of action of local factors including growth factors and neuro-regulatory factors, which have been extensively investigated for rodents, cattle human, are required to be studied for the goat. This information may help to improve the reproductive efficiency especially foliculogenesis and rate of superovulation in the goat.

The circulating level of oestrogen-17ß was 15.54 ± 1.0 and 15.30 ± 1.59 pg/ml on the day one of oestrus in Surti and Marwari goats, respectively (Pathak *et al.* 1992). The serum oestradiol level gradually declined (5.87 ± 0.93 and 5.64 ± 1.30 pg/ml) as luteal phase advanced up to day 13 of the oestrous cycle. Thereafter, the nidre of this hormone increased representing the new follicular phase and acquires a maxima (17.76 ± 1.86 and 16.51 ± 1.08 pg/ml) on day 20th (Pathak *et al.* 1992). However, Mgongo *et al.*, (1984a, b) reported slightly lower values for oestradiol-17ß in African goats.

The minimum progesterone level (0.50 ± 0.11 and 0.76 ± 0.11 ng/ml) in blood serum was recorded on day 1 of the oestrous cycle in Surti and Marwari goats, respectively. A peak in serum progesterone level was recorded on day 9 in Surti (3.32 ± 0.54 ng/ml) and on day 13 in Marwari goats (2.34 ± 0.58 ng/ml) (Pathak *et al.* 1992). Circulating progesterone was at base level in rest of the days of the cycle. A similar trend was reported for African goat (Mgongo *et al.* 1984a), Assami goat (Baruah *et al.* 1987), and for German goat (Wani 1989). The variations recorded in ovarian steroid production is a direct indication of the pulsulation in level of pituitary FSH and LH (Guraya 1997).

In vitro studies have indicated that FSH alters the biosynthesis of specific protein (Chakaraborty *et al* 1993). As the quantity of circulating FSH increases, the synthesis of specific type of protein increases, whereas, the proteins of other type decrease quantitatively. FSH stimulates the growth of large follicles by promoting the synthesis and release of stimulatory polypeptides of 370 kD MW but medium-sized follicular growth is regulated by oestrogen induced inhibitory peptides (Chakaraborty *et al.* 1993). Studies on stage specific analysis of proteins synthesized during folliculogenesis would be enlightening and results shall be directly implemented in the process of maturation and ovulation in goats *in vitro.* Principal hormones that influence follicular growth differentiation and maturation include gonadotropins (FSH, LH, prolactin) produced by the pituitary, and steroid hormones such as progesterone, androgens and oestrogens secreted by the ovary (Findlay and Carson 1980, Richard 1980, Guraya 1985, Hillier 1985, Ireland Roche 1987, Greenwald and *Terranova 1988, Inskeep et al. 1988, Gore-langton and* Armstrong 1994, Greenwald and Roy 1994). Specific role of FSH and LH in growth and differentiation of follicles has, however, not been studied in goats and buffaloes (Findlay 1993, Gore-Longton and Armstrong 1994, Greenwald and Roy 1994). It has been established that gonadotropins are required for the development of dominant follicles and production of oestrogen and for ovulation (Driancourt 1991, Driancourt *et al.* 1993). However, the mechanism that regulate the development of ovulatory and non-ovulatory dominant follicles remain to be determined.

In vitro culture of goat granulosa cells revealed time

dependent production of progesterone 0 hr, 0.92 ± 0.20, 6 hr, 1.54 ± 0.25, 12 hr, 2.15 ± 0.40, 24 hr, 2.65 ± 0.65, 48 hr, 3.95 ± 0.15, and 72 hr', 4.01 ± 0.30 ng/10^5 cells (Sharma A 1987). The estrogen release increased from 0.45 ± 0.20 at 0 hr to 3.75 ± 0.32 ng/10^5 cells at 72 hr of culture (Sharma A 1987). A similar pattern of secretion of hormones was observed in *in vitro* cultured theca cells. The estradiol production was more in theca cell culture than the granulosa cells (Sharma 1987).

Follicular dynamics

Camp *et al.* (1983) studied ovarian follicular activity by of laparotomy exteriozation of the ovaries and examination of slaughter house ovaries.In the goat, the growth and regression of ovarian follicles (≥ 3 mm) were investigated by trans rectal ultrasonography for 4 interovulatory intervals in each of 5 Saanen goats (Ginther and Kot 1994). The observed number of growing 4 mm follicles per day differed significantly ($P<0.05$) from randomness, thus indicating that follicles on the average, emerged in waves (groups). Averaged overall interovulatory intervals the number of 3 mm follicles on each day that later reached ≥ 6 mm, followed a pattern significant peak on days 0, 4, 8, 14 (Ginther and Kot 1994). A follicular wave defined by consecutive days of entry of follicles ≥ 6 mm into the wave, and the day of emergence was defined as the first day that the 6 mm follicles were 3 mm (Ginther and Kot 1994). In 75% interovulatory intervals wave emerged during each of day 2 to day 1 (wave 1), days 2 to 5 (wave 2), days 6 to 9 (wave 3) and days 10 to 15 (wave 4). Ovulation occurred during wave 4 (Ginther and Kot 1994). The mean days of emergence of waves 1 to 4 were days 1, 4, 8, 13 respectively. However, in 5 for these 15 interovulatory intervals 50% of the apparent waves emerged or were continuous so that a distinction could not be made between 2 waves (Ginther and Kot 1994). The largest follicle grew to a larger ($P<0.05$) maximum diameter for wave 1 (8.7 ± 0.3 mm), and 4 (9.7 ± 0.3 mm) than for waves 2 (7.2 ± 0.2 mm) and 3 (7.3 ± 0.2 mm) (Ginther and Kot 1994). They further suggested that the phenomenon of follicular dominance was more common during waves 1 and 4 than during waves 2 and 3. The inter wave intervals (days) were longer ($P<0.05$) for waves 1 (3.4 ± 0.2) and 4 (4.3 ± 0.6) than for waves 2 and 3 (2.5 ± 0.2 for each wave) and the correlation between maximum diameter of largest follicle and the subsequent inter-wave interval was significant for waves 1 and 4, but not for waves 2 and 3 (Ginther and Kot 1994). The remaining interovulatory intervals were irregular and involved more than 4 waves including 2 interovulatory intervals with prolonged follicular phases (day 14 and 21) and failures of ovulation (Ginther and Kot 1994). They have concluded that the predominant follicular wave pattern was 4 waves. The ovulation occurred from wave 4 and apparent follicular dominance was expressed during some follicular waves especially during waves 1 and 4.

The development of distinct groups of follicles during the oestrous cycle in cattle (Pierson and Ginther 1987a) and mares (Pierson and Ginther 1987b) was demonstrated by trans rectal ultrasonography. This technique allows the maintenance of day-to-day identity of individual follicles in specific animal and 2 or 3 waves occur during the estrous cycle in cattle (Pierson and Ginther 1988, Savio *et al.* 1988, Sirois and Fortune 1988) and one to two waves in mares (Ginther 1990, Sirois *et al.* 1988). In these 2 species, one follicle becomes dominant and far exceeds in its diameter than subordinates in the waves. In sheep dominance was equivocal, but may have occurred in association with the development ovulatory follicles (Ravindra *et al.* 1993, Schrick *et al.* 1993).

In goat ovary, some binucleate oocytes were observed in the primordial follicles. These healthy follicles were characterised by possessing 2 nuclei with 2 distinct nucleoli within 1 oocyte (Guraya *et al.* 1998). Biovular and triovular follicles were also observed at some stage. These polyovular follicles were enclosed within 1 follicle wall (Guraya *et al.* 1998). Binucleate ova may be formed during early development of the ovary when primordial germ cells proliferate by mitotic activity to form numerous female germ cells (Guraya *et al.* 1998). During this process in some primordial germ cells, the nucleus may undergo mitotic division to form 2 nuclei, which is not accompanied by cytokinesis, resulting in the formation of binucleate female germ cells or oocytes which gets associated with somatic cells to form primordial oocyte by completing early stages of meiosis in both the nuclei (Guraya *et al.* 1998). It may be possible that binucleate oocyte may be formed by the fusion of 2 adjecently placed primordial germ cells in the early stages of ovarian development. Multiovular follicles in the ovaries of sheep and goat appear to be formed by the enclosure of more than 1 primordial germ cells or primordial oocytes by the follicle wall. Morphogenetic factors involved in the formation of polyovular follicles remain to be determined at the cellular and molecular level. It also remains to be determined whether polyovular follicles can ovulate more than one egg in response to various hormonal treatments which are being used for superovulation (Guraya *et al.* 1998). These follicles generally undergo atresia.

Oocyte maturation

The caprine oocytes like those of other mammalian species remain arrested at dyctiate stage of the meiotic prophase until shortly before ovulation. It is preovulatory LH surge that triggers the resumption of meiosis. The spontaneous resumption of meiosis was demonstrated when oocytes are released from follicles *in vitro* (Edwards 1965). This phenomenon includes the germinal vesicle breakdown (GVBD) and culminates at metaphase II, where second meiotic arrest occurs. At this stage, oocyte is said to be mature and ready for fertilization (Edwards 1965). The cumulus enclosed or denuded oocytes collected from different

mammalian species and placed in suitable culture medium *in vitro* resume meiosis spontaneously and undergo germinal vesicle breakdown (GVBD) (Edwards 1965, Thibault and Gerard 1973). This phenomenon of spontaneous maturation *in vitro* was used as a model for studying the meiotic regulatory factors in oocyte maturation.

During the last decade, the importance of *in vitro* maturation has gained a great importance because this technique affords to produce a large number of embryos required for research and to get offsprings. Recently, a lot of research has been involved in the development and improvement of *in vitro* maturation (IVM) and *in vitro* fertilization (IVF) of oocytes of domestic animals (Brackett 1992). A number of factors including physical, physiological, biochemical and immunological play a significant role in IVM and IVF (Chauhan *et al.* 1997a, b, 1998a, b, 1999). The size of the follicle from which oocytes are collected (Fukui and Sakuma 1980, Iritanti 1988), the presence or absence of adhering cumulus cells (Leibfried and First 1979, Fukui and Sakuma 1980, Crister *et al.* 1986) and temperature at which oocytes are cultured (Lenz *et al.*1983) largely influence the success rate of IVM and IVF.

The follicular oocytes are harvested either by aspiration or by ovarian slicing, although the slicing of the follicles or ovary yields better oocyte recovery (Katska 1984, Wahid *et al.* 1992, Pawshe *et al.*1994b). Yet aspiration is commonly used (Gordon and Lue 1990). Experiments to improve oocyte recovery by follicular aspiration and *in vitro* maturation and fertilization and embryo culture in small ruminants were made (Keskintepe *et al.*1993). Crozet *et al.* (1995) studied the developmental competence of caprine oocytes collected from follicles of different sizes following *in vitro* maturation, fertilization and culture. They demonstrated that developmental competence of goat oocytes is acquired gradually during follicular growth. It is, therefore, only a few oocytes collected from large antral follicles develop the potential to progress to blastocyst stage following *in vitro* fertilization and maturation culture.

In bovine, important differences in proportion of oocytes were observed by splicing and aspiration method. The number was statistically significantly higher in the splicing method (Hamano and Kuwuyama 1993). The impact of collecting technique on number of oocytes per ovary and their developmental competence after maturation, fertilization and culture *in vitro* was documented in bovine (Carolan *et al.* 1994). Recently, Bungarz *et al.* (1995) collected ultrasound aided follicular aspirated gonadotropin primed or normal oocytes at different stages of development. The relative efficiency of 3 different techniques on oocyte recovery from slaughtered buffalo ovaries was made (Jain 1995). A number of investigations suggested that the type of oocyte investment and structure might be the determinants of its ability to mature in vitro (Fukui and Sakuma 1980, Madison *et al.*1992, Del Campo *et al.* 1995 Chauhan *et al.* 1997a, 1998b). A culture media is an important factor that determines the maturation of oocytes *in vitro* (Lenz *et al.* 1983, Ocana *et al.* 1994). A wide variety of culture media formulations (Fukui *et al.* 1982) and supplements such as gonadotropins (Fukui *et al.*1982, Sidhu and Cheema 1995), proteins of different origins (Sanbuissho 1989, Sanbuissho and Threlfall 1990, Lim *et al.* 1994) and co-culture with cumulus cells (Crister *et al.* 1986) were implicated to influence developmental competence of bovine oocytes matured *in vitro*. A common culture system for in vitro maturation and, *in vitro* fertilization and *in vitro* production of goat, sheep, and buffalo blastocyst embryos was prepared by supplementing TCM-199 medium with 10% foetal bovine serum (FBS) and 5 (μ/ml follicle stimulating hormone (FSH). The maturation rates ranged from 65 to 78%, with no significant difference among the species. The cleavage rates were maximum in buffalos (59%), and minimum in goats (41%). These oocytes were then cultured in TCM-199 medium supplemented with 10% FBS and 5 (μ/ml insulin for 9 days, 34% of buffalo, 8.1% goat, and 4.7% of sheep oocytes reached the blastocyst stage (Chauhan *et al.* 1997a). The protein and hormonal supplements of culture media enhance *in vitro* maturation and fertilization of domestic cat eggs. The serum supplementation actually provides hormones that increase fertilizability and developmental potential of bovine oocytes matured *in vitro* (Saeki *et al.* 1991, Chauhan *et al.*1998a). The estrus cow serum (ECS) or foetal calf serum (FCS) supplementation to the culture media significantly increase the percentage of oocyte maturation (Sanbuissho and Threlfall 1990). The effect of FCS supplement in culture media were reported on *in vitro* maturation (Willis *et al.* 1991), fertilization (Fukui and Ono 1989) and development (Fukui 1989, Fukui and Ono 1989). Experimental evidences suggest that superovulated buffalo serum is superior to buffalo oestrus serum, foetal bovine serum, and steer serum in supporting development of buffalo embryos to blastocyst stage (Chauhan *et al.* 1998b). The role of cumulus cells in oocyte maturation was studied and a positive correlation was observed in oocytes cultured with cumulus cells (Fulka and Motlic 1981, Kameyama and Yoshihiro 1994). Temperature affects the rate of oocyte maturation *in vitro* and 32 to 39°C temperature is the most ambient for oocyte maturation (Lenz *et al.* 1983). Modesit *et al.* (1994) reported that oocyte mature to a comparatively advanced meiotic stage at 38°C when compared with 20°C *in vitro* in pigs.

Gonadotropins (FSH and LH) and estradiol addition to the culture media significantly enhance the rate of oocyte maturation in cattle (Fukui *et al.* 1982, Fukushima and Fukui 1985, Saeki *et al.* 1991). Although cumulus expansion does occur without FSH, but addition of FSH enhances maturation rate many folds in goat. Totey *et al.* (1991) demonstrated the role of gonadotropin and estradiol in maturation of buffalo oocytes. Chauhan *et al.* (1997b) have demonstrated that buffalo follicular fluid has the substance(s) which promote cumulus expansion and induce nuclear and cytoplasmic

maturation, and can be used as *in vitro* maturation supplement for the production of morulae and blastocysts.

Oocyte maturation is not only resumption of meiosis, but also involves a number of changes in cellular activities and biochemistry such as transcription, translation and post-translational processing of protein (Kastrop *et al.* 1990a, b). Cytoplasmic maturation is inducted by the synthesis of several stage specific proteins (Totey *et al.* 1993). Synthesis of different patterns of proteins reported in the oocytes undergoing different stages of cytoplasmic maturation in sheep pigs cattle (Hunter and Moor 1987), buffalo (Totey *et al.* 1993) and goat (Pawshe *et al.* 1994a).

Future perspective

The critical analysis of biology of ovarian follicles in goat, besides enhancing the depth of the reproductive physiology has posed a number of questions which are still to be tackled at research level. To enhance polyembryony, the success rate of endocrinological manipulations need to established for each specific breed. Biochemistry of the proteins (nutritional and regulatory) synthesized by the oocyte and the granulosa cells need to be analysed in a stage specific manner throughout the folliculogenesis. Granulosa-oocyte interactions both anatomical and biochemical are required to be explored further to establish clearly the role of maternal proteins of granulosa cell origin in early gene expression and initiation of development. Immunological studies using lectins need to be conducted to analyse the membrane bound and free macromolecular glyco-conjugate assembly dynamics. This approach shall be of extreme value in working out the factors inducting the follicular selection. Extensive *in vitro* studies are required to establish autocrine, paracrine factors and their chemistry, and functions in relation to ovarian biology. Immuno-cytochemical studies involving the localization of specific regulatory peptides and specific nutritional requirements of the oocyte will be highly rewarding endeavour. Molecular clock of ooplasmic maturation involving in its biochemistry is yet another unexplored area. Oocyte maturation and fertilization success largely depends upon all these factors and an exhaustive research in the areas mentioned shall be directly affecting the goat production and thus economy.

ACKNOWLEDGEMENTS

Authors thank University Grants Commission, New Delhi, for sponsoring major Research Project No.F.3-7/94-SR II. This article is dedicated to Late Mrs S Chaudhury, who expired while working in this Project.

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Indian Journal of Animal Sciences **70** (4): 386-389, April 2000

Efficacy of dietary protein against monocrotophos toxicity on some carbohydrate metabolic enzymes in rats

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Received: 18 January 1999; Accepted: 7 October 1999

ABSTRACT

A study on carbohydrate metabolic hepatic enzymes was conducted to establish the beneficial effect of dietary protein in alleviating the toxic stress of a single or repeated intubation of monocrotophos (2 mg/kg body weight) in rats maintained on formulated isoenergetic diets under normal, protein deficient and protein supplemented dietary conditions at 20 and 5% protein levels. Acute and chronic administration of organophosphate at varying intervals induced variable responses. An accelerated rate of anaerobic glycolysis with a concomitant decreased rate of gluconeogenesis was indicated in malnourished rats after acute dosage.

Chronic monocrotophos poisoning induced increased energy demand in normal rats. In protein deficient rats, the rate of glycogenolysis and glycolysis elevated to meet the primary stress response; the activity of gluconeogenic enzymes also got stimulated. However, the animals were able to counteract the toxicity of pesticide on hepatic enzymes, when fed on high protein diet.

Key words: Dietary protein, Hepatic enzymes, Monocrotophos, Rats

Experimental data revealed the efficacy of dietary protein in determining the susceptibility to pesticide toxicity (Pal *et al.* 1989, Bulusu and Chakraborty 1992, Sharma and Kushwah 1997, Sharma *et al.* 1997). In developing countries like India, a large proportion of protein malnourished population is exposed to health hazards of pesticides during their manufacture, transport and agricultural needs.

Monocrotophos, one of the most commonly used organophosphorus pesticides in agriculture in India, is indicated to be a potent toxicant (Ramulu and Rao 1987). A single or repeated oral dose of monocrotophos is reported to affect the level of certain clinical enzymes (Sharma *et al.* 1999) and some biochemical constituents (Kushwah *et al.* 2000) in blood and liver of malnourished male albino rats that appeared to affect carbohydrate metabolism significantly. However, protein supplementation in the diet proved efficient in combating its toxic stress (Sharma *et al.* 1998, 2000). The present investigation was, therefore, aimed at evaluating the impact of toxicity of this organophosphate on the activity of certain key regulatory enzymes of carbohydrate metabolism in malnourished rats and its alleviation under high protein diet.

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MATERIALS AND METHODS

Adult male albino rats, weighing 150-200 g were procured from the animal colony of Veterinary College, Mhow. These were maintained on a standard diet and were offered water *ad lib.* The study was divided into 3 phases as described earlier (Sharma *et al.* 1998).

The animals were divided into 2 dietary groups and were maintained on formulated isoenergetic diets at 20% (phase 1) and 5% (phase 2) protein levels. After 30 days, some animals of phase 2 were supplemented with standard protein diets at 20% level for 7, 15, 30 and 60 days and formed a third group (phase 3).

The animals in each group (6 each) were exposed to an oral administration of monocrotophos (dimethyl-cis-1-methyl-2-methyl carbonyl-vinylphosphate) @ 2 mg/kg body weight in water (approximately 1/10th LD_{50} oral dose). Animals were intubated with monocrotophos either once (6, 12, 24 hr) or repeatedly (7, 15, 30 and 60 days, once daily). The corresponding animals of control groups were also kept on similar diets at low and high protein levels and were given a calculated dose of vehicle alone.

For short term experimentation, the animals were kept on fasting overnight. Concurrently, these were administered with

a single dosage of insecticide at variable intervals so as to be sacrificed as per experimental schedule. For long term experimentation, too, the animals were fasted overnight before sacrifice. Liver was quickly excised and placed in 0.15M saline. It was cleared off all the extraneous materials and weighed. Liver homogenate was prepared (10% w/v, in 0.15M saline) below 4°C using homogenizer. The activity of phosphorylase (E.C. 2.4.1.1, Sutherland 1955), hexokinase (E.C. 2.7.1.1., Crane and Sols 1955), glucose 6-phosphatase (E.C. 3.1.3.9., Swanson 1955), fructose 1, 6-diphosphatase (E.C. 3.1.3.11., Freeland and Harper 1959) and adenosine triphosphatase (E.C. 3.6.1.3, Post and Sen 1967) were estimated colorimetrically after determining the content of inorganic phosphorus in supernatant (Fiske and Subbarow 1925). Protein content was estimated by the method of Reinhold (Oser 1965). The enzymatic activities were described in their respective units/mg protein. The data were subjected to Students "t" test after calculating mean and standard error using conventional formulae.

RESULTS AND DISCUSSION

A single sublethal oral dose of monocrotophos induced no changes in the hepatic activities of some key regulatory enzymes, viz. phosphorylase, hexokinase, fructose 1, 6 diphosphatase and adenosine triphosphatase in rats maintained on 20% protein diet (Table 1) implying that the rate of glycogenolysis, glycolysis and gluconeogenesis remained unaffected. There seemed to be no change in fluid permeability, too. Phosphorylase activity was stimulated after an acute dosage of dichlorovos (Teichert and Szymczyk 1979) or diazinon (Husain and Matin 1987) without any change in glucose-6-phosphatase activity. However, the latter enzymic activity suffered a nonsignificant reduction in the present study but significantly at 24 hr post treatment reflecting the rate of glucose formation from hepatic glycogen or from glucose 6-phosphate. However, the activity was reported to be unchanged in malathion treated rats (Matin and Husain 1987).

An acute administration of pesticide to the malnourished animals elevated the levels of phosphorylase and hexokinase at 12 hr. Despite stimulation of glycogenolytic enzyme in inducing hyperglycemia, after release of glucose from liver to the circulation, blood glucose level seems to be maintained (Sharma *et al.* 1998). Enhanced hexokinase activity (Table 1) with a concommitant raised hepatic lactic acid level and a fall in pyruvic acid level (Sharma *et al.* 1998) points to an accelerated rate of anaerobic glycolysis as part of *in vivo* tissue response to monocrotophos exposure. A decreased rate of gluconeogenesis was also indicated after the suppression of fructose 1, 6-diphosphatase and glucose-6-phosphatase in these animals. Pesticide intoxication seemed to derange cation transport system by inhibiting ATPase activity at 6 hr. However, at later exposures, an adaptive response of the tissue against pesticide poisoning was reflected.

Long-term administration of monocrotophos produced a significant stimulation in hepatic phosphorylase activity on

Table 1. Effect of a single oral administration of monocrotophos on some hepatic enzymes of rats under varying dietary regimes

Dietary Status	Period of sampling (hr)	Enzymes				
		Phosphorylase	Hexokinase	Glucose-6 phosphatase @	Fructose-1, 6 di-Phosphatase @	Adenosine triphosphatase @
Normal (20% protein level)	0	0.023 ±0.003	0.048 ±0.002	9.723 ±0.526	1.093 ±0.076	9.016 ±1.001
	6	0.023 ±0.001	0.046 ±0.001	0.804 ±0.379	1.207 ±0.093	9.014 ±0.001
	12	0.023 ±0.009	0.048 ±0.003	8.148 ±0.328	1.091 ±0.103	9.014 ±0.924
	24	0.023 ±0.0018	0.052 ±0.004	7.326* ±0.626	1.229 ±0.111	9.016 ±1.023
Malnourished (5% protein level)	0	0.019 ±0.0019	0.035 ±0.0011	5.818 ±0.345	0.453 ±0.079	11.575 ±0.683
	6	0.019 ±0.001	0.034 ±0.001	3.414*** ±0.139	0.315 ±0.056	9.362* ±0.009
	12	0.246* ±0.002	0.051** ±0.004	4.955 ±0.510	0.288* ±0.039	12.332 ±1.282
	24	0.016 ±0.0001	0.035 ±0.002	4.225* ±0.336	0.316 ±0.046	10.339 ±0.856

Values are mean ± SE of 6 observations; enzyme units expressed as specific activity (u moles) (@ n moles)/min/mg protein); *P<0.05, **<0.01, ***P<0.001.

15th and 60th day in rats maintained on high protein diet reflecting an increased energy demand during this period. This was clearly evidenced by an accelerated activity of hexokinase at these intervals (Table 2). Coult *et al.* (1979) indicated an accumulation of c-AMP in organophosphorus poisoned animals that may activate phosphorylase activity (Husain and Matin 1987). The activities of glucose 6-phosphatase and fructose diphosphatase were also enhanced at 15th and 60th day pesticide treated rats, indicating an accelerated conversion of glucose 6-phosphate to meet out the excess energy demand in defence to pesticide intoxication. Strikingly, the activities of phosphorylase, glucose 6-phosphatase and fructose 1, 6 diphosphatase were suppressed at 30th day post treatment. However, the activity of hexokinase remained unaffected (Table 2).

The activity of ATPase was markedly suppressed on both 30th and 60th day after exposure (Table 2) pointing to maximum dysfunction of the enzyme system at these intervals due to the derangement of cation transport system. However, the activity remained unchanged at early stage of exposure (Table 2).

In contrast, hepatic activities of all the enzymes under study

Table 2. Effect of repeated oral administration of monocrotophos on the activity of some hepatic enzymes of rats under varying dietary regimes

Dietary Status	Period of sampling (days)	Enzymes				
		Phosphorylase	Hexokinase	Glucose-6 phosphatase @	Fructose-1, 6 di-Phosphatase @	Adenosine triphosphatase @
Normal (20% protein level)	0	0.023 ±0.003	0.048 ±0.002	9.723 ±0.526	1.093 ±0.076	9.016 ±1.801
	7	0.023 ±0.002	0.046 ±0.002	8.069 ±0.596	1.386 ±0.338	9.013 ±0.802
	15	0.034* ±0.002	0.079 ±0.002	13.317* ±0.939	1.616* ±0.140	9.019 ±0.902
	30	0.019* ±0.004	0.043 ±0.002	7.591* ±0.188	0.649** ±0.089	6.007*** ±0.801
	60	0.034 ±0.002	0.064** ±0.002	11.311* ±0.323	1.863* ±0.092	6.007*** ±0.801
Malnourished (5% protein level)	0	0.028 ±0.005	0.056 ±0.007	9.239 ±0.799	0.337 ±0.022	11.075 ±1.839
	7	0.039 ±0.002	0.083* ±0.007	9.775 ±0.799	0.423 ±0.022	14.912 ±1.839
	0	0.246 ±0.002	0.055 ±0.003	3.264 ±0.183	0.260 ±0.029	9.958 ±0.920
	15	0.048* ±0.003	0.096* ±0.004	5.304* ±0.2416	0.437 ±0.024	13.023* ±1.235
Protein supplemented (20% protein level)	0	0.044 ±0.002	0.184 ±0.013	4.133 ±0.571	0.641 ±0.081	8.332 ±.531
	7	0.024*** ±0.002	0.124** ±0.007	3.234 ±0.077	0.528 ±0.083	6.321* ±0.944
	0	0.023 ±0.002	0.122 ±0.007	0.899 ±0.077	0.641 ±0.083	8.641 ±0.234
	15	0.019 ±0.001	0.119 ±0.008	1.025 ±0.135	0.647 ±0.027	8.888 ±0.174
	0	0.018 ±0.001	0.122 ±0.019	2.018 ±0.214	0.410 ±0.063	13.747 ±0.359
	30	0.016 ±0.001	0.136 ±0.019	2.116 ±0.214	0.472 ±0.063	13.673 ±0.359
	0	0 024 ±0.002	0.148 ±0.011	4.039 ±6.466	0.329 ±0.042	17.729 ±1.989
	60	0.024 ±0.002	0.154 ±0.006	3.829 ±0.226	0.232 ±0.046	17.977 ±1.591

Values are mean ± SE of 6 observations; enzyme units expressed as specific activity (n moles) (@ n moles)/min/mg protein); *P<0.05, **<0.01, ***P<0.001.

were elevated in rats fed on low protein diet. A significant stimulation in phosphorylase activity points to an increased rate of tissue glycogenolysis and corroborated well with our earlier findings on the decreased content of glycogen (Sharma *et al.* 1998). Likewise, hexokinase activity was also increased at both intervals (Table 2) indicating an increased energy demand for enhanced muscular activity to be met with an increased rate of glycolysis. An elevation in lactate to pyruvate ratio testifies to an enhanced muscular activity (Sharma *et al.* 1998) that accentuates the primary stress response in protein deficient animals, establishing the toxic impact of insecticide, more so in these groups of animals (Sharma *et al.* 1997, 1998, Kushwah *et al.* 2000). The activity of both the gluconeogenic enzymes was also stimulated (Table 2) to meet the extra energy demand to counteract the toxic hazards of monocrotophos, since their capability of resistance to toxicity was reduced when fed on low protein diet (Sharma *et al.* 1997, 1998). An increased activity of ATPase inferred to an increased rate of transport associated with enzymic proteins for energy purpose.

Monocrotophos dosage to the malnourished animals, when fed on a high protein diet (20%), decreased phosphorylase markedly on seventh day post treatment (Table 2). The activity of hexokinase and ATPase were also suppressed at this interval signifying nonalleviation of pesticide toxicity till this period. Later, protein diet seemed to have a beneficial impact in counteracting the toxicity of pesticide. This was evidenced by nonsignificant alterations in all the enzymic activities (Table 2). This might be due to rapid detoxification of monocrotophos by microsomal drug metabolising enzymes activated in the liver under high protein diet (Sato and Nakazima 1984).

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Indian Journal of Animal Sciences **70** (4): 390-391, April 2000

Pathogenicity of Newcastle disease virus in guinea fowl

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Received: 13 October 1998; Accepted: 22 October 1999

Key words: Guinea fowl, Newcastle disease virus, Pathogenicity

Newcastle disease virus (NDV), a member of genus Paramyxovirus of the family Paramyxoviridae causes major losses to poultry industry due to its high morbidity and mortality. The importance and impact of a particular NDV isolate is directly related to its virulence. Depending on the pathogenicity in field, the virus isolates are broadly classified into 3 categories viz. velogenic (virulent), mesogenic (intermediate in virulence) and lentogenic (avirulent) strains. Pathogenicity tests are useful markers to differentiate the NDV strains. Various laboratory tests which have been used by several workers for assessing the pathogenicity of the virus are mean death time (MDT) in embryonated chicken egg (ECE), intracerebral pathogenicity index (ICPI) in day-old chick, intravenous pathogenicity index (IVPI) in 6-week-old chicken, cloacal and conjunctival mean death time (CCMDT) in 8-week-old chicken (Martone *et al.* 1976, Allan *et al.* 1978, Alexander and Parsons 1984).

Guinea fowl is native to Savannah zone of West Africa and the bird is seen mostly in wild. At present there is an increasing tendency to domesticate and commercialise its production. The birds are susceptible to both natural and experimental infection with NDV (Durojaiye and Adene 1988, Okaeme *et al.* 1988, Agoha *et al.* 1992, Rampin *et al.* 1993). Therefore, the present study was undertaken to compare the pathogenicity tests conducted in chicken with those in guinea fowl.

Three Newcastle disease (ND) virus isolates: 1 from chicken, 1 from guinea fowl, and 1 from pigeon were passaged in 11-day-old ECE and then subjected to various pathogenicity tests (Table 1). From the results it is evident that the MDT, ICPI and IVPI of chicken isolate were 56 hr, 1.75 and 2.51, respectively, whereas these values for guinea fowl isolate were 48 hr, 1.50 and 2.57 respectively. These values fall within the range given for velogenic strain, as has been reported by various workers (Singh and Singh 1970, Allan *et al.* 1978).

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Table 1. Pathogenicity index of different Newcastle disease virus isolates conducted in chicken

	MDT (hr)	ICPI	IVPI	CCMDT (days)
Chicken isolate	56	1.75	2.51	4
Guinea fowl isolate	48	1.50	2.57	6
Pigeon isolate	84	1.16	1.01	–

The MDT, ICPI and IVPI of pigeon isolate conducted in chicken were 84 hr, 1.16 and 1.01 respectively. These values fall within the range prescribed for mesogenic strains (Allan *et al.* 1978, Alexander and Parsons 1984). Similarly, Allan *et al.* (1978) reported that mesogenic strains with MDT 60-90 hr, ICPI 0.5-1.5 and IVPI 0-1.5 were intermediate in virulence.

Ahmed and Reda (1968) used pigeons to determine the neuropathic index (ICPI) and they reported that the index was directly correlated with the pathogenicity of the strains used. In the present study, the IVPI test was conducted in 6-week-old guinea fowl. The IVPI was 2.36, 2.42 and 0.96 for chicken, guinea fowl and pigeon isolate respectively. These figures were very close to the IVPI conducted in 6-week-old chickens. This indicated that guinea fowl could be used as an alternative to chicken to study IVPI. Ahmed and Reda (1968) also reported that intracerebral inoculation of pigeon provided additional reliable means for assessing the pathogenicity of strains of NDV as well as distinguishing virulent strains from vaccine strains. Cloacal and conjunctival mean death time test was conducted to distinguish between velogenic strains, viz. viscerotropic / neurotropic. The guinea fowl and chicken isolates upon inoculation on to conjunctiva and into cloaca of 8-week-old chickens caused diarrhoea and death of all the 4 birds within 4-6 days post infection (DPI) (Table 1). Therefore, both the isolates were classified as viscerotropic velogenic virus. No mortality was recorded in the chickens following inoculation of pigeon isolate, however, mild clinical signs were observed in all the 4 birds that confirmed the isolate to be mesogenic strains. Alexander and Allan (1974) reported CCMDT (4-8 days) to be a valuable method to differentiate between viscerotropic and neurotropic strains of NDV. The

CCMDT was also conducted in 8-week-old guinea fowl for comparison. All the 4 birds inoculated with chicken isolate developed dullness and depression on second day and there was diarrhoea and death of all the birds within 6 DPI. Out of 4 guinea fowls inoculated with guinea fowl isolate, there was diarrhoea and death of 3 birds within 4 days of inoculation and fourth one died on sixth day. In the birds of both the groups, haemmorhage in proventriculus and caecal tonsils was observed on PM examination. In contrast to the above 2 isolates, all the birds inoculated with pigeon isolate became dull and remained like that till the end of the experiment. PM examination of sacrificed birds did not reveal any gross changes. The symptoms and lesions produced by chicken and guinea fowl isolates were characteristics of viscerotropic velogenic NDV, whereas pigeon isolate was mesogenic strain.

The results of IVPI and CCMDT in guinea fowl were comparable with the IVPI and CCMDT of chicken. This seems to be the first report of conducting the pathogenicity test in guinea fowl which indicated that guinea fowl could be taken as an alternative to chicken for these tests and it might provide additional reliable means for assessment of pathogenicity of the virus.

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Indian Journal of Animal Sciences **70** (4): 392-393, April 2000

Incidence of spastic paresis in bullocks in Gujarat

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Received: 21 October 1998; Accepted: 6 October 1999

Key words: Bullock, Hock, Spastic paresis

A survey was carried out to record the incidence of bovine spastic paresis in villages and Panjarapoles of 3 talukas, viz. Jamkhambhaliya, Jamkalyanpur and Bhanvad of Jamnagar district in Gujarat.

The details of field survey are given in Table 1. A large number of salvage animals were gathered in Panjarapoles due to drought conditions prevailing during 1993-94. In all 59 cases of spastic paresis were recorded in bullocks only and not a single case was seen in female. The incidence of spastic paresis based on total cattle population was 0.05% (1: 2 000), while on the basis of bullock population, it was 0.11% (1: 1 000). All the bullocks were used for draught purpose.

Table 1. Incidence of spastic paresis in bullocks of Jamnagar district in Gujarat

Taluka/ Panjarapole	Total cattle population	Number of bullocks	Number of spastic animals
Jamkhambhalia	39162	16425	11
Bhanvad	44870	19702	9
Jamkalyanpur	35524	18606	10
H.V.O.S.Panjarapole	2599	555	17
Movan Panjarapole	3000	630	12
Total	125155	55918	59
Per cent	–	44.68[+]	0.05[+]
	–	–	0.11[++]

[+]Per cent on the basis of total cattle population,
[++]Per cent on the basis of bullock population.

Age of onset and breed-wise distribution of spastic paresis in bullocks is given in Table 2. Age of onset was noted during anamnesis based upon the wisdom of owners in percepting the clinical signs like reduced work performance, difficulty in getting up in the morning, straightness of the limb, tremulous muscle contractions and alternate lifting of hind legs. Age at onset of spasticity ranged from 6 to 18 years,

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Table 2. Age of onset and breed-wise distribution of spastic paresis in bullocks (n=59)

Age (years)	Kankrej	Gir	Nondescript	Total
6-8	9	2	2	13
	(15.2)	(3.4)	(3.4)	(22.0)
8-10	11	1	8	20
	(18.6)	(1.7)	(13.6)	(33.9)
10-12	6	2	2	10
	(10.2)	(3.4)	(3.4)	(17.0)
12-14	7	1	3	11
	(11.9)	(1.7)	(5.1)	(18.6)
14-16	1	0	1	2
	(1.7)	(0.0)	(1.7)	(3.4)
16-18	1	1	1	3
	(1.7)	(1.7)	(1.7)	(5.1)
Total	35	7	17	59
	(59.3)	(11.9)	(28.9)	(100.0)

n = Number of spastic animals.
Figures in parentheses indicate per cent values based on number of spastic animals.

with maximum number of cases (33) occurring in the age group of 6 to 10 years. The results are in accordance with the reports of Rao (1967) and Gadgil *et al.* (1970, 1972).

Since the disease affects the bullocks in their prime working life it causes enormous losses to the farmer. In western countries, the condition occurs commonly in calves (Elliot and Hopkins 1966), but cases involving animals of 5 to 11 years have also been reported (Bradley and Wijeratne 1980).

The incidence of spastic paresis was maximum in the Kankrej breed (59.3%) followed by non-descript (28.8%) and the least in Gir (11.9%). The incidence of spastic paresis is most frequently reported in Friesian cattle (Huffel *et al.* 1986). Other affected breeds include Ayrshire (Roberts 1965), Beef Shorthorn (Leipold *et al.* 1967), Charolais (Love and Weaver 1963). In India, spastic lameness was reported for the first time in a non-descript bullock and a cow (Rao 1967), subsequently Gadgil *et al.* (1970, 1972) reported spastic lameness only in bullocks. Contrarily, spastic paresis has been

reported by others in adult cows (Deppe *et al.* 1976) and young calves of both sexes (Leipold *et al.* 1967). The high incidence in working bullocks of only a specified region in our study is indicative of some environmental factors, which might have played a role in the phenotypic expression of genetically predisposed animals as was postulated by Gadgil *et al.* (1970). Further investigations on this are essential to arrive at a conclusion. The higher incidence of spastic paresis in Kankrej breed may have been influenced by many factors. There is a tendency amongst farmers to select the animals with a fairly straight hocks than the typical sickle shaped hocks and this might have forced the breeders to select the bulls with favourable hind limb confirmation. Further most of the Kankrej bullocks originate from Kutchh region where large Kankrej herds are being reared and bred with a few selected bulls. The main income of these cattle owners is from sale of castrated male calves. In the present study, however, it is not possible to ascertain the pedigree records of individual affected animal, the possibility of inbreeding can not be ruled out. In 1 study on Kankrej bullocks with spastic paresis, the pedigree data of the affected animals showed that they were inbred animals with an inbreeding coefficient of 12 to 17% (Gadgil *et al.* 1970). The non-descript cattle also possess some inheritance of Kankrej and hence they also might have been influenced simultaneously.

Breed and limb-wise distribution of spastic paresis in bullocks is given in Table 3. Bilateral cases of spastic paresis (41) accounted for 69.5% of the total spastic cases. The involvement of the right limb was more (37%) as compared to the left (19%), in the both unilateral (11%) and bilateral (26%) severely affected limbs.

According to Formston and Jones (1956) and Wheat (1960) spastic paresis is normally a bilateral condition, wherein the signs are usually more pronounced in 1 limb. However, Leipold *et al.* (1967) and Keith (1981) observed the condition most frequently to be unilateral involving right hindlimb, while Boyd and Weaver (1967) recorded higher involvement of left leg. Unilateral hindlimb hyperextension with a straight hock was a more common finding in older animals (Gadgil *et al.* 1970), and when severely affected limb was operated upon the characteristic signs developed in the opposite normal or mildly affected limb (Gadgil *et al.* 1970, Vestweber and Noordsy 1971). This was also seen in a few operated bullocks under the present study.

During anamnesis, it was reported that the signs of spastic paresis were seen after episodes of ephemeral fever in 4 cases and foot-and-mouth disease in 2 cases. This might be a coincidence as their relationship has not been established (Baird *et al.* 1974). In 4 animals in spite of presence of straight hock, there was no obvious diminution in work efficiency, though it simulated initial signs of spastic paresis.

Table 3. Breed and limb distribution of spastic paresis in bullocks (n = 59)

		Unilateral	Bilateral	Total
Breed	Kankrej	10 (16.9)	25 (42.4)	35 (59.3)
	Nondescript	5 (8.5)	12 (20.3)	17 (28.8)
	Gir	3 (5.1)	4 (6.8)	7 (11.9)
	Total animals	18 (30.5)	41 (69.5)	59 (100)
Limbs	Severely affected			
	Left	7 (7.0)	12 (12.0)	19 (19.0)
	Right	11 (11.0)	26 (26.0)	37 (37.0)
	Total	18 (18.0)	38 (38.0)	56 (56.0)
	Mildly affected			
	Left	–	29 (29.0)	29 (29.0)
	Right	–	15 (15.0)	15 (15.0)
	Total	–	44 (44.0)	44 (44.0)
Total affected limbs		18 (18.0)	82 (82.0)	100 (100)

n = Number of spastic animals.

Figures in parentheses indicate per cent values based on total animals/limbs.

During anamnesis, it was reported that the signs of spastic paresis were seen after episodes of ephemeral fever in 4 cases and foot-and-mouth disease in 2 cases. This might be a coincidence as their relationship has not been established (Baird *et al.* 1974). In 4 animals in spite of presence of straight hock, there was no obvious diminution in work efficiency, though it simulated initial signs of spastic paresis.

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Indian Journal of Animal Sciences **70** (4): 394-395, April 2000

Sedative, analgesic and some cardiopulmonary effects of romifidine in dogs

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Received: 18 May 1998; Accepted: 22 October 1999

Key words: Analgesia, Dog, Romifidine, Sedation

Romifidine has been used for sedation in dogs intravenously at wide range of dosage (20-120 µg/kg) (England *et al.* 1996). The present article describes the sedative and analgesic effects of 3 dose levels of romifidine injected intramuscularly in dogs.

Clinically healthy adult mongrel dogs (12) of either sex weighing between 11 and 16 kg were divided into 3 groups of 4 animals each. All the dogs were kept off feed and water for 24 and 12 hr, respectively, before administration of any drug. After recording the base values, the animals were injected with atropine sulphate @ 0.65 mg/animal intramuscularly. It was followed, 15 min later, by intramuscular administration of romifidine @ 50, 75 and 100 µg/kg body weight in animals of groups A, B and C, respectively, after diluting the drug in normal saline to make the injectable solution up to 1 ml.

Heart rate (beats/min), respiration rate (thoracic excursion/min), and rectal temperature (°F) were recorded in all the animals just before the administration of atropine sulphate and at 5, 10, 15, 20, 30, 45, 60, 75 and 90 min after the administration of romifidine. Various reflexes like pedal, pin prick, cough reflex (response to intubation), relaxation of jaw and posture of the animal during the study were also recorded at the same intervals to assess the extent of sedation, analgesia and muscle relaxation. All these parameters were recorded using a 0-3 or 0-4 score scale (Amarpal *et al.* 1996), so that the extent of effect could be quantified. The animals were also observed for pain at injection site and any other untoward sign during the study.

The data for heart rate, respiration rate and rectal temperature were analysed using paired 't' test for within the group comparison of the means and analysis of variance for between the group comparison of means. The data for various reflexes were analysed using Wilcoxson sign rank test as per Snedecor and Cochran (1967).

Mean weak time (time between injection of drug and onset of sedation) was 4.57 ± 0.63, 6.00 ± 0.91 and 4.00 ± 0.58 min in animals of groups A, B and C respectively. It was, however, not significantly ($P<0.05$) different among the groups. The increase in dose of romifidine from 50 to 100 µg/kg body weight seemed to have no effect on the speed of onset of effects of the drug given intramuscularly. Rapid onset of sedation (within 1 min) after intravenous administration of romifidine has been reported in dogs, but no difference in onset time was found between the dose rate of as low as 20 µg/kg and as high as 120 µg/kg (England *et al.* 1996). Animals of all the groups appeared sleepy with lowered head and sat down calmly within 10-20 min of drug administration irrespective of the dose rate used. Apparently, animals appeared moderately sedated but when disturbed, they got up, moved away without much inco-ordination and again-resumed sternal recumbency when left undisturbed. The duration of effect was also not different among the different groups and by the end of the observation period, all the animals appeared alert and walked without inco-ordination.

Pedal reflex was only mildly abolished in all the groups viz A, B and C. Though depression of pedal reflex was slightly lesser in group A as compared to groups B and C, the difference was not statistically significant among the groups at any interval. Maximum depression of pedal reflex was observed at 45 min interval in group C but by 75 min interval all the animals of different groups had normal (score 0) pedal reflex. Moderate analgesia (pin prick reflex score = 2) was recorded in all the groups.

The peak effect was recorded between 15 and 30 min in all the groups and the difference among the groups was not statistically significant. Very mild to mild analgesia (Scores 0. 50 ± 0.29 group A to 1. 25 ± 0.25 group C) persisted even at 90 min interval. The status of cough reflexes was measured by recording the response to intubation on a 0-3 scale. The results indicated only very mild depression of cough reflex in all the groups which were not significantly different between the groups.

On the basis of scores recorded for pedal reflex, pin prick reflex and cough reflex, muscle relaxation and incoordination, it was evident that romifidine produced only mild to moderate analgesia and moderate sedation. Though the difference was not significant between the groups, the dose rates of 75 and

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100 μg/kg produced more consistent results which were almost similar in both the groups. However, analgesia in animals of group A was relatively lesser as compared to groups B and C.

In the present study, no appreciable changes in heart rate was recorded during first 20 min in any of the groups. A slight fall in HR was recorded after 30 min in all the groups which was significant ($P<0.05$) only at 20 min and 75 min intervals in groups B and C respectively.

Respiratory rate showed a significant ($P<0.05$) fall in all the groups. In group A, the significant fall was recorded between 15 and 16 min of intervals, however, in groups B and C significant fall was recorded even at 5 min after injection and persisted for a longer time. Reduction in respiratory rate and periodic apnoea have also been reported after intravenous administration of romifidine (England *et al.* 1996). No significant difference in RR was seen between B and C. The rectal temperature did not change significantly ($P>0.05$) in any of the groups.

The results of the present study indicated that romifidine administered intramuscularly @ 50 μg/kg produced only very mild sedation and analgesia. However, romifidine @ 75 and 100 μg/kg produced mild to moderate sedation and analgesia.

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Indian Journal of Animal Sciences **70** (4): 396-398, April 2000

Reduced responsiveness of oxyclozanide and tetramisole hydrochloride combinations to gastrointestinal parasites of sheep

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Received: 8 September 1999; Accepted: 7 October 1999

Key words: *Haemonchus contortus*, Paramphistomes, Oxyclozanide, Teteramisole hydrochloride

Parasitic gastroenteritis, caused by immature paramphistomes and *Haemonchus contortus*, results into high mortality and morbidity in sheep (Dutt 1980, Gupta *et al.* 1985, 1987, 1988). The combined formulation of anthelmintics, oxyclozanide and tetramisole hydrochloride, is being used for the control of parasitic gastroenteritis in sheep of eastern Haryana since last 2 decades. The efficacy of oxyclozanide and teteramisole hydrochloride, when used individually, was quite high (100%) against immature paramphistomes (Misra and Ruprah 1972) and *H.contortus* (Chaudhri *et al.* 1983). Only 2 years before, a few reports from this region were made about the reduced efficiency of the combined formulation in improving the clinical conditions of sheep. This necessitated the re-evaluation of the anthelmintics in the combined formulation and the results are reported in this communication.

Nine healthy lambs, 3-4 month-old, of either sex, were procured locally and examined on 3 occasions for the presence of parasitic infections. They were treated orally with oxyclozanide @ 15 mg/kg.b.wt. and levamisole hydrochloride @ 7.5 mg/kg.b.wt. Thereafter, they were kept on the concrete floor and precautions were taken to avoid accidental parasitic infection. Animals were provided wheat straw, green fodder, water *ad lib.* and pelleted feed @ 250 g /lamb daily. They were divided into 3 groups as per experimental design after 3 weeks of maintenance (Table 1). Each lamb of infected treated and infected control groups was experimentally infected with paramphistome metacercariae, collected from naturally infected *Indoplanorbis exustus*. The experimental lambs of infected treated group were orally administered oxyclozanide @ 15 mg/kg b.wt. 20 days post-infection. All the lambs were killed on day 30 post-infection(PI)/on day 10 post-treatment (PT).

In a field trial, 8 flocks of sheep (50-100 in each) in Karnal, Kurukshetra and Yamunanagar districts of Eastern Haryana were selected during September, 1998 to March 1999 based on the history of reduced responsiveness in sheep exhibiting clinical signs like bottle jaw condition, dysentery, pica, partial anorexia, progressive emaciation etc. to the recommended doses of the combined anthelmintic formulation of oxyclozanide 3% w/v and tetramisole hydrochloride 3% w/v. Additionally, 3 sheep among the selected flocks, which were earlier treated with the combined formulation and died, were also found to have *H.contortus* worms (1450, 1890, 970) in their abomasii. All sheep of these flocks were treated orally with oxyclozanide @ 15 mg/kg b.wt. for the removal of immature paramphistomes, if any. Fifteen days post-treatment, they were faecal sampled individually on day 0 and again on day 10 after treatment. Faecal egg counts per gram (EPG) were determined by modified Mc Master technique.

Sheep (30), 6-month to 2-year-old, ranked from 1-30 on the basis of higher to lower EPG counts, from each flock were selected, numbered and divided into 2 comparable groups, infected treated (15) and infected control (15), on the basis of EPG counts on day 0. The sheep of the infected treated group were treated orally with tetramisole hydrochloride @

Table 1. Efficacy of oxyclozanide in sheep experimentally infected with *Cotylophoron* spp.

Lambs		No. of mc used for inf.	Dose of drug	Worms recovered		%
Grs.	No.			Abo	SI	(E)
1	12	5500	15 mg/kg	0	96	
	13	5500	kg. b.wt	0	3	97.7
	18	5500	per os	0	18	
2	3	5500	nil	88	2164	
	6	5500		31	1761	0
	14	5500		32	1120	
3	7	nil	nil	0	0	
	9			0	0	0
	10			0	0	

mc, Metacercariae; E, efficiency; Abo, abomasum; SI, small intestine; os, orally.

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15 mg/kg.b.wt. Pooled faecal cultures from each group were also made to obtain infective larvae by Baerman's technique.

The recovery of immature paramphistomes from the killed lambs was done as per technique of Horak (1967). They were identified (Dutt 1980) and counted (Reincke *et al.* 1962). The per cent efficacy was calculated as per formula:

$$\frac{\text{Worm recovered from infected control- worms recovered from infected treated}}{\text{Worms recovered from infected control}} \times 100$$

The faecal egg count reduction (FERC) in sheep under field trial was calculated by the method using arithmetic mean (Coles *et al.* 1992). Less than 95% reduction in average EPG with a 95% confidence interval extending below 90% level was considered to be indicative of resistance whereas either of the 2 criteria suggested partial resistance (Coles *et al.* 1992).

Oxyclozanide, one of the anthelmintics of the combined formulation, was highly (97.7%) effective in reducing the average immature *Cotylophoron* spp. recovery in the infected treated lambs (Table 1). Fifteen years ago, oxyclozanide was also found highly effective (99.5-100.0%) against mature and immature paramphistomes of sheep in eastern Haryana (Chaudhri *et al.* 1983, 1985). Therefore, presently observed efficiency of this anthelmintic clearly indicates that this drug can still be used successfully for the control of paramphistomosis in sheep of this region.

The field trial with the other anthelmintic, tetramisole hydrochloride, of the formulation, revealed variable responses in the examined flocks of sheep from different areas of eastern Haryana (Table 2). The interpretation of EPG data showed prevalence of resistance in 5 flocks of sheep from 2 districts (Kurukshetra and Yamunanagar) of eastern Haryana. Of 3 flocks from Karnal, 1 was partially resistant and the remaining 2 were susceptible to the recommended dosage of tetramisole hydrochloride. The results of pooled coproculture on day 10 post-treatment revealed that per cent composition of *H.contortus* L3 in tetramisole resistant sheep of infected treated group was 55%, as compared to 62% in the infected control group. The sheep, which were partially resistant to tetramislole, had 12% *H.contortus* L3 in the pooled cultures of infected treated groups in comparison to 94% in the infected control group. The tetramisole susceptible sheep of infected group were, however, free from *H.contortus* L3 when compared with 86% recovery from the infected control group. This suggested that H.contortus worms were the main nematode parasites, which were resistant to tetramisole hydrochloride.

Table 2. Interpretation of egg per gram (EPG) of faecal counts on day 10 post-treatment with tetramisole hydrochloride

Sheep flocks	Mean EPG	FECR %	FECR Variance	UCL	LCL
T1/C1	1110/1807	39*	0.06	63*	-1.45*
T2/C2	553/1127	51*	0.33	85*	-59.13*
T3/C3	223/1597	86*	0.19	94*	66.00*
T4/C4	412/825	50*	0.09	73*	8.00*
T5/C5	150/1120	87*	0.05	92*	79.00*
T6/C6	13/1170	99***	1.10	99***	98***s
T7/C7	20/473	96**	0.56	99**	80**
T8/C8	5/475	99***	1.40	99***	98***s

FECR, Faecal egg count reduction; UCL, upper 95% confidence limit; LCL, lower 95% confidence limit.

The treated and control groups had 15 sheep in each, T 1-8: Treated with tetramisole hydrochloride @ 15 mg/k.g b.wt. os., C1-8: Control groups; * resistant; **partially resistant; ***susceptible.

In the early seventies, tetramisole hydrochloride was found highly effective (100%) against both mature and immature *H.contortus* (Mishra and Ruprah 1972), the most prevalent and highly pathogenic gastrointestinal nematode of sheep (Gupta *et al.*1986, 1988). Resistance to tetramisole and another imidazothiazole, levamisole, which is an l-isomer of tetramisole, has also been recorded in sheep and goats of Uttar Pradesh (Srivastava *et al.* 1995) and western Haryana (Kumar 1992, Yadav and Uppal 1993). It appears that continuous indiscriminate use of tetramisole in combination with oxyclozanide in many formulations for the control of mixed parasitic infections in sheep and goats for the last many years had also selected for resistant strains of *H.contortus* in the eastern Haryana.

Since immature paramphistomes and gastrointestinal nematodes dominated by *H.contortus* in small ruminants are usually tackled with anthelmintic combinations, therefore, prevalence of tetramisole resistance warrants the cautious use of this anthelmintic in combined formulations.

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Indian Journal of Animal Sciences **70** (4): 399-400, April 2000

Disposition kinetics of sulfadoxine following oral administration in poultry

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Received: 26 August 1999; Accepted: 27 September 1999

Key words: Sulfadoxine, Kinetics, Poultry

The disposition kinetics of different sulfonamides have been described in various species of animals (Silvestri *et al.* 1967, Nielson and Rasmussen 1977, Reddy *et al.* 1988, Jain *et al* 1992). There is no data available on the pharmacokinetics of sulfadoxine in poultry. Therefore, this investigation was undertaken to determine the pharmaco, kinetic profiles of sulfadoxine in White Leghorn (WLH) chicken following oral administration in a semi-arid tropical climate.

Six WLH chickens of about 1½ years of age were used. Sulfadoxine was administered orally 100 mg/ kg b. wt. Blood samples were collected from the cannulated wing vein in the heparinized glass centrifuge tubes at different time intervals i.e. 0 (before drug administration) and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 9, 12, 24, 48 and 72 hr post-administration. Plasma was separated after centrifugation at 3 000 rpm for 15 min at the room temperature. The day temperature of the animal house was recorded between 37-40°C during the experiment. All the samples were stored at -10°C until analysis. The samples were analysed spectrophotometrically by the method of Bratton and Marshall (1939) at 545 nm. The plasma sulfadoxine concentration-time data were fitted by a 2-exponential equation and different pharmacokinetic parameters were determined as described by Gibaldi and Perier (1982) and using computer p ogramme (Tallarida and Murray 1987).

The sulfadoxine plasma concentrations at different time intervals are presented in Table 1. The maximal level of sulfadoxine was observed to be 127.0 µg/ ml at 1.5 hr, which declined to 6.83 µg/ ml at 72 hr. A concentration above the minimum effective therapeutic concentration (>40 µg/ ml) considered to be effective against most of the organisms, was immediately observed at 0.25 hr following oral administration of sulfadoxine and was maintained up to 12 hr in plasma.

The various disposition kinetic parameters are summarized in Table 2. The pharmacokinetic behaviour of sulfadoxine in poultry is best described by a two-compartment open model. Similar observations were reported for sulfadoxine in cows (Davitiyananda and Rasmussen 1974) and goats (Nielson and Rasmussen 1976). The drug was quickly absorbed into the blood (Ka; 1.557 hr^{-1}). The elimination half-life of sulfadoxine following oral administration in poultry as established in this

Table 1. Plasma levels (µg ml^{-1}) of sulfadoxine in poultry following single oral administration (100 mg kg^{-1})

Time (hr)	Plasma level (µg ml^{-1})
0.25	91.83±3.81
0.50	99.50±6.17
0.75	108.83±7.74
1	115.75±8.85
1.5	127.00±11.03
2	116.14±8.83
3	98.25±6.44
4	83.08±5.74
6	73.10±6.40
9	58.20±3.82
12	44.58±2.32
24	28.33±2.27
48	14.85±2.09
72	6.83±1.18

Values are mean ± SE of 6 animals.

Table 2. Kinetic parameter estimates for the 2-compartment model of sulfadoxine in the plasma after a single oral administration in poultry (100 mg kg^{-1})

Parameters	Unit	Values
Ka	hr^{-1}	1.557
A	µg ml^{-1}	70.12
t½ Ka	hr	0.448
Kel	hr^{-1}	0.115
B	µg ml^{-1}	140.18
t½ Kel	hr	6.036
C^{max}	µg ml^{-1}	170.88
t^{max}	hr	1.81
Vd	1 kg^{-1}	0.475
Vd^{area}	1 kg^{-1}	0.740
AUC	µg ml^{-1} hr	1526.30

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study (6.036 hr), was comparatively lower to that of sulfamethoxazole (8.77 hr) and higher in comparison to sulfadiazine (4.5 hr) reported in poultry by Reddy *et al.* (1988). The calculated maximum concentration (C_{max}) of sulfadoxine in plasma was 170.88 μg ml^{-1} which was achieved at 1.81 hr (t_{max}). The apparent volume of distribution (Vd) was 0.475 l. kg^{-1} indicating that the drug is distributed in the volume of 47.5% of the body weight. A volume of distribution above 1 indicates higher concentration of the drug in tissues than in plasma (Nielson and Rasmussen 1975). In the present case, Vd was less than 1, indicating that the distribution and concentration of sulfadoxine in tissues was lesser than in plasma in poultry. These results are in agreement with the findings of Reddy *et al.* (1988) who also reported Vd less than 1 for various sulfonamides, viz. sulfadiazine, sulfamethoxazole, sulfadimethoxine and sulfadimidine following their oral administration in poultry. The total area under the plasma concentration curve (AUC) was 1526.30 μg ml^{-1}. hr. Thus, the results of this study revealed marked differences in the kinetic behaviour of sulfadoxine in comparison to other sulfanomides in poultry.

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Indian Journal of Animal Sciences 70 (4): 401-402, April 2000

Serum proteins in crossbred (Friesian × Hariana) pregnant cows and its calves

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Received: 24 February 1999; Accepted: 27 September 1999

Key words: Calves crossbred (Friesian × Hariana), Cows serum protein, Preparturient

The present experiment was conducted to study circulatory levels of serum proteins in preparturient F_2 generation crossbred cows and its F_3 generation calves with a view to have a reference base of the circulatory protein profile among the neonatal and prepubertal crossbred female calves having 87.5% exotic genotype with reference to its dam of F_2 generation having 75.% exotic genotype in tropical climate. Six crossbred cows (Friesian 75% × Hariana 25%; inseminated with frozen semen) at 280 days of gestation as verified from AI date and its crossbred (Friesian 87.5% × Hariana 12.5%) female calves were selected from animal herd of Animal Production Research Institute, Pusa, Bihar. The newly born female calves were allowed to suck colostrum 40 to 60 min after birth and, thereafter, they were allowed to suck milk for 3 min twice daily at 12 hr intervals. The calves started to take in feed and fodders between day 15 and 20 after birth.

Jugular venous blood from each pregnant cow (at 280 days of gestation) and female calves at 30 to 40 min (before feeding colostrum), 120 min and 360 min (after feeding colostrum), on day 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, and also at 4 to 5 months and 10 to 11 months after birth were collected. Serum was harvested from all the samples and biochemical constituents were analysed in fresh serum on the day of blood collection. The total serum proteins, albumin and globulin were estimated by using diagnostic kits. The statistical analysis to find the effect of age and sample variation was done using analysis of variance (Snedecor and Cochran 1967).

The data of serum protein, albumin and globulin are presented in Table 1. The total serum proteins and globulin in preparturient cows were higher ($P<0.01$) than its calves at birth while these were lower ($P<0.01$) than the calves of 4 to 5 months and 10 to 11 months of age. The serum albumin concentration ranged from 2.31 ±0.01 g/dl to 3.00 ±0.17 g/dl from birth to 72 hr. The value increased ($P<0.01$) on day 5, thereafter, it again decreased on 10th day ($P<0.05$) and 20th day ($P<0.01$) maintained at similar value up to 30th day. The serum albumin concentration in preparturient cows and calves of 4 to 5 months was similar while it was lower ($P<0.05$) than the serum albumin recorded in calves at birth and between 10 and 11 months of age. Following feeding colostrum the total serum protein and serum globulin increased from 2 hr up to third day. Three to four times increase in serum protein occurred on 5th, 10th and 15th day was associated with 6-to 7-times increase in serum globulin on respective day. Thereafter, the values declined significantly ($P<0.01$).

Table 1.Total serum proteins, serum albumin and serum globulin in crossbred cows and calves

Animals	Total serum protein (g/dl)	Serum albumin (g/dl)	Serum globulin (g/dl)
Preparturient			
Cows	6.78 ±0.76	2.26 ±0.07	4.51 ±0.79
Neonates			
30-40 min before feeding colostrum	4.58 ±0.04	2.83 ±0.02	1.75 ±0.02
120 min after feeding colostrum	5.60 ±0.13	2.31 ±0.01	3.28 ±0.10
360 min after feeding colostrum	5.68 ±0.23	2.36 ±0.06	2.31 ±0.19
Day			
Day 1	6.16±0.80	2.65 ±0.18	3.51±0.66
Day 2	8.03 ±0.40	3.00 ±0.17	5.03 ±0.71
Day 3	9.75±0.72	3.00±0.17	6.75±0.67
Day 5	17.31 ±0.94	3.78 ±0.26	13.53 ±0.77
Day10	13.60 ±1.20	2.58 ±0.24	11.01 ±1.17
Day 15	13.76 ±1.23	2.80 ±0.05	10.96±1.23
Day 20	8.50±0.66	2.78 ±0.03	5.71 ±0.81
Day 25	9.81 ±0.07	2.80 ±0.05	7.01±2.13
Day 30	7.10±0.55	2.73 ±0.26	4.36±0.40
Months			
4-5	9.03 ±0.56	2.66±0.12	6.36 ±0.47
10-11	9.98 ±0.20	3.01 ±0.19	6.96 ±0.29

The total serum protein recorded in preparturient cows of

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present experiment was lower (Ghosh *et al.* 1991) but comparable (Dutta and Dugvekar 1983) to the total serum protein concentration during advanced stage of gestation in cows. The total proteins and globulin determined in calves at birth in our experiment was lower than the total serum protein and globulin reported earlier in purebred calves of identical age (Kaneko and Mills 1970). The pattern of total serum proteins, albumin and globulin recorded from birth to day 3 in calves were similar to those recorded in cow (Kurz and Willett 1991) and buffalo calves (Joshi *et al.* 1993).

The increase in total serum proteins and globulin on fifth day after birth in calves was similar to the increase in serum globulin concentration within 3 days after birth among crossbred calves (McMurray *et al.* 1978). The increase in total serum globulin between 5 and 20 day after birth might be the consequence of the fact that calves after developing a completely aseptic uterine environment got exposed to the environment rich in microorganisms after birth and/or possibility to ingest some unidentified organism through sucking of teats and ingestion of different types of proteins/ epithelial cells and other antigens through colostrum. The above factors probably had cumulative effect to synthesize increased amount of immunoglobulin by the immune system of the neonatal calves and produced primary immune response to combat the situation in new born calves as confirmed earlier (Tizard 1996). Besides, the newborn calves having serum globulin less than 2 g/dl at birth have synthesized more serum globulin than the calves having more than 2 g/dl serum globulin at birth (Gaikwad *et al.* 1992, Lamotte 1977, Sridhar *et al.* 1988). Such mechanism to synthesize more immunoglobulins by hypogammaglobulinemic calves by 1-week –old supports the increase in serum globulin concentration between day 5 and day 15 in calves of present experiment as these calves were also having lower globulin (1.75 ±0.02 g/dl) at birth (Table 1). It has also been confirmed that the youngs of domestic animals are capable of mounting primary immune response with a prolonged period, and hypogamaglobulinemic calves at birth begins, to synthesize their own immunoglobulins by about 1- week of age (Devery *et al.* 1979, Roy 1980, Tizard 1996). The above possibility have been confirmed if the different fractions of globulin would have been estimated. The value of serum proteins, albumin and globulin recorded in calves from birth to 20 day (Swenson and Reece 1996, Schalms *et al.* 1975) and day 20 to 11 months of age (Gaikwad *et al.* 1992, Kitchenham *et al.* 1975, Kurz and Willett 1991, Rekwot *et al.* 1989) were similar to the protein, albumin and globulin determined at respective age in calves.

ACKNOWLEDGEMENTS

Authors thank the Dean-cum-Principal, Bihar Veterinary College, Patna, and Director (Research), Rajendra Agricultural university, Pusa, Bihar for providing adequate facilities.

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Indian Journal of Animal Sciences 70 (4): 403-404, April 2000

Circulatory level of follicle stimulating hormone (FSH) in Murrah buffalo heifers (*Bubalus bubalis*)

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Received: 7 July 1999; Accepted: 27 September 1999

Key words: Buffalo heifers, FSH

Information is very limited on endocrine profile in prepubertal buffaloes. It is not established whether in buffaloes the expression of first oestrus is delayed due to inadequate growth rate, poor oestrus expressivity at the start of the ovarian cyclicity, incoordination of hypothalmo-hypophyseal-gonadal axis or due to poorly developed receptors at pituitary and ovarian level. The estimation of circulatory level of hypophyseal hormone at different ages of buffalo heifers will indicate the release pattern of this hormone from prepubertal to pubertal and late maturing buffaloes and relate the concentration of hypophyseal hormone with onset of puberty. The present experiment was conducted to study the circulatory level of follicle stimulating hormone (FSH) in Murrah buffalo heifer *(Bubalus bubalis)* of different age groups.

Non-cycling Murrah buffalo heifers (6) at each age group of 12 months and 24 months while 5 heifers of 30 months and 4 heifers of 42 months (late maturing) were selected from institute's herd. A potent teaser bull with trained attendant was used 6 hr daily from the day they attained their particular age to detect the oestrus. Genitalia were palpated per rectum to observe ovarian condition. Blood samples were collected from each animal through jugular catheter between 7.00 AM and 9.00 AM once daily on day 0 (the day when animals attained its particular age), 3, 5, 7, 9, 13, 18 and 21 in chilled heparine-treated test tubes. Plasma was separated and preserved at –20°C for estimation of FSH. FSH was estimated by sensitive radioimmunoassay within 30 days of blood collection. The radioiodenation of FSH using chloramin T, its radiochromatography of labelled preparation and radioimmunoassay of FSH was carried out (Singh and Madan 1998). The sensitivity of FSH was 0.08 ng/tube. The intra- and inter-assay coefficient of variation for FSH were 4.5% and 4.9% respectively. Pair T test and two-way analysis of variance to compare the difference of means of hormone between the samples and groups of animals were obtained (Snedecor and Cochran 1967).

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The genitalia of all heifers were normally developed and heifers were not detected in oestrus at start of blood sampling. The circulating plasma FSH concentration (mean ±SE) in 12 months and 42 months old buffalo heifers was significantly lower ($P< 0.05$) than 24 months and 30 months. However, FSH values between 12 months and 42 months and 24 months and 30 months old heifers were nearly identical.

The age of puberty in buffalo is highly variable and depends on breed and different climatic conditions. However, in India the age of puberty in Murrah buffaloes ranged from 2.5 to 3.5 years (Madan and Raina 1984). The significantly higher plasma FSH level in 24 and 30 months of buffalo heifers than 12 months heifers indicate that sexual maturation of buffalo heifers requires the elevation of FSH in circulation. Our observation also get support from earlier report on cattle heifer that content of FSH in pituitary tissue (Desjardin and Hafs 1968) and in circulating blood (Gonzalez-Padilla *et al.* 1975) remain stable at lower level during prepubertal stage and the event of sexual maturation are associated with elevation of FSH in circulation of sheep (Foster *et al.* 1975) and cattle (Desjardin and Hafs 1968). The significant higher plasma FSH concentration in 24 months and 30 months than in 12 months buffalo heifers in present experiment revealed that buffalo heifers at 24 months of age reached at certain stage of sexual maturation when ovarian feed back action initiates the events of onset of puberty in coordination with hypothalamic-hypophyseal axis, since a significant increase in circulatory plasma FSH concentration associated with follicular development and resumption of sexual cyclicity in mature buffaloes (Razdan *et al.* 1984, Galhotra *et al.* 1985) has been reported. The FSH level observed in 24 months and 30 months buffalo heifers are similar to the basal FSH concentration in parous Murrah buffaloes during postpartum when follicular activity is suspended (Palta and Madan 1995). This suggests that FSH concentration recorded in 24-and 30-month-old buffalo heifers has reached at higher threshold of secretion akin to parous buffaloes, though not in full measure to activate the hypothalamic-hypophyseal and gonadal axis.

The lower plasma FSH concentration in 12-month and 42-

Table 1. Circulating levels of follicle stimulating hormone (ng/ml) in buffalo heifers (*Bubalus bubalis*)

Day of sample	12 Months (6)	24 Months (6)	30 Months (5)	42 Months (4)
1st day	7.64±0.36	1.27 ± 0.98	11.32 ± 0.54	7.43 ± 0.83
3rd day	6.91 ± 0.15	11.27 ±0.45	11.06 ± 1.08	7.22 ± 0.67
5th day	7.32 ± 0.65	11.12 ± 1.12	10.00 ±0.4	8.13 ± 1.09
7th day	7.86 ± 0.67	11.43 ± 0.60	10.85 ±1.1	7.86 ± 0.85
9th day	8.16 ± 0.26	10.45 ± 0.90	11.49 ±0.46	6.81 ± 0.85
13th day	7.38 ±0.35	11.25 ± 1.37	11.88 ± 0.86	7.58 ± 1.03
18th day	7.89 ± 0.81	10.62 ± 1.14	12.23 ±1.77	7.54± 1.23
21st day	6.83 ± 1.37	10.45 ± 1.13	11.09 ± 1.48	7.59 ± 0.91

Figures in parentheses indicate number of animals.

month-old buffalo heifers might be because of either poor maturation or incoordination of the hypothalamo-hypophyseal axis as the major cause for reduced secretion of hypophyseal hormone and delayed puberty has been ascertained to be the incoordination or dysfunction of hypothalamic-hypophyseal axis (Wilson and Foster 1985).

The difference in basal circulating gonadotrophin level observed among buffalo heifers of different age groups indicated that process of sexual maturation of buffaloes was associated with increase in FSH release up to 30 months of age.

However, a possible lack of intrinsic hypothalamic-hypophyseal interplay resulting in lower secretion of FSH was observed in late maturing buffalo heifer of 42-month of age.

ACKNOWLEDGEMENT

Authors thank the Director, National Dairy Research Institute, Karnal 132 001, for providing facilities for this experiment.

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Indian Journal of Animal Sciences 70 (4): 405-406, April 2000

Clinicopathological changes in pigs experimentally infected with *Sarcoptes scabiei*

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Received: 18 January 1999; Accepted: 1 November 1999

Key words: Mange, Pig

The present study was planned to see the effect of *Sarcoptes scabiei* in experimental infection on clinical signs and histopathological changes of skin in pigs maintained at Pig Breeding Farm of Ranchi Veterinary College, Ranchi (Bihar). Before experimental infection of pig, infected scraping materials were collected from the natural cases confirmed for *S. scabiei*. Hairs from the selected site were clipped and then slightly scratched both horizontally and transversely with the pointed edge of scalpel. The infected scraping materials containing the adult mites of both sexes and their developing stages were applied at the site. Few drops of tap water sprinkled to the site to keep the scrapings remained adhered to the site of application.The applied scraping materials were loosely covered with the pieces of bandage attached to the body with adhesive tape. On third day observation was made to know the establishment of infection at the site. Daily observations were made to study the progress of lesion, course and clinical signs of the disease. From 14th day of the infection, the lesions were studied. Skin biopsies were taken from the site of infection and histopathological changes were studied (Culling 1963) on appearance of clinical mange infections.

The present investigation also included 223 pigs naturally infected with sarcoptic mange and showing the clinical signs. There was a marked irritation with intense itching and scratching, exudation from inflammatory parts. Later, excessive keratinisation and proliferation of connective tissue lead to thickening and wrinkling of the skin. Loss of hair, thick brown scab, scratching and biting were the common clinical observations seen in the study, as reported by Blood *et al.* (1994). The maximum number of cases were observed in February to March.

In experimental infection, on third day the lesion developed with marked keratinisation of skin and crust formation at the site. The infection was localized and progressive. Pruritis, itching, biting at the site and scratching of the body were observed. Slow progressive development of lesion all over the body was also seen. On 14th day of infection, generalised lesions were observed and apparent clinical signs were marked

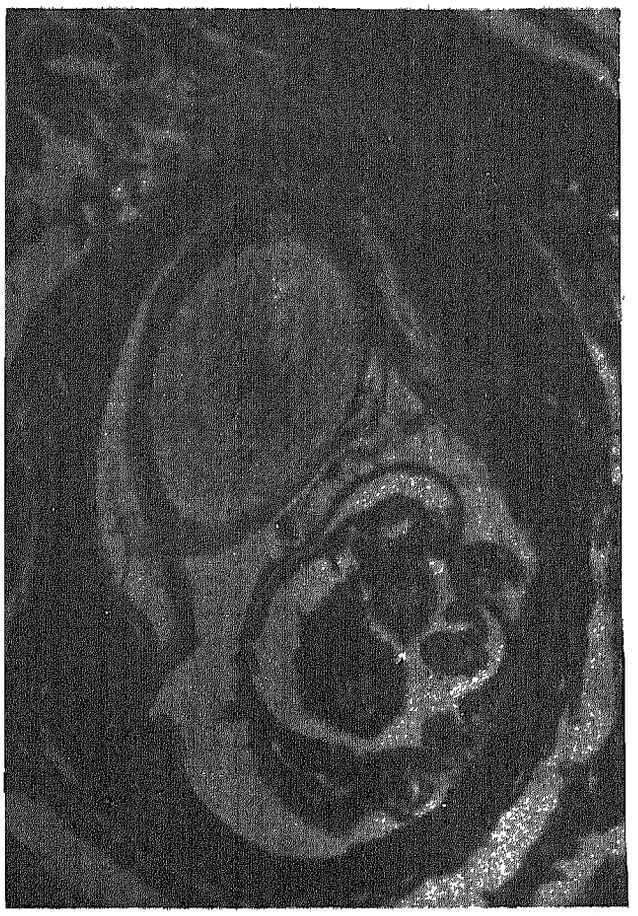

Fig. 1. Microphotograph showing magnified view of hair follicle infected with *Sarcoptes scabiei*.

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on 17th day onwards. Similar clinical observations were made by Penny and Muirhead (1986).

The histopathological study of infected skin biopsy revealed different stages of mite in stratum corneum. Bunches of ova were seen in superficial horny layer of epidermis. Few mites were also observed in stratum malpighii. Similar observations were reported by Sahai *et al.*(1984) and Morsy *et al.*(1989). In epidermis varying degree of changes like hyperkeratoses, acanthosis of parakeratosis were observed under the microscopic examinations of histopathological slides. Other changes like mononuclear cell infiltration of inflammatory cells were observed. There was hyalinization of the stratum corneum where mites were located. At places dysplastic reaction was observed in the stratum malpighii. Occasionally, the mites penetrated into the dermis and localised in the hair follicles (Fig.1).

In the case of investigation, fungi, viz. *Trichophyton montagrophytes*, *Canadida* spp. and *Altenaris* spp. were also isolated.

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Indian Journal of Animal Sciences **70** (4): 407-408, April 2000

Observations during parturition in Black Bengal goats

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Received: 5 June 1998; Accepted: 30 September 1999

Key words: Black Bengal goat, Parturition observations

Safe delivery and expulsion of foetal membrane have great influence on the overall production and reproductive efficiency of a female. Information on parturition characteristics of Black Bengal goats is meagre. An attempt was made to study different physical parameters and behavioural symptoms during parturition.

Pregnant Black Bengal goats (40; first to fourth parity) of the institute goat farm, due for kidding were carefully watched 10 days before the expected date of parturition. Symptoms of approaching parturition and during the different stages of parturition were watched carefully from a distance so that the animals were not disturbed. The duration of kidding was arbitrarily divided into 3 stages. The period from the beginning of labour to rupture of water bag was considered as first stage. The period from the rupture of water bag to expulsion of foetus was considered as the second stage in the single birth and in multiple birth, the second stage ranged from rupture of first water bag to the expulsion of last foetus. The third stage starts from the expulsion of the first/last foetus to the dropping of the placenta. Sex, presentation of foetus, type of kidding and weight of the placenta were recorded. Data were analysed statistically (Snedecor and Cocharn 1967).

The symptoms of approaching parturition included restlessness (90%), pronounced development of udder (92.5%), relaxation and loosening of sacrosciatic ligaments (82.5%), widening and swelling of vulva (97.5%) and vaginal mucus discharge (50%). The symptoms of parturition were almost identical to that reported by Verma *et al.* (1990 a, b). However, symptoms like loss of appetite and segregation from the flock were seen in 10 and 40% cases, respectively.

Most of the goats exhibited symptoms like restlessness, bleating, sitting down and getting up, looking back and loss of appetite during the first and second stage of parturition. Observations of Verma *et al.* (1990 b) and John (1990) are in agreement with the present findings. During parturition 65% goats assumed sitting position whereas 35% kept standing. During third phase, 90% animals showed restlessness and bleating was observed in 15% animals. The symptoms might be due to individual difference, breed and topography (*Bhattacharyya et al. 1983, Kuriakose et al. 1983*). For male birth, mean duration of first, second, third stages of parturition and total duration of parturition was 91.7±6.22, 17.48±2.04, 132.52±9.78 and 241.7±10.41 min, whereas in female birth, it was 94.17±8.09, 16.08±1.89, 122.16±12.53 and 232.42 ±15.84 min respectively. The difference was nonsignificant though more time was taken by the male kid for parturition than female. The mean time taken in single birth for first, second, third stage of parturition and total duration of parturition was 93.05±7.68, 16.63±1.86, 127.34±7.35 and 237.13±11.54 min, whereas in twin kidding it was 98.85±17.61, 18.62±3.15, 134.61±22.38 and 252.07±30.62 respectively. Out of 40 kidding 1 triplet kidding was recorded and the time taken for first, second, third stages of parturition and total duration of parturition was 102, 25, 129 and 256 min respectively. In general, more time was taken for twin and triplet kidding than single kidding but there was no significant differences between duration of single and twin kidding which is in agreement with the report of Verma *et al.* (1990 a) in local goat of Madhya Pradesh. Kuriakose *et al.* (1983) reported that more time was required for completion of parturition in the twin or multiple births as compared to single birth because expulsion of single foetus takes less time than the expulsion of consecutive foetuses. Out of 63 kids born in 40 kiddings, 5 (7.94%) kids were born with posterior and 58 (92.06%) with anterior presentation, which is nearer to normal presentation (Jindal 1984). The average weight of placenta was 224.46±5.36, 398.13±6.96 and 495 g in single, twin and triplet births respectively. Single, twin and triplet kidding percentage were recorded as 45, 52.5 and 2.5, respectively, which clearly showed the tendency of more twin kidding and this observation was supported by Singh (1980). Male and female kid ratio was recorded as 4: 5.

ACKNOWLEDGEMENTS

Authors thank the Director, ICAR Research Complex for NEH Region, Umiam, Meghalaya, and Joint Director, ICAR,

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Tripura Centre, Lembucherra, for providing necessary facilities for the study.

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Indian Journal of Animal Sciences **70** (4): 409-414, April 2000

Performance of crossbred cattle and comparison of sires evaluation methods under organized farm condition

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Received: 8 April 1999; Accepted: 30 September 1999

ABSTRACT

The efficiency of sire evaluation methods in organized herd with small progeny group size was examined along with performance evaluation of crossbred cattle. The data on 1 224 first lactation performance records of Karan Fries (Holstein Freisian × zebu) cows born out of 129 sires and spread over 21 years were used to examine the efficiency, accuracy and repeatability of 5 sire evaluation methods, viz. simple daughter average index (I), contemporary comparison method (CC), least squares method (LSQ), simplified regressed least squares method (SRLS) and best linear unbiased prediction (BLUP). The least squares of variance showed that genetic group had highly significant ($P<0.01$) effect on age at first calving (AFC), first lactation 305-day or less milk yield (305MY) and first lactation total milk yield (LTMY). The season of calving had highly significant effect on 305MY, LTMY, first service period (FSP) and first calving interval (FCI). The period of calving had highly significant effect on 305MY, FSP, and FCI. The CC method was observed to be the most efficient sire evaluation method and I the least efficient. The F_1 (HF × Tharparkar) crosses had highest 305MY among all the genetic groups. The rank correlations of CC method with other 4 methods ranged between 0.77 with SRLS and 0.85 with BLUP. The repeatability of sires' breeding value estimation in CC, LSQ, SRLS and BLUP was lower with less number of progeny record (5-8), however, increased with the increase of progeny group size.

Key words: Breeding value, Crossbred cattle, Sire evaluation method (SEM)

Serious limitation of using advanced sire evaluation methods exists in India because most of the progeny testing programmes are limited within organized farms with small herd size resulting small progeny number/sire. Besides this spread of data over a longer period is another important limitation in estimating sire breeding values. The information on the efficiency of various sire evaluation methods, change in accuracy of sire evaluation with the change of progeny group size, evaluation of the phenotypic performance of crossbred cattle and the suitability of sire evaluation methods in organized herd condition was meagre. Hence present study was designed to evaluate breeding value of bulls besides genetic parameters on the number of progeny available.

MATERIALS AND METHODS

The present investigation was carried out on Friesian × zebu crossbred cattle (Karan Fries) maintained at the National Dairy Research Institute (NDRI), Karnal. The data comprised 1 224 first lactation performance records of Karan Fries cows born out of 129 sires and the data were spread over 21 years from 1974 to 1994.

The animals were classified into 5 genetic groups, viz. (i) HT (F_1: Holstein × Tharparkar), (ii) HS (F_1: Holstein × Sahiwal), (iii) HJT (Holstein (50%) + Jersey (25%) + Tharparkar (25%), (iv) HBT (Holstein (50%) + Brown Swiss (25%) + Tharparkar (25%), (v) KF (crossbred-*inter se* mated). The year was divided into 5 seasons based on climatological factors, viz. winter (December-January), spring (February-March), summer (April-June), rainy season (July-September) and autumn (October-November).

The data utilized for the present study were spread over 21 years. Therefore, there might be difference in performance of animals from year to year due to the change of various nongenetic factors like feed and fodder availability, management practices and environmental factors. However, the variation might not be significant enough to detect the effect of each year separately because of small number of records available per year. Therefore, all the data were grouped into 7 periods taking 3 years in each period. This was done with the aim to minimize within period variance and to maximize between period variation.

The effect of various factors like genetic group, period and season of calving on various first lactation production

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and reproduction traits of Karan Fries cattle were studied to ascertain the contribution of these factors towards variability in various economic traits. The data were subjected to least squares analysis as described by Harvey (1990). The mathematical model used to study the effect of different factors was as follows.

$$Y_{ijklm} = \mu + S_i + P_j + G_k + B_l + b\,(X_{ijklm}-A) + e_{ijklm} \quad \text{.....(i)}$$

Where, Y_{ijklm} is the observation associated with mth animal of l^{th} sire of kth genetic group in the jth period and ith season; μ is population mean; S_i is the effect of ith season; P_j is the effect of jth period; G_k is the effect of kth genetic group; B_l is the effect of lth sire; b is the regression of Y on age at first calving (X); X_{ijklm} is age at first calving corresponding to Y_{ijklm}; A is the average age at first calving, and e_{ijklm} is the random error associated with mth observation assumed to be NID (0, σ_e^2).

Sire evaluation methods (5) were used to estimate the breeding value of sires. The record of sires having at least 5 or more daughters with first lactation recorded was used for sire evaluation. The method used were simple daughter average index (I), contemporary comparison method (CC), least squares method (LSQ) (Harvey 1990), simplified regressed least squares method (SRLS) (Harvey 1979) and best linear unbiased prediction (BLUP) (Henderson 1973). The I and CC method was followed as given by Jain and Malhotra (1971). In LSQ method the mathematical model used was as described in equation (i) where, season, period, genetic group and sire effect was considered as fixed variable and age at first calving was taken as a covariable.

In SRLS sire's breeding value was estimated as:

$$S_s = \frac{\sigma^2_s}{\sigma^2_s + A_{ii}\sigma^2_e}\ S_i$$

where, S_s or S_i are breeding value of ith sire by SRLS and LSQ method respectively; σ^2_s and σ^2_e are sire and error variance respectively obtained by LSQ method; A_{ii} was diagonal element of the inverse of the coefficient matrix corresponding to its sire.

In BLUP the mathematical model used was same as in LSQ except the sire effect was taken as random. The effectiveness of sire evaluation method was judged using 2 criteria, *(i)* efficiency i.e. inverse of error (within sire) variance, and *(ii)* rank correlation among various methods. The simple correlation among the breeding values estimated by various method were also calculated. The repeatability of sire evaluation methods were estimated as correlation between breeding values estimated taking variable progeny group sizes with that of 'true breeding value' (based on 15 or more progeny record) by the same method.

RESULTS AND DISCUSSION

The least-squares means for first lactation economic traits and standard errors of various sub groups are presented in Table 1.

Age at first calving (AFC)

The effect of genetic group was highly significant (P<0.01) on age at first calving. The age at first calving was minimum

Table 1. Least squares means for first lactation production and reproduction traits in Karan Fries cattle

Effects	AFC (days)	305-MY (kg)	LTMY (kg)	FLL (days)	FSP (days)	FDP (days)	FCI (days)
Overall mean	968.5±7.9 (1224)	2919.2±44.7 (1212)	3338.1±69.5 (1196)	343.7±5.8 (1196)	127.5±6.1 (965)	65.1±2.9 (964)	406.8±6.0 (966)
Genetic group							
HT	856.9±17.5^{a} (119)	3549.2±99.1^{a} (119)	3956.7±153.4^{a} (119)	328.6±12.7 (119)	122.9±12.3 (108)	61.8±6.0 (155)	400.2±12.2 (116)
KF	987.1±5.3^{b} (908)	3101.0±33.5^{b} (892)	3590.8±51.9^{b} (878)	351.4±4.3 (878)	139.4±4.4 (714)	70.4±2.1 (703)	421.1±4.4 (703)
HS	1058.9±20.1^{c} (57)	2730.1±113.6^{c} (67)	2951.0±176.0^{c} (67)	320.2±14.6 (67)	113.9±15.2 (51)	71.9±7.3 (53)	400.3±15.0 (53)
HJT	935.5±15.4^{d} (83)	2585.0±87.4^{e} (82)	3065.6±136.0^{c} (81)	359.5±11.3 (81)	138.7±12.4 (58)	63.6±5.9 (60)	416.8±12.1 (60)
HBT	1004.2±18.0^{b} (57)	2630.5±106.5^{c} (52)	3126.6±166.3^{c} (51)	359.0±13.8 (51)	122.6±15.7 (34)	57.7±7.7 (33)	395.5±15.6 (34)
Season							
Winter	971.3±10.6 (263)	3042.7±56.9^{a} (291)	3412.9±88.4^{a} (289)	335.5±7.3^{a} (289)	116.2±7.5^{a} (241)	65.7±3.7 (242)	396.4±7.5^{a} (243)
Spring	978.2±10.9 (224)	2836.1±53.9^{b} (335)	3394.2±84.1^{a} (328)	357.9±7.0^{b} (328)	139.8±7.5^{b} (254)	66.0±3.7 (249)	418.8±7.5^{b} (249)
Summer	970.9±10.8 (251)	2911.7±57.4^{bc} (311)	3476.1±89.5^{a} (307)	362.4±7.4^{b} (307)	158.1±7.9^{b} (246)	72.4±3.8 (249)	438.4±7.7^{b} (249)
Rainy	964.5±9.9 (323)	2798.1±70.9^{b} (155)	3098.3±110.1^{b} (154)	326.9±9.1^{a} (154)	116.7±9.5^{a} (127)	60.1±4.6 (128)	392.8±9.3^{a} (129)
Autumn	957.8±12.3 (163)	3007.2±77.0^{ac} (120)	3309.1±120.0^{ab} (118)	336.0±9.9^{a} (118)	106.6±10.3^{a} (97)	61.4±5.0 (96)	387.4±10.3^{a} (96)
Period							
1974-76	966.0±13.8^{a} (155)	3017.8±85.3^{ac} (138)	3383.3±132.0 (138)	350.3±10.9^{ac} (138)	114.9±11.1^{a} (116)	62.2±5.3 (125)	396.4±10.9^{a} (126)
1977-79	1035.0±10.6^{b} (178)	2800.2±65.0^{ab} (132)	3485.7±100.6 (132)	381.1±8.32^{b} (132)	156.4±8.7^{c} (107)	65.6±4.2 (107)	435.0±8.6^{b} (108)
1980-82	1043.7±12.0^{b} (190)	2713.2±68.3^{b} (202)	3309.9±106.2 (200)	366.9±8.8b^{c} (200)	149.1±9.3^{bc} (142)	60.8±4.5 (144)	429.9±9.3^{b} (144)
1983-85	974.9±13.4^{a} (211)	3038.0±77.6^{c} (213)	3391.4±120.3 (213)	335.8±10.0^{a} (213)	119.8±10.4^{ab} (167)	60.8±5.0 (168)	399.0±10.3^{a} (168)
1986-88	888.5±13.6^{c} (192)	3031.5±79.6^{c} (198)	3454.4±123.3 (196)	340.3±10.2^{a} (196)	126.8±10.4^{ab} (174)	65.0±5.1 (168)	403.9±10.4^{a} (168)
1989-91	966.6±13.6^{a} (207)	2917.3±85.2^{ac} (163)	3229.8±132.0 (163)	321.9±10.9^{ad} (163)	114.9±11.4^{a} (128)	69.2±5.6 (125)	393.0±11.4^{a} (125)
1992-94	904.9±17.0^{d} (91)	2916.2±83.7^{ac} (168)	3112.5±132.2 (154)	309.8±10.9^{d} (154)	110.7±11.3^{a} (131)	72.0±5.5 (127)	390.4±11.3^{a} (127)

Means with similar superscripts do not differ significantly (P<0.05) from each other.

Figures in the parentheses are number of observations.

(856.9±17.5 days) in HT (F_1) crosses and maximum (1058.9±20.1 days) in HS crosses. The change of AFC in crossbred cattle with the change of exotic breed and level of exotic inheritance was also reported by Gill (1979). Parmar *et al.* (1984) observed that halfbreds had the lowest age at first calving. Similarly, the effect of exotic inheritance level on AFC was found to be significant by Reddy and Basu (1985).

The season of birth of the animal did not show any significant effect on age at first calving. Similar non-significant effect of season of birth on AFC was reported by Polastre *et al.* (1987). On the contrary, Singh *et al.* (1989) reported that AFC was significantly affected by season of birth of the animal.

The effect of period of birth was highly significant (P<0.01) on AFC in this study. The animals born during 1990-1992 had the lowest AFC. Significant effect of period on AFC was also reported by Chaudhary *et al.* (1994).

305-day or less milk yield (305MY)

The genetic group was observed to have highly significant effect (P<0.01) on first lactation 305-day or less milk yield. The HF (F_1) crosses had highest average 305MY. The imported semen of progeny tested HF bulls were used to produce F_1 animals. This might be the reason of highest 305MY in F_1 animals. The HJT crosses had lowest 305MY. Jadhav *et al.* (1991) and Datt and Joshi (1992) reported highly significant effect of genetic group on first lactation 300-day-milk yield. Jadhav *et al.* (1991) observed that halfbred had highest milk production.

The season of calving had significant (P<0.05) effect on 305MY. This may be due to variation in availability of feeds and fodder and also the climatic factors in different seasons. The highest average milk production was found in those animals which calved during winter and, similar level of milk production was also found in animal calved in autumn. The animals those calved in rainy season had lowest 305MY. Similar effects of season on 305MY were reported by Nagarcenkar and Rao (1982).

The period of calving had highly significant (P<0.01) effect on 305MY. The lowest average 305MY was found in cows calved during 1980 to 1982. The highest average 305MY was in animal calved during the fourth period (1983-85). The period of calving, although had significant effect on milk yield, did not show any specific trend.

Lactation total milk yield (LTMY)

The effects of genetic group and season of calving were found to be highly significant (P<0.01) on LTMY. Similar to that of 305MY, HT (F_1) had highest and HS crosses had the lowest LTMY. Singh *et al.* (1993) observed that genetic group had significant effect on first lactation milk yield. Similar to this study, the effect of genetic group was found to be highly significant on lactation milk yield (Jadhav *et al.* 1991) and also observed that halfbreds had higher lactation milk production. The results showed that animals those calved during rainy season had lower LTMY. However, the animals that calved in summer, winter, spring and autumn did not differ significantly as far as LTMY was concerned. Singh *et al.* (1981) reported that in HF × Sahiwal crossbreds, winter calvers had higher LTMY in comparison with the cows those calved in other seasons.

The period of calving had nonsignificant effect on LTMY. Similar nonsignificant effect on period of calving on LTMY was reported by Basu *et al.* (1979).

First lactation length (FLL)

The analysis of variance indicated that the genetic groups did not have significant effect on first lactation length (FLL). Milagres *et al.* (1988) observed that the breed of the animal did not affect the lactation length. Dalal *et al.* (1991) and Singh *et al.* (1993) reported that first lactation length was not significantly influenced by genetic group. However, Tajane and Rai (1989) and Jadhav *et al.* (1991) observed significant variation in lactation length in various genetic groups.

The season of calving had highly significant (P<0.01) effect on first lactation length. The animals that calved during summer had longest average FLL. The rainy season calvers had shortest FLL. Milagres *et al.* (1988) and Jadhav *et al.* (1991) also reported significant effect of season on FLL.

The period of calving also had highly significant (P<0.01) effect on FLL. There was a gradual decrease in FLL over the periods. This may be due to improvement of reproductive management of the herd over the years. This was also evident from decrease in FCI of the animals over the years (Table 1). This had resulted into force drying the cows about 2 months before the next calving. The significant effect of years or period of calving on FLL was also reported by Tajane and Rai (1989) and Jadhav *et al.* (1991).

First service period (FSP)

The genetic group had nonsignificant effect on FSP. However, the season of calving had highly significant (P<0.01) effect on first service period. The animals calved in summer had highest service period and the autumn calvers had lowest service period. The significant effect of season of calving on FSP were reported by Bhat *et al.* (1978), Duc and Taneja (1984) and Jadhav *et al.* (1991). Similarly the period of calving also had highly significant effect on FSP. There was a decreasing trend in average FSP over the periods. Similar to this study, significant effect of period of calving FSP was reported by Duc and Taneja (1984) and Rao and Sunderseran (1982).

First dry period (FDP)

The effect of genetic group, season of calving and period of calving were found to have nonsignificant effect on FDP. This may be due to the reason that animals are allowed to dry about 2 months before the next calving. This is evidence from the population average of FDP (65.09±2.95 days). Therefore

Table 2. Average and range of breeding values (BV) in various sire evaluation methods

Methods	Average BV (kg)	No. of sires above the average	No. of sires below the average	Maximum BV(kg)#	Minimum BV(kg)#	Range of BV(kg)
D	3095	35	37	4065 (39.25)	2330 (20.18)	1735
CC	2918	33	39	3894 (33.39)	1879 (35.63)	2015
LSQ	2886	37	35	3578 (22.57)	2108 (27.79)	1470
SRLS	2895	35	37	3335 (14.24)	2473 (15.29)	862
BLUP	2905	36	36	3437 (17.74)	2480 (15.05)	957

Values in the parenthesis are per cent above/ below the herd average.

this FDP was mostly decided by management based on the probable date of subsequent calving rather than allowing the cows to dry naturally. Singh *et al.* (1993) also reported that the genetic group, the season of calving and the period of calving did not affect FDP significantly.

First calving interval (FCI)

The influence of various genetic groups on first calving interval was nonsignificant. However, the effect of season of calving on FCI was highly significant (P<0.01). The autumn calvers had shortest FCI as also observed in case of FSP. The summer calvers had longest FCI. Parmar *et al.* (1984) and Jadhav *et al.* (1991) also reported significant effect of season on FCI.

The effect of period of calving on first calving interval was observed to be highly significant. There was an overall decrease in FCI over the periods. Significant decrease in FCI over the periods indicates improvement in reproduction management. The significant effect of year/ period of calving in FCI was also reported by Dalal *et al.* (1991) and Jadhav *et al* (1991).

Average breeding values and their ranges

The average breeding values and range of breeding values estimated by various methods are presented in Table 2. The average breeding values ranged from minimum of 2 886 kg in LSQ to 3 095 kg in I method. The range between maximum and minimum breeding was lowest (862 kg) in SRLS method and highest (2 015 kg) in CC method. The top ranking sire had breeding value 33.39% above herd average in CC method. Out of the 5 methods used in this study, CC method discriminated among the bulls better which was evident from the range of breeding values. The percentage of sires whose BV was 5, 10 and 20% above herd average are given in Table 3. The results in CC and LSQ methods were very similar. The percentage of sire whose breeding value was above 20% of herd average was very low (1.39%) in SRLS and BLUP methods.

Efficiency of various sire evaluation methods

The breeding values for 305-day or less milk yield of Karan Fries bulls were estimated by sire evaluation methods, as per materials and methods. The relative efficiency of different sire evaluation methods were estimated in comparison to the most efficient method observed in this study and the results are presented in Table 3. The efficiency of 5 sire evaluation methods was judged by error (within sire) variance. Out of the 5 methods, contemporary comparison method (CC) had the lowest error variance. Therefore, CC method was adjudged to be the most efficient method for estimating the sires breeding value in this study.

Table 3. Relative efficiency of various sire evaluation methods and the superiority of sires over herd average

Method	Error variance	Relative efficiency	Percentage of sires' BV 5% above herd average	Percentage of sires' BV 10% above herd average	Percentage of sires' BV 20% above herd average
CC	282215	100.00	30.56	15.28	11.11
D	2143648	13.17	29.17	18.06	9.72
LSQ	484160	58.00	27.78	22.22	11.11
SRLS	483761	58.34	20.83	4.17	1.39
BLUP	514898	54.81	19.44	4.17	1.39

The relative efficiency of different sire evaluation methods ranged from minimum 13.17% in simple daughter average index (I) to maximum 58.34% in simplified regressed least squares (SRLS) methods. The relative efficiency in case of LSQ and BLUP were 58.29 and 54.81% respectively. Jain and Malhotra (1971) observed that relative efficiency of contemporary comparison method was higher than that of simple daughter average index. However, Gurnani and Nagarcenkar (1982) reported that BLUP method was more efficient than contemporary comparison method when more than 10 daughters/sire was used. Singh (1992a) and Singh (1992b) reported that contemporary comparison method was more efficient than LSQ and BLUP methods. The results obtained in the present investigation were contrary to the reports from the developed country that Henderson's BLUP method was most efficient (Danell 1982 and Anacker and Diete 1990).

From the above results, it appeared that although theoretically, the BLUP method is considered to be the most efficient method followed by LSQ method, but in practice, data obtained under Indian farm conditions, these methods may not be the most efficient and accurate methods due to

Table 4. Rank correlation and simple correction between breeding values by different methods

Method	D	CC	LSQ	SRLS	BLUP
D	1.0	0 83	0.86	0 86	0.87
CC	0.79	1.0	0.82	0.84	0.90
LSQ	0.84	0.77	1.0	0.98	0.95
SRLS	0.85	0.79	0.99	1.0	0 96
BLUP	0.86	0.85	0.94	0.94	1.0

Values below the diagonal are rank correlation and values above the diagonal are simple correlation.

small data size and spread of data over longer period and the CC method might be more efficient in estimating the breeding value of sire when data from a single herd is used.

Accuracy of various methods

Accuracy of various methods of sire evaluation with respect to the most efficient method (CC) observed in this study was estimated by determining the simple and rank correlation and presented in Table 4. The rank correlation among various methods ranged between 0.77 (CC and LSQ) and 0.99 (LSQ and SRLS). The rank correlation between CC method and other methods ranged from 0.77 in LSQ to 0.85 in BLUP. All the rank correlation were highly significant ($P<0.01$). The high and significant rank correlations between the prevalent sire evaluation methods indicated that all these methods did not differ significantly in ranking of sires. The simple correlation between breeding values estimated by various methods (Table 4) were also high and ranged between 0.82 (CC and LSQ) and 0.98 (LSQ and SRLS). Similar to this observation Cordovi *et al.* (1984) also found that there were slight difference in ranking of bulls by CC, LSQ and BLUP methods. Tajane and Rai (1990) found that correlation of breeding values by contemporary comparison, least squares and BLUP with auxiliary sire index ranged from 0.597 to 0 681. Singh (1992a) observed that the rank correlation between CC with LSQ and BLUP were 0.872 and 0.866 respectively.

Repeatability of breeding values with change in progeny group size

The breeding values of sires were estimated separately on the basis of 4 progeny group sizes, viz. 5, 8, 12 and 15 or above daughters performance. The correlation between the breeding values of the sire based on different progeny group size and the 'true breeding value' *i.e.* based on 15 or above daughters using same methods were estimated and presented in Table 5.

The results showed that in simple daughter index the repeatability between breeding values when progeny size were small with that of 'true breeding value' were higher in comparison to that of other sire evaluation methods. However, the increase in repeatability values with the increase in progeny group size from 5 to 12 were maximum in CC method

Table 5. Repeatability of sire evaluation correlation between breeding value of bulls on the basis of varying progeny group size with that based on "15 or above" daughters records by same method in Karan Fries cattle

Method	Progeny group size		
	5	8	12
D	0.53	0.62	0.82
CC	0.42	0.57	0 73
LSQ	0.32	0.46	0.54
SRLS	0.33	0.46	0 54
BLUP	0.40	0.58	0 69

(74%) followed by BLUP (73%), LSQ (69%), SRLS (64%) and I (55%). These results indicated that the repeatability of breeding value estimation was low in improved sire evaluation methods when progeny group size was small. However, with the increase in progeny size the increase in repeatability of sire evaluation was higher in these methods. Therefore, number of daughter records available per sire may be the deciding factor of sire evaluation method appropriate for a specific data.

Moller (1976) considered progeny tests with less than 20 daughters of little value and increasing progeny group size above 40 as unnecessary. Singh (1992b) observed that the accuracy of sire evaluation with 5, 8 and 12 progeny group size in Karan Fries cattle were higher in simple daughters average index than CC, LSQ and BLUP method of sire evaluation.

Contemporary comparison was most efficient method among all the methods studied. The BLUP method though considered to be the most efficient method, had lower efficiency than CC method under the given Indian farm conditions. The repeatability of sires' breeding value estimation in CC, LSQ, SRLS and BLUP was lower with less number of progeny record (5-8), however, increased with the increase of progeny group size.

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Indian Journal of Animal Sciences **70** (4): 415-418, April 2000

Effect of urea supplementation to urea molasses mineral block (UMMB) lick on nutrient utilization and rumen fermentation pattern in crossbred cattle

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Received: 26 March 1999; Accepted: 29 September 1999

ABSTRACT

The effect of incremental urea supplementation on intake of urea-molasses mineral blocks (UMMB), nutrient utilization and rumen fermentation was investigated on 16 adult crossbred cattle. They were divided into 4 equal groups following a completely randomized design. Animals were offered wheat straw and UMMB lick *ad lib.* and crushed maize grains to meet out the energy requirements. In addition to this, animals in groups 1 to 4 were offered urea @ 0, 15, 30 and 45 g/ animal a day, respectively. A metabolism trial of 6 days duration was conducted. Results revealed that intake and digestibility of all the nutrients were alike except that CP intake was significantly ($P<0.05$) more in urea supplemented groups. However, the intake of UMMB was highest in group 2 where urea was supplemented @ 15g/day. Balances of N linearly increased due to various levels of urea supplementation. A fermentation study was carried out on 4 rumen fistulated adult cattle following 4 × 4 latin-square design. Strained rumen liquor was collected and analyzed for pH, ammonia nitrogen, total-N, TCA precipitable-N, rumen fluid volume and rumen fluid flow rate. Total N, ammonia-N and TVFA were significantly affected due to urea supplementation. There was no significant change in various blood biochemical constituents. It can be concluded that 15g urea supplementation is sufficient to maintain the optimum intake of UMMB and fermentation pattern.

Key words: Nutrient utilization, Rumen fermentation, UMMB, Urea

Ammonia-N is utilized by the ruminal bacteria and the maximum microbial synthesis takes place when the rumen ammonia concentration is as high as 15-25 mg N/ml rumen liquor depending on nature of diet (Krebs and Leng 1984, Hidman *et al.* 1986). When ammonia concentration in the rumen liquor falls below about 20 mg N/100 ml, the rumen microorganisms are inefficient and are likely to respond to NPN supplements particularly through UMMB (Tiwari *et al.* 1989, Song and Kennely 1990).

Considering the above facts a study was planned to observe the effect of different levels of ammonia-N concentration in the rumen (created by different levels of urea supplementation) on nutrient intake and their utilization by crossbred cattle fed on wheat straw and UMMB.

MATERIALS AND METHODS

The commercially available UMMB was prepared by the hot process of mixing various ingredients thoroughly with molasses at 70°C which was then poured into specially designed moulds to form blocks of 23 × 14 × 5 cu. cm in size weighing about 3 kg each. The UMMB contained molasses, urea, cottonseed-meal, mineral mixture, common salt, calcite powder and bentonite in the proportions of 450, 150, 100, 150, 80, 40 and 30 g/kg, respectively.

The study was conducted on 16 adult crossbred cattle weighing approximately 300 kg and randomly allotted to 4 equal groups of 4 animals each. All the animals were kept in a similar dietary regime for 15 days before starting actual experimental feeding. Animals were offered wheat straw *ad lib.* and had free access to UMMB lick. Crushed maize grain was offered to meet their energy requirement (NRC 1989). In addition, the animals of group 1 to 4 were offered fertilizer grade urea @ 0, 15, 30 and 45 g/animal a day, respectively, in solution form, sprinkled and mixed with wheat straw to obtain 4 different levels of N in the ration. At the end of 60 days of experimental feeding a metabolism trial of 6 days duration was conducted by conventional method by harnessing all the animals in metabolism cages. Representative samples of feed offered and residues left were taken daily for dry matter estimation and proximate analysis. The intake of UMMB was estimated as the difference between the initial and final weight of the block on a dry-matter basis during the metabolism trial. The rumen fermentation study was carried out with 4 adult rumen fistulated animals of

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Table 1. Effect of dietary urea levels on intake of UMMB, dry matter, nitrogen balance and nutrient digestibility

Attributes	Groups			
	1	2	3	4
Body weight (kg)	309.7	307.2	319.1	308.6
(kg $W^{0.75}$)	73.82	73.37	75.49	73.62
DM intake through				
UMMB (g/day)	116.0	152.0	94.0	87.0
Maize (kg/day)	1.4	1.4	1.3	1.5
Wheat straw (kg/day)	3.0	3.0	3.2	2.7
Total intake (g/kg$W^{0.75}$/day)	61.4	62.2	59.8	57.5
Crude protein				
Intake (g/day)*	313.9[a]	376.7[b]	383.3[bc]	435.5[c]
(g/kg$W^{0.75}$/day)	4.25	5.13	5.08	5.91
Digested (g/day)*	136.9[a]	178.8[a]	178.1[a]	251.1[b]
DCP intake (g/kg$W^{0.75}$/day)*	1.9[a]	2.4[a]	2.1[a]	3.1[b]
TDN intake (g/kg$W^{0.75}$/day)	32.3	33.7	32.0	31.9
Nutrient digestibility (%)				
DM	51.7	52.6	51.2	51.3
OM	54.2	55.6	53.9	53.9
CP	42.9	47.4	46.1	57.5
EE	61.2	51.9	54.7	58.2
TCHO	56.5	57.8	56.9	56.7
NDF	41.7	41.8	43.2	39.1
ADF	44.2	38.9	41.9	38.1
Cellulose	58.9	56.9	59.1	55.1
Nitrogen intake (g/day)	50.2[a]	60.3[b]	61.3[bc]	69.6[c]
N-losses (g/day) through				
Faeces	28.3	31.7	32.8	29.5
Urine*	9.7[a]	11.3[a]	10.4[a]	15.2[b]
Total N losses (g/day)	38.0	42.9	43.2	44.7
N balance (g/day)	12.2	17.3	18.1	24.9
N balance as% of intake	23.7	28.3	28.8	35.5
Intake of rumen fistulated animals				
Body weight (kg)	271.0	280.6	293.0	286.6
UMMB (g/day)	163.3	151.0	125.0	117.7
Maize (kg/day)	1.4	1.4	1.4	1.4
Wheat straw (kg/day)	3.1	3.1	3.1	3.3
Dry matter (kg/day)	4.6	4.6	4.7	4.8
Organic matter (kg/day)	4.3	4.6	4.3	4.4
Nitrogen (g/day)*	54.7[a]	60.6[a]	65.9[b]	72.9[b]

Figures bearing different superscripts in a row differ significantly ($P<0.05$).

comparatively lower body weight following 4 × 4 latin-square design. Animals were fed individually the experimental diet. After 21 days of experimental feeding strained rumen liquor (SRL) samples were collected with the help of a metallic probe covered with nylon cloth, from each individual animal for 3 consecutive days at 0, 2, 4, 6 and 8 hr post feeding, after collection, pH was recorded immediately and samples were preserved in plastic vials in a deep freeze until analyzed. The rumen fluid volume and the flow rate were determined by the method of Smith and McAllan (1970) by infusing polythene glycol (PEG, Mol. Wt 6000) solution 25 g PEG in 100 ml of distilled water into rumen through fistula. After thorough hand mixing of the rumen contents the estimation of PEG was done by collecting SRL at hourly interval up to 4 hr post feeding. The SRL was analyzed for ammonia-N (Conway 1957), total volatile fatty acids (Barnett and Reid 1957), total-N and TCA precipitable-N. The data were subjected to a test of significance between the diets using the analysis of variance technique (Snedecor and Cochran 1967) and treatment means were compared using Duncan's multiple range test (Steel and Torrie 1980).

RESULTS AND DISCUSSION

The UMMB contained organic matter 63.5%, crude protein 49.3%, ether extract 0.2%, total carbohydrate 14.0%, neutral detergent fibre 8.2%, acid detergent fibre 4.9% and cellulose 2.1%. Apparently, the intake of block was in decreasing order with increasing level of urea supplementation alongwith the

basal roughage. The intake of UMMB decreased with higher level of dietary urea supplementation, probably the ammonia-N is one of the factors responsible to control the intake of block, as the rumen ammonia-N was significantly increased with increasing level of dietary urea. Similar reduction in UMMB intake was observed in buffalo calves when ammonia-treated straw 2.5% (w/w) was fed (Mangat Ram *et al.* 1990) and with incremental levels of urea supplementation (Hosamani *et al.* 1995). The total dry-matter intake and the digestibility coefficients of all the nutrients were alike statistically in all the 4 groups (Table 1). Similar trend was recorded by Hosamani *et al.* (1998).

The total nitrogen intake increased linearly with UMMB and urea supplementation (Table 1). CP intake in different groups had linear increase may be due to high solubility and degradability of urea in the rumen leading to high concentration of ammonia in the rumen (Slyter *et al.* 1979, Song and Kennely 1990). The liberated ammonia might have been utilized up to certain extent by micro-organisms and excess was absorbed through the rumen wall. Similar trend was also observed in buffaloes (Tiwari *et al.* 1990, Hosamani *et al.* 1995). As there is no significant change in the intake of various nutrients expect CP, ultimately resulting into insignificant difference in TDN intake. Data on nitrogen balance indicated that loss of N through urine was significantly ($P<0.01$) more in urea supplemented groups, indicating that ammonia-N could not be utilized completely due to less available energy or lack of synchronisation urea nitrogen with available energy. Similar N-excretion was reported in crossbred calves and buffaloes fed diets containing higher than optimal amount of protein (Dass and Arora 1989) and with various levels of urea with molasses (Ali and Mirza 1986).

The data on blood biochemical constituents and rumen fermentation pattern are presented in Table 2. The concentration of ammonia-N and urea in blood plasma were within the physiological range in spite of additional urea supplementation in different groups may be due to the physiological adjustment. In plasma total protein level there was no significant difference due to additional urea supplementation as dietary urea had no sparing effect on ruminal degradation. The plasma calcium values were slightly lower than normal physiological values (9.7 to 12.4 mg/dl) but the plasma phosphorus values were within the normal physiological limits (5.6 to 6.5 mg/dl).

The rumen fermentation pattern at different hours of post-feeding indicated that higher pH obtained at the sixth hour post feeding. The peak concentration of total-N, TCA-ppt-N and ammonia-N in urea supplemented groups were at 2 hr post feeding. The immediate increase in nitrogen fraction in the SRL is attributed due to rapid hydrolysis of urea alone and as well as through UMMB lick. The ammonia-N utilization depends on the activity of rumen microflora and rumen microbes are maximum in quantity when there is matching supply of energy to the available-N. The concentration of TVFA was significantly ($P<0.01$) lower in group 1 and comparable with group 2. Similarly, it was comparable in groups 2 and 4 indicating that supplementation of dietary urea is beneficial up to 30g in addition to UMMB lick. However, UMMB intake was maximum in group 2 (where urea supplementation was 15g) among the urea supplemented groups. The variability in VFA concentration was attributed to carbohydrate and protein in the diet of different groups (Pant and Roy 1971). There was a progressive increase in the concentration of TVFA after feeding and attained a peak level at 4 hr in groups 1, 2 and 3 but in 2 hr in group 4. Rumen fluid volume and flow rate of liquid digesta were not affected due to dietary urea supplementation. Similar observations have already been reported in cattle and buffalo (Mangat Ram and Gupta 1987). In the present experiment, the rumen fluid volume were between 21.74 to 24.26% of the body weight of animals, which is similar to the values reported earlier (Toppo *et al.* 1997).

Table 2. Rumen fermentation pattern and blood biochemical constituents in adult cattle fed on wheat straw supplemented with urea and UMMB lick

Attributes	Groups 1	2	3	4
Fermentation pattern				
Rumen fluid volume (litre)	62.2	68.1	63.7	67.7
Rumen fluid flow rate (litre/hr)	4.3	3.7	4.0	3.9
pH	6.87	6.82	6.92	6.92
Total N (mg/dl of SRL)**	50.9^{a}	52.7^{a}	54.0^{a}	62.6^{b}
Ammonia-N (mg/dl of SRL)**	8.7^{a}	15.7^{b}	17.9^{b}	23.5^{c}
NPN (mg/dl of SRL)	18.9	21.2	23.3	25.9
TCA-ppt-N (mg/dl of SRL)	32.0	31.5	30.0	36.7
TVFA (mM/dl of SRL)**	7.8^{a}	8.4^{ab}	9.5^{c}	9.3^{bc}
Blood biochemical constituents				
Hb%	7.82	10.37	10.50	7.40
Ammonia-N (mg/dl)	0.27	0.31	0.29	0.43
Urea (mg/dl)	16.84	25.68	25.36	18.84
Total protein (g/dl)	7.10	7.24	7.53	7.54
Calcium (mg/dl)	7.04	8.09	8.77	10.17
Phosphorus (mg/dl)	6.94	6.18	6.14	6.77

Figures bearing different superscripts differ significantly ($P<0.01$).

It is concluded that 15 g urea supplementation is sufficient to maintain the optimum intake of UMMB and fermentation pattern.

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Indian Journal of Animal Sciences **70** (4): 419-422, April 2000

Intake, digestibility and growth of roaster rabbits fed graded dietary levels of neem *(Azadirachta indica)* seed kernel-cake

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Received: 17 March 1999; Accepted: 6 October 1999

ABSTRACT

Male broiler rabbits (24) of 12-week-old were assigned equally to 4 isonitrogenous and isocaloric composite (25 roughage: 75 concentrate) diets containing 0 (D_1), 5 (D_2), 10 (D_3) and 20 (D_4)% neem seed kernal-cake (NSKC), fed *ad lib* up to 24 weeks of age. The intake and digestibility of most of the nutrients and retention of nitrogen and energy depressed with increasing dietary levels of NSKC. The nutritive value of diets having NSKC beyond 10% was lower than the diets containing deoiled groundnut-cake and 5% NSKC. However, the rabbits on all the diets grew without any significant difference with similar efficiency of nutrient utilization (unit intake/unit gain). Incorporation of NSKC at higher dietary levels for longer periods appears to affect the nutritional performance of roaster rabbits adversely.

Key words: Growth, Neem *(Azadirachta indica)* seed kernel-cake, Nutrient utilization, Rabbit

Rabbit husbandry is being recognized as crucial in meeting the critical meat shortage in developing countires (Owen 1981) owing to its high potential in producing quality meat. Like other livestock species, economic rabbit production is, however, constrained by chronic shortage of feeds and fodders. Under these circumstances, most of the animal production systems in the tropics are gradually relying more on unconventional feed resources. Neem *(Azadirachta indica)* seed kernel-cake (NSKC), a by-product of neem oil industry, is one such product showing great potential for livestock feeding (Nath *et al.* 1974). Full fat or processed NSKC can be fed to rabbits in limited amount without any adverse effect (Fuzinimi *et al.* 1990, Salawu *et al.* 1994, Bhosale 1994). However, vital information is lacking on feasibility of long-term feeding of raw NSKC. This study was therefore designed to investigate intake, nutrient utilization and growth response of roaster rabbits to the inclusion of varying levels of raw NSKC as a substitute for conventional protein supplement in their diets.

MATERIALS AND METHODS

Mixed group of Soviet Chinchilla (SC, 8) and White Giant (WG, 16) rabbits (24), were randomly allotted equally to 4 dietary treatments in a randomized block design. All the rabbits were reared under hygienic and uniform managerial conditions by housing them individually in clean metallic cages, fitted with feeders and waterers, located in well ventilated cement floored shed. Clean drinking water was provided *ad lib*. Rabbits were dewormed at fortnightly intervals.

Rabbits were fed iso-nitrogenous and isocaloric composite [25 parts ground maize *(Zea mays)* hay, 75 parts concentrate] diets (Table 1) in mash form containing graded levels (0, D_1; 5, D_2; 10, D_3; 20, D_4% in the diet) of NSKC by replacing 0, 25, 50 and 100% of deoiled groundnut-cake (DGNC) nitrogen of concentrate moiety.

Weighed quantities of feed were offered daily so as to allow *ad lib*. intake throughout the experimental period of 12 weeks (between 12 and 24 weeks of age). Leftovers of feed residues were weiged 24 hr post feeding to ascertain daily food consumption. A digestion cum metabolic trial consisting of 4 days total collection of faeces, urine, feed and residues preceded by 3 days preliminary period was conducted during the last week of the experiment.

The rabbits were weighed individually before feeding and watering at weekly intervals to find out the live weight changes and growth rate throughout the experimental period of 12 weeks (between 12 and 24 weeks of age). Efficiency of feed and nutrient utilization was calculated as unit intake per unit gain. Feed cost per kg gain was derived by multiplying feed intake per unit gain with cost (Rs) of kg feed.

The pooled and ground representative samples of feed offered, residues and dried wet faeces were subjected to the

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Table 1. Ingredient and chemical composition of diets

Ingredient/constituent	Diet*			
	D_1	D_2	D_3	D_4
Ingredients (% air dry feed)				
Maize hay (ground)	25	25	25	25
Yellow maize	35	35	35	35
Deoiled groundnut-cake	18	13.5	9	0
Neem seed kernel-cake	0	5	10	20
Meat-meal	8	8.5	8.5	9.5
Wheat bran	5.5	4	3	0
Molasses	5	5	5	5
Tallow	1.0	1.5	2.0	3.0
Mineral mixture	2.0	2.0	2.0	2.0
Sea salt	0.5	0.5	0.5	0.5
Chemical composition (% dry matter)				
Organic matter	87.0	86.3	86.2	87.3
Crude protein	16.3	16.8	16.5	16.4
Ether extract	4.5	5.5	6.5	8.6
Crude fibre	10.9	10.9	10.8	10.9
Nitrogen free extract	55.2	52.9	52.3	51.4
Ash	13.0	13.7	13.7	12.6
Gross energy (kcal/gDM)	4.3	4.1	4.1	4.3

*Added 30g mineral mixture containing 50 000 IU and 5 000 IU of vitamin A and D_3 per g, respectively, 50g tetracycline HCl (50 µg/g) and 50g Coccidiostat (sulpha-quinoxaline, 16.67%; amprolium HCl, 16.67%).

analysis of proximate principles (AOAC 1980). Gross energy content of feed, fodder, residues and faeces were estimated with the help of Gallenkemp ballistic bomb calorimeter as per the Gallenkemp mannual.

The data were subjected to analysis of variance as per Snedecor and Cochran (1967) by programming and processing in a Micro-32 Computer.

RESULTS AND DISCUSSION

Nutrient digestibility and retentions

Dietary incorporation of graded levels of NSKC significantly ($P<0.01$) influenced the digestibility of nutrients except ether-extract (Table 2). Like any other species, the nutrient digestibility in rabbits is influenced by dietary ingredient composition, forage quality, level of intake (Miller *et al.* 1954), energy, protein and fibre density, rate of passage and caecal retention (Aguilera *et al.* 1970). As the dietary level of NSKC increased, the intake of DM decreased significantly ($P<0.01$, Table 3) but resulted in depressed ($P<0.01$) DM digestibility probably because of low energy density of diets (Fekete *et al.* 1990). Crude protein (CP) digestibility depressed beyond 5% NSKC incorporation. Similarly, digestibility of CF, NFE and energy depressed significantly ($P<0.01$) due to graded levels of NSKC in the diet and resultant depression in the dietary density of energy (Table 3) as other factors were similar. However, observed

Table 2. Nutrient digestibility, nutritive value and retention by rabbits

Variable	Diets				SEM
	D_1	D_2	D_3	D_4	
Digestibility (%)					
DM	65.04^a	60.76^{ab}	57.52^b	50.26^c	2.43
CP	64.87^a	62.32^a	53.24^b	48.80^b	3.29
EE	80.34	78.29	73.93	82.05	3.11
CF	26.09^a	22.30^b	19.20^{bc}	17.95^c	1.81
NFE	70.61^a	66.42^{ab}	64.93^b	56.80^c	2.40
Energy	64.83^a	58.30^b	57.70^b	52.03^c	2.73
Nutritive value (% DM or Mcal/kg DM)					
Digestible crude protein (DCP)	10.68^a	10.18^a	8.76^b	8.16^b	0.55
Total digestible nutrients (TDN)	60.56^a	55.63^b	54.36^b	49.20^c	0.19
Digestible energy (DE)	2.75^a	2.36^b	2.36^b	2.25^b	0.11
Metabolizable energy	2.51^a	2.19^b	2.00^{bc}	1.83^c	0.12
DCP : DE ratio	25.73^a	23.26^b	27.27^a	27.65^a	1.08
DCP: TDN ratio	5.67^{bc}	5.48^c	6.28^b	7.29^a	0.33
Nutrient retention (as % of absorbed)					
Nitrogen	50.58^{ab}	65.15^a	56.80^{ab}	27.15^c	7.56
Energy	90.97^{ab}	92.50^a	82.57^{bc}	81.06^c	2.90

Means marked with unlike superscripts are significantly different ($P<0.01$).

Table 3. Growth rate and efficiency of nutrient utilization

	Diets				SEM
	D_1	D_2	D_3	D_4	
Initial body weight (g)	1240.00	1283.33	1276.66	1268.33	96.47
Final body weight (g)	1818.00	1770.00	1663.33	1605.00	97.58
Total weight gain (g)	578.00	483.33	386.66	336.66	115.01
Average daily gain (g)	6.88	5.75	4.60	4.01	1.36
Nutrient intake (g or kcal/kgW$^{0.75}$)					
DM	56.18^{ab}	64.65^a	48.88^b	44.28^b	6.83
CP	9.26^{ab}	10.65^a	8.07^b	7.30^b	1.13
DCP	6.00^b	6.67^a	4.27^b	3.75^b	0.81
TDN	34.10	36.28	26.61	26.25	4.31
DE	154.80^{ab}	154.00^{ab}	115.50^{bc}	102.16^c	19.47
Nutrient efficiency (intake, g or kcal/g gain)					
CP	2.01	2.67	2.79	3.83	1.21
TDN	7.58	8.89	9.15	11.57	2.09
Feed cost/kg gain (Rs)*	58.12	72.50	74.26	96.71	162.76

*Based on prevailing market price (Rs/kg) of various ingredients (maize hay, 0.84; yellow maize, 4.37; deoiled GNC, 5.30; NSKC, 1.50; meat-meal, 4.00; wheat bran, 3.34; molasses, 0.40; tallow, 8.00; mineral mixture, 4.99, sea salt, 1.20 and feed additive @ Rs 131.0 per 100 kg diet.)

Means marked with unlike superscripts are significantly different ($P<0.01$).

range of nutrient digestibility was close to values reported for rabbits and lambs fed with variously processed NSKC (Bhosale 1994, Gowda 1994, Musalia 1994). The rabbits digested EE similarly irrespective of diet. It appears that saponification of fat in the gut of rabbits leads to its lesser recovery in the faeces which enhances the apparent EE digestibility.

Though the nitrogen retention as percentage of absorbed nitrogen was comparable in rabbits fed DGNC and 10% NSKC containing diets, it was significantly higher in rabbits on 5% and lower on 20% NSKC diets. Similarly, energy retention from absorbed energy was significantly ($P<0.01$) lower in rabbits fed diet D_4 and comparable among rabbits on all other diets (Table 2). Nitrogen retention in rabbits was better on diets containing 23.42 Kcal DE/g DCP (Swarooparani 1993). The DCP: DE ratio in diet D_2 having 5% NSKC was 23.26 (Table 3) which was nearer to the suggested ratio. This might be the probable reason for better nitrogen and energy retentions by rabbits on this diet than on other NSKC containing diets as DCP: DE ratio in those diets were either wider or narrower than the suggested ratio.

Nutritive value and plane of nutrition

The DCP and TDN contents (% DM) of composite diets offered to rabbits decreased with increase in NSKC incorporation especially beyond 10% (Table 3). The DE and ME which ranged from 2.25-2.75 and 1.83-2.51 Mcal/kg DM respectively, were significantly ($P<0.01$) lower in all the NSKC containing diets as compared to diet D_1 containing DGNC.

The observed TDN and DCP of composite diets were lower than the recommended values (65% TDN, 10.7-13.5% DCP) of NRC (1977) and Santoma *et al.* (1989), respectively. The intake of all the nutrients differed significantly between diets (Table 3). In general, the intake was higher on control (D_1) and 5% NSKC containing diet (D_2) but lower on D_4 containing 20% NSKC. However, the intake of protein and energy in rabbits was lower than the stipulated requirements of NRC (1977) for growing rabbits of similar weight irrespective of dietary treatment.

Growth rate, nutrient efficiency and economics

The rabbits from all the diets grew without any significant difference (ADG: 4.01-6.88g) between 12 to 24 weeks of age (Table 3). Similarly dietary inclusion of NSKC did not significantly influence the efficiency of utilization of various nutrients (Table 3). Though nonsignificantly, the rabbits fed DGNC containing diet (D_1) were more efficient by 29.3, 36.3 and 90.4% in utilizing DM than those fed D_2, D_3 and D_4 diets, respectively. The feed conversion efficiency (12.3-23.5g DM/g gain) was far below the efficiency (5.4 to 8.4 g DM/g gain) reported by Swarooparani (1993). However, Khan (1994) observed lowered efficiency (8.6-10.0 g DM/g gain) in WG rabbits even on water-washed or alkali-treated NSKC containing diets. The energy concentration in terms of DE and DCP (%) content of all the experimental diets was far below the recommended requirements (Santoma *et al.* 1989) which resulted in poorer perforamance of rabbits irrespective of dietary vegetable protein supplement. The poor growth rate and feed efficiency in general might be because of the poor genetic make up of rabbits alongwith tropical environmental conditions of present study. Under temperate climates, Lukefar and Cheeke (1991) observed 10-20 g ADG in tropics as against 35-40 g due to the heat stress and subsequent lowered feed intake. Moreover, it could also be attributed to the effect of long-term feeding of NSKC.

The feed cost per kg gain was comparable among rabbits fed different diets. The lower cost of composite diets having NSKC was not reflected in terms of feed cost per unit gain because of compensatory higher intake of these low energy density diets especially in older rabbits due to diversion of partitioned nutrients for maintenance with advancement of age (Huang *et al.* 1989).

It is concluded that incorporation of raw NSKC beyond 5% in rabbit diets for longer periods adversely affects the nutritional performance.

ACKNOWLEDGEMENT

This study has been supported with funds provided by the Indian Council of Agricultural Research (AP Cess fund).

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Indian Journal of Animal Sciences **70** (4): 423-425, April 2000

Evaluation of lustre and physical attributes of wool in Marwari and Nali sheep breeds*

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Received: 8 April 1999; Accepted: 7 October 1999

Key words: Heritability, Lustre, Marwari, Nali, Selection, Sheep, Wool quality

ABSTRACT

Marwari and Nali are two important carpet wool producing breeds of sheep in India. The wool of Nali breed was more lustrous and finer than that of Marwari. The least-squares means for wool quality traits i.e. tenacity, extension, reflectance, fluorescence, total, ratio, diameter and medullation of wool fibre have been estimated to be 6.48±0.167 g/tex, 23.37±0.53%, 176.43±3.899 √T, 111.20±2.664 √T, 287.79±6.402 √T, 1.08±0.103, 32.91±0.528 μ and 51.42±2.052% in Marwari and 6.42±0.144 g/tex, 21.55±0.464%, 215.67±3.359 √T, 140.08±2.295 √T, 355.67±5.516 √T, 0.6±0.088, 28.33±0.455 μ and 50.80±1.768% in Nali.

The heritability estimates for tenacity, extension, reflectance, fluorescence, total (r+f) and medullation revealed that all the traits had moderate to high heritability, indicating that phenotypic selection for individual trait would be quite effective. The phenotypic correlations among the quality traits indicate that the selection index would be a better choice if simultaneous selection in quality traits is desired.

Marwari and Nali are important breeds of sheep in North western India. The wool duced by Marwari is approximately 7.2 million kg per annum and is very good for carpet manufacture. Nali, though less in number, produces higher quantity of wool per sheep.

The demand of wool is growing significantly due to the fast growth of the carpet industry in the country. An excellent carpet, having desired resiliency, durability and appeal, can only be manufactured with wool having fibre diameter— 30-35μ, medullation— 40-50% and a good lustre.

This study was aimed to evaluate and compare the lustre and other physical attributes of Marwari and Nali wool. The heritability, genetic and phenotypic correlations among wool quality traits were also estimated for further genetic studies and improvement of the 2 breeds.

MATERIALS AND METHODS

The data on wool samples of 132 lambs of both the breeds at the age of 6 months were collected from the Central Sheep and Wool Research Institute, Arid Region Campus, Bikaner.

The lustre was measured in terms of reflectance and fluorescence of the wool fibres. The tenacity was measured as g/tex to reflect the tensile strength of the wool fibre and the extension as percentage.

The analysis of wool samples was carried out at fibre physics section of the CSWRI, Avikanagar, where diameter and medullation percentage were estimated using a projection microscope. The bundle strength and extension were measured by using fibre bundles at gauge length 1/16" on stelometer and the reflectance and fluorescence using the fluorimeter (Parthasarathy *et al.* 1995).

Least-squares technique using LSMLMW computer programme (Harvey 1990) was utilized to estimate the effect of different factors.

RESULTS AND DISCUSSION

The least-squares means for wool quality traits i.e. tenacity, extension, reflectance, fluorescence, total, ratio, diameter and medullation of wool fibre were estimated in Marwari and Nali (Table 1). The effect of breed was significant in all the parameters except tenacity and medullation. The lustre parameters indicated that Nali wool was significantly ($P<0.01$) more lustrous than that of Marwari. Also, the Nali wool fibres were relatively more fine. However, the bundle strength and medullation in the wool of 2 breeds differed nonsignificantly

*Part of M.V.Sc. Thesis submitted to Rajasthan Agricultural University, Bikaner.

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Table 1. Least-square means with standard errors for wool traits

Effect	N	Tenacity (g/tex)	Extension (%)	Reflectance (√T) (R)	Fluorescence (√T)(F)	Total (√T) (R+F)	Ratio (F/R)	Diameter (μ)	Medullation (%)
Overall	132	6.45±0 110	22.45±0.356	196.05±2 573	125.64±1.758	321.73±4.220	0.863±0.068	30.628±0.348	51 111±1.354
Breed		NS	*	**	**	**	**	**	NS
G1	55	6.48±0.167	23.3690.539	176.43±3.899	111 20±2.664	287.79±6.402	1.086±0.103	32.919±0.528	51 418±2 052
G2	77	6.42±0.144	21.55±0.464	215.67±3.359	140.08±2.295	355.67±5.516	0.640±0.088	28.337±0.455	50.804±1 768
Sex		NS	NS	NS	NS	NS	NS	NS	*
Sex 1	59	6.45±0.161	22.43±0.521	191.56±3 769	122.36±2.575	314.58±6.188	0.966±0.099	30.100±0 510	48.270±1 983
Sex 2	73	6.45±0.150	22.48±0.485	200.53±3.504	128.92±2.394	328.88±5.755	0.760±0 092	31.155±0.475	53 953±1.844
Interaction		NS	NS	NS	NS	NS	NS	NS	NS
G1 × Sex 1	28	6.30±0 234	22 87±0.756	171.84±5.464	107.96±3.733	281.24±8.972	1.301±0.144	32.595±0.740	48 844±2.875
G1 ×Sex 2	27	6.66±0 238	23 85±0.770	181.04±5.564	114 43±3.802	294.34±9.136	0.870±0.147	33.242±0 754	53 992±2 928
G2× Sex 1	31	6.59±0.222	21.99±0.718	211.28±5.193	136.75±3.548	347.92±8.527	0.631±0.137	27.605±0 703	47 695±2 733
G2 × Sex 2	46	6.24±0.182	21.11±0.590	220.05±4.263	143.40±2.912	363.41±7.00	0.649±0.112	29.069±0 577	53 913±2.243

NS Nonsignificant, *P<0.05, **P<0.01; G1, Marwari; G2, Nali;sex 1, male; sex 2, female.

(P>0.05). The findings of Parathasarthy *et al.* (1997) for Nali and Singh *et al.* (1998) for Marwari breed are quite consistent with the present findings.

The effect of sex was nonsignificant in all traits except medullation. The present results are in agreement with the findings of Nehra (1991), Ganai (1992) and Pannu (1994) who reported nonsignificant effect of sex of lamb on the fleece quality traits in Marwari. The interaction between breed and sex was found nonsignificant in all the wool quality traits.

Heritability estimates

The heritability estimates (Table 2) for tenacity, extension, reflectance, fluorescence, total (r+f) and medullation revealed that all the traits had moderate to high heritability. The estimates for all the parameters obtained here are relatively higher than those reported in the literature. Mehta *et al.* (1998) reported lower heritabilities for reflectance, fluorescence and total in Magra sheep. Chopra and Chopra (1972) reported heritability of medullation percentage to be 0.17±0.15 in Nali breed. Nehra (1991), Ganai (1992) and Pannu (1994) estimated heritability of medullation percentage as 0.03±0.10, 0.02±0.12 and 0.10±0.212 in Marwari respectively. Present results indicate that phenotypic selection would be quite effective for improvement in the wool quality traits.

Genetic and phenotypic correlations

Genetic correlations among different traits are mainly attributed to pleiotropic gene action and linkage of genes governing different traits. These are useful for estimating correlated response to selection (Table 2). The phenotypic correlation among the traits is the combined effect of genetic and environmental correlations among them along with heritabilities of the concerned traits.

The tenacity is phenotypically negatively correlated with all parameters except extension but genetically it is positively correlated with lustre parameters and negatively with extension and medullation. The difference of sign in genetic and phenotypic correlations for lustre parameters and extension indicates that the environmental correlation is probably negative for lustre parameters and positive for extension. This indicates that the genetic and environmental sources of variation are probably affecting lustre and medullation through different physiological mechanisms (Falconer 1989).

The extension is phenotypically negatively correlated with all the parameters except ratio, whereas genetically it is positively correlated with the lustre parameters as has been observed and explained in tenacity.

The lustre parameters are phenotypically as well as

Table 2 Heritability, phenotypic and genetic correlation of wool quality traits

Trait	Tenacity	Extension	Reflectance	Fluorescence	Total	Ratio	Diameter	Medullation
Tenacity	0.308 ± 0.295	-0.225± 0.611	0.427 ± 0.668	0.424± 0.570	0.422± 0.619	N.E.	N E.	-0 287± 0 595
Extension	0 443	0.412 ± 0.311	0.770± 0.579	0.457 ± 0.493	0.581± 0 535	N.E.	N.E.	-0.304 ± 0.506
Reflectance	-0.094	-0.035	0.468 ± 0.319	1.014 ± 0.014	0.999± 0.012	N.E	N.E.	-0.799 ± 0.426
Fluorescence	-0.103	-0.068	0.933	0 758 ± 0.355	1.005± 0.012	N.E.	N.E.	-0.579 ± 0.352
Total	-0.113	-0.068	0.983	0.974	0.596± 0.336	N.E.	N E.	-0.675± 0.384
Ratio	-0.049	0.003	0.182	0.147	0.160	N.E.	N.E.	N E.
Diameter	-0.085	-0.033	-0.005	0.010	N.E.	0.063	N.E.	N.E.
Medullation	-0.245	-0.134	0.34	0.085	0.049	0.049	0.552	0.746± 0.354

Diagonal values present heritabilites, upper right triangle present genetic correlations, lower left triangle present phenotypic correlations. N.E. Not estimated.

genetically very highly correlated positively. Similar results were obtained by Mehta *et al.* (1998) and they recommended the use of total (r+f) as a single reliable measure of lustre. The present findings also recommend the use of total as a single reliable measure of lustre. The ratio (f/r) cannot be taken as a reliable measure of lustre and should be discouraged.

The diameter and medullation are a little correlated phenotypically with other parameters but are significantly correlated positively with each other. Similar results were reported by Nehra (1991) in Marwari and Mehta *et al.* (1998) in Magra sheep. The phenotypic correlations among the quality traits indicate that the selection index would be a better choice if simultaneous selection in quality traits is desired.

The wool of Nali breed was more lustrous and finer than that of Marwari. The heritability estimates indicated that phenotypic selection for individual traits would be quite effective. The phenotypic correlations among the quality traits indicated that selection index would be a better choice if simultaneous selection in quality traits is desired.

ACKNOWLEDGMENT

The senior author is thankful to Director, CSWRI, Avikanagar, for the permission to utilize the data for M.V.Sc.thesis.

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Indian Journal of Animal Sciences **70** (4): 426-427, April 2000

Reproductive performance of Muzaffarnagri sheep and its crossbred progeny

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Received: 21 September 1998; Accepted: 20 September 1999

Crossbreeding of Muzaffarnagri sheep with exotic sheep breeds, viz. Corriedale, Suffolk and Dorset, was taken up under the aegis of the All-India Co-ordinated Research Project *(AICRP) for augmenting mutton production. The reproductive* studies on these crossbreeds are conducted mainly on the F1 generation (Sinha *et al.* 1979a, 1979b and Dogra and Misra 1993). Three-breed crossing was used for introduction of 2 exotic inheritance representing different desirable characteristics. No study has been undertaken, so far, to find out the reproductive performance of Muzaffarnagri crossbred arising from 3-breed crossing. The aim of this work was to compare the reproductive performance of Muzaffarnagri and their 3-breed crosses.

Reproductive data of 110 Muzaffarnagri sheep and 125 ¼ Suffolk × ¼ Dorset × ½ Muzaffarnagri ewes (F2 generation) were used in the study. Animals were reared under semi-intensive management system at the Institute farm since birth. In addition to pasture grazing they were provided concentrate @ 250-450 g/animal/day and green fodder *ad lib.* in housing sheds. Daily heat detection was conducted throughout the year. Before the non-pregnant ewes were sent for grazing in the morning, they were kept in a closed paddock for 1 hr and 2 vasectomized rams were allowed to run with the ewe flock. Whenever an ewe was detected for oestrus, it was immediately removed from the flock and mated with the selected ram. Oestrus detection was resumed 2 months after lambing. Lambs were weaned at 3 months of age. Post-weaned lambs were reared separately up to 6 months of age and thereafter they joined the adult flock. Data pertaining to reproductive traits viz. age at first oestrus (AFE), body weight at first oestrus (BWFE), age at first lambing (AFL) and lambing interval (LI) were analysed according to least square analysis of variance technique (Haravey1966).

Muzaffarnagri and its 3-breed crosses expressed first oestrus at the age of 448 and 456 days respectively (Table 1). Koul (1978) and Sinha *et al.* (1979a) also found similar AFE in Muzaffarnagri sheep (429 to 438 days), but in F1 progenies AFE was found shorter (304 to 397 days). Season of birth had no significant effect on AFE. This finding was contrary to the finding of Sinha *et al.* (1979a). Ewe lambs born in 1983 had lowest AFE (373 days), whereas AFE of ewe born during 1984 to 1988 varied from 440 to 490 days, the differences being significant ($P < 0.05$). But Koul (1978) did not observe any effect of year on AFE in Muzaffarnagri sheep.

Table 1. Least squares means with standard errors of reproductive traits of ewes

Factors	AFE (days)	BWFE (kg)	AFL(days)	LI (days)
Overall (μ)	452±10	29±0.4	634±17	277±12
	(229)	(212)	(196)	(331)
Breed group				
Muzaffarnagri	448±12	31±0.5[a]	609±20[a]	256±14[a]
	(107)	(104)	(98)	(190)
Crossbred	456±13	28±0.5[b]	663±20[b]	297±14[b]
	(122)	(108)	(98)	(190)
Season				
Winter	486±18	31±0.7	668±30[a]	285±16
(Dec to Feb)	(38)	(35)	(28)	(103)
Dry-hot	411±13	29±0.5	622±20[b]	301±17
(April to June)	(83)	(78)	(74)	(65)
Dry-humid	412±31	27±1.3	576±54[c]	258±19
(July to Sep)	(13)	(11)	(8)	(43)
Comfortable	468±12	30±0.5	657±18[a]	261±15
(March, Oct,Nov)	(95)	(88)	(88)	(119)

Figures in the parenthesis indicate the number of observations. Means with different superscript under same classification differ significantly,AFE age at first oestrus, BWFE body weight at fust oestrus, AFL age at first lambing, LI lambing interval.

BWFE of Muzzaffarnagri sheep (31 kg) was significantly ($P < 0.01$) higher than that of crossbred sheep (28 kg). Season of birth did not influence the BWFE but year had significant ($P < 0.05$) effect on BWFE. Koul (1978) also reported similar effect of year on this trait in F1 crossbred sheep.

First lambing in Muzaffarnagri sheep occurred 54 days earlier ($P < 0.05$) than crossbred sheep (Table 1) and this was comparable to the finding of Koul (1978) and Sinha *et al.* (1979a). AFL was about 3 months less in ewes born during the hot-humid season than those born during winter and comfortable season ($P < 0.05$). Similar findings were reported earlier by Koul (1978) and Dogra and Misra (1993). Lambing was significantly earlier ($P < 0.05$) in ewes born during 1983

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(565 days) than those born during 1984 to 1988 (601 to 706 days). Environmental variation from year to year might be responsible for this observed effect. This finding was in agreement with the finding of Koul (1978) and Dogra and Misra (1993). Lambing interval observed in the present study was remarkably low (277 days) in comparison to that recorded in other Indian sheep and crossbreeds (ranged from 304 to 406 days; Sinha *et al.* 1979b, Amble and Malhotra 1968, Narayanaswamy *et al.* 1976, Reddy *et al.* 1984 and Singh and Gupta 1986). Practise of daily heat detection in ewe flock throughout the year followed in the present study (instead of usual practise of seasonal mating in sheep) might be responsible for reduction in the lambing interval. Season of lambing had no significant effect on next lambing interval. But Sinha *et al.* (1979b) in their study on Muzaffarnagri ewes reported significant effect of season on lambing interval.

From this study it can be concluded that the reproductive performance of 3-breed Muzaffarnagri crosses was inferior compared to native breed indicating the poor adaptation of crossbred ewes in the Indian environment. It is possible to obtain 9 months lambing interval (i.e. 4 lambings in 3 years) by adopting the practise of daily heat detection in the ewe flock.

ACKNOWLEDGEMENT

I thank the Director, Indian Veterinary Research Institute, Izatnagar for providing the necessary facilities for this work.

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Indian Journal of Animal Sciences **70** (4): 428-430, April 2000

Effect of feeding rumen protected and unprotected protein on nutrients intake and reproductive performance of lactating buffaloes

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Received: 18 August 1997; Accepted: 1 November 1999

Key words: Buffalo, Nutrients intake, Protected protein, Service period

Amino acids absorbed from the small intestine of ruminant animals are supplied by microbial protein synthesized in the rumen, undegraded or protected food protein which bypass the rumen, and mobilisation of body reserves (Annexstad *et al.* 1987). When protein requirement of milk production and maintenance exceeds the amount of protein synthesised by rumen microorganism a supply of dietary undergradable protein is essential to attain high milk production (Folman *et al.* 1981). Methionine and lysine are considered important amino acids in ruminant nutrition and were reported to be most limiting or co-limiting amino acids for milk and milk protein synthesis (Schwab *et al.* 1976). Feeding a combination of protein sources that have complementary amino acids profile may increase chances of increasing post ruminal supply of lysine and methionine (Blake and Stern 1988). Higher level of lysine and methionine were reported in soybean-meal and sesame-cake, respectively, compared to groundnut-cake and mustard-cake (Johri *et al.* 1998) and sesame-cake may be added up to 10% in concentrate ration for lactating animals (Lennerts 1990).

The present study was, therefore, designed to investigate the effect of feeding of combination of dietary protein involving soybean-meal and sesame-cake rich in lysine and methionine, respectively, and partial replacement of conventional mustard-cake (control) with a commercially available bypass protein feed on nutrients intake and reproductive performance of lactating buffaloes.

The experiment was conducted in the Department of Livestock Production and Management, College of Animal Sciences, Haryana Agricultural University, Hisar, India, for 150 days. Murrah buffaloes (18) in early lactation were randomly divided into 3 groups of 6 buffaloes each on the basis of nearness in their body weight, parity, lactation length and daily milk yield following a factorial completely randomized design. The buffaloes in control group were fed a concentrate-mixture containing mustard-cake as a major source of dietary protein (mustard-cake, 30%; barley, 40%; deoiled rice polish, 27%; mineral mixture, 2% and salt, 1%), while buffaloes in treatment group 1 fed a concentrate mixture containing soybean-meal and sesame-cake in the ratio of 2: 1 as a source of protein (soybean-meal, 16%; sesame-cake, 8%; barley, 46%; deoiled rice polish, 27%; mineral mixture, 2% and salt, 1%). The buffalo in treatment group 2 were fed a concentrate ration containing mustard-cake at 50% level to that of control group, the remaining protein requirement being met by supplementing the ration with commercially available bypass protein feed (mustard-cake, 15%; bypass protein feed, 24%; barley, 31% deoiled rice polish, 27%; mineral mixture, 2% and salt, 1%). The commercially available bypass protein feed comprised formaldehyde-treated mustard-cake, 20%; groundnut-cake, 10% and untreated cotton seedcake, 6%; bone-meal, 2.5%; broken wheat, 12%; barley sprout, 18%; wheat bran, 10%; deoiled rice bran, 12.5%; molasses, 8%; and urea spray, 1%. The concentrate rations in 3 groups were formulated to be isoproteinous having 16.6% crude protein. It was given individually 2 times a day at the time of milking as per production level, recommended by ICAR (1985) and the amount offered was revised fortnightly on the basis of animals actual production level. Apart from feeding a concentrate mixture, the animals in 3 treatment groups were fed green sorghum or green maize and wheat straw *ad lib.* to meet the total dry matter requirement. The total dry matter of individual animal of different treatment groups was assessed. Representative samples of green fodder, wheat straw and concentrate mixture were analysed for chemical composition (AOAC 1980). The TDN intake was calculated on the basis of published nutritive values of the various ingredients of concentrate mixture, dry and green fodder (Ranjhan and Pathak 1979). All the experimental buffaloes were housed in a loose house with covered and open area except during milking.

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Table 1. Mean values of dry matter, crude protein and total digestible nutrients intake of lactating Murrah buffaloes under different dietary treatments

Particulars	Control	Treatment 1	Treatment 2
DM intake (kg/day)	14.68±0.19	14.73±0.09	14.87±0.11
DMI (kg)/100kg BW	2.80±0.04	2.79±0.03	2.82±0.03
DMI (kg)/kg FCM	1.63a±0.04	1.64a±0.05	1.39b±0.02
CP intake (kg/day)	1.40a±0.02	1.41a±0.02	1.47b±0.02
TDN intake (kg/day)	8.78±0.12	8.81±0.08	8.95±0.08

Treatment means with different superscripts differ significantly ($P<0.01$) from each other.

Nutrients intake

No significant change in dry matter intake expressed either in absolute amounts (kg/day) or per 100 kg body weight was observed in lactating buffaloes following feeding of either single or combined dietary protein source or protected protein (Table 1). The results are in agreement with previous work where no difference in DM intake was observed when buffaloes were fed protected protein in the concentrate mix (Chopra *et al.* 1982, Tiwari and Yadava 1990). However, dry matter intake per kg FCM yield in T2 group was significantly ($P<0.01$) lower than control and T1 group. The buffaloes fed protected protein required only 1.39 kg of dry matter for producing 1 kg of FCM as compared to control and treatment 1 group animals that consumed 1.63 and 1.64 kg of dry matter, respectively, for producing the same quantity of FCM. This was probably due to significantly higher net milk production and higher fat content of the milk produced by buffaloes fed protected protein, since the DM intake was more or less similar in the different treatment groups. The present results are in agreement with reports of Crawford and Hoover (1984) where higher efficiency of milk production was observed by feeding formaldehyde-treated soybean-meal to dairy cows, but differs from the work of Rogers *et al.* (1989) who could not find any improvement in the efficiency of milk production when diets containing soybean-meal were supplemented with rumen protected methionine and lysine.

Significantly ($P<0.01$) higher CP intake was observed in T2 group offered protected protein in concentrate mixture. This could be due to slightly higher intake of concentrate mixture in T2 group because of their relatively higher milk production compared to the control, since the schedule of concentrate mixture feeding to animal in different treatment groups revised fortnightly in accordance with he level of individual animal's milk production. When the CP intake was calculated in terms of per kg fat corrected milk produced it was less (136g vs 153g) in the protected protein fed group. This suggests that the feeding of protected protein to lactating buffaloes brings about improved nitrogen utilization. Our present findings of CP intake vis-a-vis protected protein feeding to lactating buffalo are at variance with earlier reports in dairy cows (Crawford and Hoover 1984) and buffalo calves (Hosmani and Srivastava 1984) fed protected protein or dairy buffaloes (Kar 1993) fed a combination of yeast culture and bypass protein. The mean TDN intake was 8.78±0.12, 8.81±0.08 and 8.95±0.08 kg for control T1 and T2, respectively, and the differences were nonsignificant.

Table 2. Body weight changes (kg) of lactating Murrah buffaloes under different dietary treatments at monthly intervals

Periods	Control	Treatment 1	Treatment 2
Initial	503.00±21.48	509.17±14.24	500.00±20.85
I	519.67±22.07	523.00±15.15	518.50±19.18
II	524.17±25.67	524.17±15.40	516.17±17.00
III	530.83±28.10	539.33 ±11.51	535.33±16.17
IV	527.67±27.35	532.83±10.83	532.83±15.88
V	534.00±26.89	530.33±10.98	542.83±16.88

Period I-V monthly intervals.

Body weight changes

There was no significant difference in body weight changes in 3 treatment groups across successive periods (Table 2). In fact, the animals in all the treatment groups showed net increase in body weight over the experimental period indicating that the animals in 3 groups were not in negative energy balance. However, the extent of body weight gain was maximum (43 kg) in the T2 group. In the earlier reports, no change in body weight in lactating dairy cows was found following feeding of different source of protein with varying degradability (Annexstad *et al.* 1987) or protected soybean-meal (Crawford and Hoover 1984). Similarly, almost equal body weigh were recorded through out the experiment period when lactating buffaloes were given a diet with less degradable protein (Sengar *et al.* 1990) or live yeast culture plus bypass protein (Kar 1993). In contrast, Papas *et al.* (1984) found that cows receiving rumen protected methionine for 3 weeks gained more weight than control cows, and Kaim *et al.* (1987) observed that cows given diet containing soybean-meal treated with formaldehyde lost only 6 kg body weight as against a loss of about 22 kg in cows given untreated soybean-meal. Hosmani and Srivastava (1984) found that gain in body weight was more in buffalo calves offered formaldehyde treated soybean than untreated soybean in concentrate mix.

Postpartum reproductive performance

There was no significant difference in postpartum oestrus interval and service period under different feeding regimes (Table 3). However, protected protein group exhibited

Table 3. Postpartum oestrus and service period in Murrah buffaloes under different dietary treatments

Treatment	Postpartum oestrus (day)	Service period (day)
Control	86.0±15.0	105.0±17.7
Treatment 1	89.5±17.8	127.3±25.2
Treatment 2	78.5±9.5	96.7±12.4

postpartum oestrus 8-11 days earlier and required marginally shorter time to conceive than the control group given mustard-cake and T1 group given soybean-meal and sesame-cake as main dietary protein sources. Earlier, Folman *et al.* (1981) also observed nonsignificant lower service period in cows fed protected soybean-meal and Panciroli (1986) found that supplementation of protected methionine improved fertility in cows. On the other hand Vincent *et al.* (1985) did not find any effect on the behavioural oestrus of cows fed concentrate containing rapeseed-meal or soybean-meal to a basal ration of straw. Similarly, Rogers *et al.* (1989) reported that feeding rumen protected methionine and lysine did not affect herd reproductive performance. The longer postpartum oestrus and service period in buffalo fed unprotected protein source could because of excess of NH_3, which overtaxes the liver and impairs fertility (Kaufmann 1979).

Replacement of the conventional mustard-cake by a combination of soybean-meal and sesame-cake, which are rich in lysine and methionine, respectively, did not produce any tangible favourable effect. However, partially replacing conventional dietary unprotected protein by bypass protein resulted in improved nitrogen utilization and marginal shorter service period.

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Indian Journal of Animal Sciences 70 (4): 431-433, April 2000

Utilization of tumba (*Citrullus colocynthis*) seed-cake of desert for goat production

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Received: 17 December 1998; Accepted: 22 October 1999

Key words Arid zone goat, Supplementary feeding, Tumba seed-cake

Goat flocks are mostly kept by marginal farmers who are unable to afford supplementary feeding with costly concentrate mixture. Simultaneously, the grazing/browsing areas are progressively shrinking with the encroachment by ever growing human population necessitating to evolve cheaper feed mixtures for higher and sustained animal production (Mathur 1996). In this experiment efforts are made to reduce the cost of supplementary feeding of goats by the introduction of a non-conventional tumba (*Citrullus colocynthis*) seed-cake (TSC) a low cost (Rs 2/kg) feed supplement. The objective of the study is to enhance goat production with low cost feeding inputs, affordable by marginal and landless farmers.

Marwari male kids (10) of 9 to 11 months old were randomly divided into 2 groups of 5 each forming control and treatment group with average initial body weight of 19.45 and 19.52 kg respectively. These animals were maintained at central research farm, of this Institute, from August to December under experiment after rainy season when much of green is available in the field of arid region. Before experiment all animals were dewormed with broad spectrum anthelmintic, albendazole as per standard dose. The dry matter intake and water consumption of experimental animals was determined during pre-experimental stall feeding trial for 7 days, conducted to determine the dry matter intake and water consumption of experimental animals. Thereafter, animals were offered a mixture of chaffed *Cenchrus ciliaris* grass and *Zizyphus nummularia* dried leaves (50: 50) *ad lib.* Leftovers were weighed the next day in the morning after 24 hr. Water was made available in marked buckets *ad lib.* and 24 hr consumption was noted in the morning daily. The animals of both the groups, viz. control and treatment, were allowed 4-5 hr of grazing on Dhaman grass (*Cenchrus* Spp., CP 6.6%) dominated protected silvipasture. Animals were housed in a side open enclosure. The browsing top feed material comprised *bordi* (*Zizyphus numnularia;* CP 12.0%), *khejri (Prosopis cineraria;* CP 14.0%), *ardu* (*Atlanthus excelsa*; CP 18.5%), Israili *babool (Acacia tortalis*; CP 13.9%), and *kumut* (*Acacia senegal*; CP 9.9%) as the main top feed sources in the grazing area, the proximate composition and nutritive value of silvipasture area is given in Table 1 as reported by Acharya and Patnayak (1977), and Harsh and Bohra (1985), which were lopped by grazier as per need. Immediately after returning from grazing, animals were watered *ad lib* The control group animals were not supplemented, however, in the afternoon each animal of treatment group was given 300g concentrate mixture comprising pelleted cattle feed and TSC in the ratio of 2: 1. The quantity of concentrate was increased to 375 g after 60 days. In addition 1% mineral mixture and 1% of common salt was also added in the concentrate mixture of treatment group. Body weight changes were recorded at weekly intervals. Health examination of individual animals was done daily. Blood was collected by jugular veinepuncture at monthly intervals in the morning before sending them for grazing. The data was analysed as per Snedecor and Cochran

Table 1. Percentage proximate composition and nutritive value of some tree leaves and grass of the grazing area

Botanical name	Common name	Crude protein	Crude fibre	Nitrogen free extract	Ether extract	Total ash	Calcium	Phosphorus	DMI (intake/100 kg b wt)	DCP	TDN
Zizyphus nummularia	Bordi	14.20	15.70	57.1	4.30	8.60	2.4	0.14	2 30	5.7	58.0
Prosopis cineraria	Khejri	15 29	17.52	54.09	4.52	5 11	1.61	0 30	—	—	—
Atlanthus excelsa	Ardu	16.25	21 85	47.48	3.00	11.43	1.43	0.17	4.26	13.00	64.00
Acacia tortalis	Israili Babul	12.3	22 40	57.90	1.80	5.6	—	—	—	5 68	62.00
Acacia senegal	Kumut	16 6	—	—	—	—	—	—	2 20	—	64.3
Cenchrus Spp	Dhaman grass	5.50	33.20	53 81	1 11	8.79	1 00	0.15	3.0	2.2	40.00

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Table 2. Chemical composition and cost of feed ingredients

	DM	*CP*	*EE*	*CF*	*NFE*	*Ash*	*Cost/kg* Rs	*Offered per* animal/day	*Feeding cost/* animal/day
Tumba (*Citrullus colocynthis*) seed-cake	90.7	16.9	3.95	42.0	25.95	11.2	2.00	100 g	0.20
Pelleted cattle feed	88.5	18.9	3.1	15.9	51.7	10.4	4.00	200 g	0.80

(1967).

The mean values for chemical composition of supplementation feed ingredients are given in Table 2, and blood metabolites and haematocrit in Table 3. The treatment group consumed readily the mixture of TSC + pelleted cattle feed completely showing good acceptability of concentrate mixture by the goats. This may be first hand report on this aspect on goats. Mathur *et al.* (1989), however, fed tumba-cake to growing heifers.

The daily DM intake and water consumption during pre experiment trial was 3.07 kg and 17.08 litres/100 kg body weight of male Marwari kids respectively. The kids of both the groups gained higher body weights than that reported by Patel *et al.* (1992), whereas, Parthasarthy *et al.* (1983) and Singh (1997) recorded higher live weights in male kids of Marwari goats by providing creep ration up to 3 months of age. Trend in body weight changes in control and treatment group are shown in the Fig. 1. From first week of experiment the increase in body weight in treatment group was on higher side as compared to control group, showing positive effect of cheaper supplementation on the growth rate of growing male Marwari kids. The ADG of kids at 6, 12 and 18 weeks of experiment was 48, 44 and 51 g in control and 76, 65 and 76 g in treatment group respectively.

The treatment group constantly maintained higher body weight gain than control group up to tenth week, however, during eleventh week the body weights of both the groups were more or less stationary. Subsequently, from 12th to 16th week (November) in the control group the ADG in body weight was very low i.e., 39 g, whereas in the treatment group, there was maximum feed conversion efficiency during the period. It is pertinent to mention here that up to eleventh week of the experiment (i.e. October) much of the browse/grazing material was available in the field in the form of monsoon

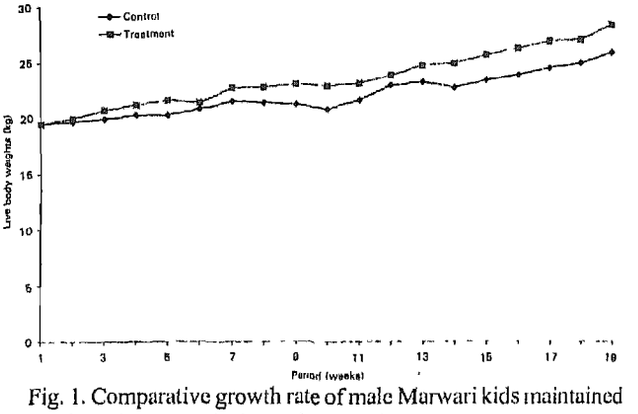

Fig. 1. Comparative growth rate of male Marwari kids maintained on grazing v/s economical supplementation.

vegetation. The monsoon vegetation thereafter, withered off, from the grazing area, therefore, control group animals maintained exclusively on grazing attained lower ADG. The ADG for complete 126 days experimental period was significantly ($P < 0.01$) higher in treatment group (71 g) supplemented with concentrate mixture of tumba (*Citrullus colocynthis*) seed-cake and pelleted cattle feed as compared to control group (51 g). The increase in body weight over initial in control and treatment groups after 18 weeks period was 33 and 46% respectively. Thus by utilizing tumba seed-cake in concentrate a saving of Rs 126/- over conventional concentrate can be achieved without affecting the health and increasing body weight. The market cost of live animal also increased from Rs 224 to Rs 312.

The pre and post experimental mean value ± SE of the blood parameters analysed for control and treatment group, were given in the Table 2. The haemoglobin and PCV values observed in the study were lower than the normal values reported for goats by Ghosal and Kataria (1995), however,

Table 3. Mean ± SE values of different blood constituents of male Marwari kids

	Whole blood		Plasma			
	Haemoglobin (g/dl)	PCV (%)	Glucose (mg/dl)	Urea (mg/dl)	Total protein (g/dl)	Albumin (g/dl)
Control						
Pre experiment	8.16 ± 0.06	24.4 ± 0.86	31.18 ± 1.17	47.18 ± 1.62	5.31 ± 0.08	2.98 + 0.05
Post experiment	6.16 ± 0.14	22.2 ± 1.24	53.28 ± 4.86	36.26 ± 1.01	6.10 ± 0.32	3.00 ± 0.11
Treatment						
Pre experiment	8.20 ± 0.12	24.25 ± 1.09	30.3 ± 0.86	48.19 ± 1.72	5.21 ± 0.22	2.85 ± 0.10
Post experiment	6.35 ± 0.12	23.6 ± 0.45	53.87 ± 2.80	37.70 ± 0.71	6.08 ± 0.23	3.38 ± 0.21

the haematological and biochemical parameters are in agreement with the values reported by Kaushish *et al.* (1999) for Marwari kids. There were no significant differences in blood parameters between control and treatment groups.

Study showed that higher live weight gain can be achieved without effecting physiological status and health of Marwari male kids by utilizing low cost-TSC, a locally available feed resource.

ACKNOWLEDGEMENTS

We thank the Director, CAZRI, Jodhpur, for providing the facilities, and to Shri Ramesh Chandra Bohra, Technical Officer for assisting in the haemotogical studies.

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Indian Journal of Animal Sciences **70** (4): 434-435, April 2000

Body composition and mineral status of some common fish varieties from village ponds

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Received: 11 November 1998; Accepted: 7 October 1999

Key words: Body composition, Nutrient status, Fish varieties

Catch fish forms a part of the food to the people in rural and tribal areas and serve as a potential source of nutrients. However the body nutrient composition of fish vary to a great extent on the level and status of nutrients in their feed. The live samples of different varieties of fish, viz. *Clarius magur, Channa punctatus, Amphinous cuchia, Mystus* spp. *Hypopthalmichthyes molitrix, Anabas testiduens, Barbus* spp. *Wallogo attu, Bagarius* spp. *Oxgaster bacaila, Catla catla, Labeo rohita, Cirrhinus mrigala* and *Mystus* spp. were collected from 10 ponds of Durg district of Chhattisgarh plains. The samples were steeped in hot distilled water at a temperature of 60°C for 5 min to remove all the extraneous material. They were dried in an oven at 60°C for 72 hr. Four dried samples of each varieties were milled into homogenous fine powder and stored in air tight containers until analysed.

Table 1. Body composition of different varieties of fish (% DM basis)

Varieties	Moisture	Crude protein	Crude fat	Total carbo-hydrates	Total energy	Acid in-soluble ash
C.magur	79.51	68.56	13.8	3.60	14.0	1.31
C punctatus	81.60	64.70	16.9	0.30	18.6	1.67
A. cuchia	76.40	68.90	18.3	2.20	10.6	0.90
Mystus spp.	80.70	64.10	27.0	0.18	12.9	0.51
H.molitrix	78.40	53.50	22.8	9.70	13.5	0.33
A.testiduens	83.10	70.50	15.5	1.50	22.9	0.97
Barbus spp.	77.40	68.60	31.2	4.90	18.6	1.35
W.attu	78.60	58.50	23.6	8.20	11.7	0.88
Bagarius spp.	77.10	48.30	33.1	4.10	17.0	0.35
O.bacaila	79.70	52.90	26.9	3.20	16.9	1.02
C.catla	76.30	65.60	10.7	7.70	15.9	--
L.rohita	78.30	59.60	21.2	5.50	13.6	--
C.mrigala	79.80	63.60	14.5	8.90	12.9	--
Mystus spp.	76.80	56.30	31.2	4.90	14.4	1.48
Mean	78.84	61.69	21.91	4.63	15.25	--
SE(±)	0.54	1.85	1.93	0.83	0.88	--
CV(%)	11.24	11.24	34.43	67.06	21.5	--

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The proximate principles, calcium and phosphorus were determined (AOAC 1980). Determination of copper, cobalt, maganese and zinc was carried out after the wet digestion with triacid mixture using atomic absorption spectrophotometer. The data were analysed as per Snedecor and Cochran (1968).

The crude protein (CP) content was higher in *A. testiduens* (70.5%) and lowest in *Bagarius* spp. (48.3%) (Table 1). The CP was comparable (Mba, 1980) with the values obtained for snail (63.3%), cray fish (69.4%) and smoked fish (70.4%). Consumption of fish reared under traditional feeding system may provide a satisfactory supply of animal protein for growing children, pregnant and breast fed women. The average nutrient requirement (ICMR 1989) of human being were reported to 70g, 50g, 440g and 2 500 kcal for the protein, fat, carbohydrate and energy, respectively, which can meet out the daily need if fish is consumed adequately alongwith the normal diet. The fats and total carbohydrates were higher in *Mystus spp.* and *Barbus* spp. (31.2%; 4.9%) and lowest in *C. catla* with a little variation in respect of Ca and P content amongst fishes.

The carbohydrates values were generally low and comparable with the values reported for blue crab (Akpan 1997). The ash content was higher in all varieties of fish (10.55 to 22.87%) indicating higher mineral content. The diet should

Table 2. Mineral composition of different varieties of fish (mg/100g DM)

Varieties	Ca*	P*	Cu	Co	Mn	Zn
C.magur	79.51	68.56	13.8	3.60	14.0	1.31
C punctatus	9.5	3.20	0.54	1.41	7.58	8.89
A cuchia	5.1	2.20	1.20	2.05	5.35	8.35
Mystus spp.	10.1	2.90	0.37	1.48	1.20	10.40
H.molitrix	6.1	2.90	0.70	1.61	2.16	8.30
A.testiduens	12.1	2.80	0.35	1.08	2.07	14.90
Barbus spp.	9.1	3.40	1.00	1.09	4.38	14.53
W.attu	7.5	2.90	0.47	1.56	1.30	10.36
Bagarius spp.	8.8	3.20	0.42	1.15	6.59	9.11
O.bacaila	8.3	3.50	1.58	1.94	1.99	9.63
C.mrigala	-		0.41	1.33	2.41	13.69
Mystus spp.	6.75	2.85	-	-	-	-

therefore contain high level ash, particularly calcium along with trace elements. Lall *et al.* (1985) reported that dietary imbalance of minerals in certain diets predisposed the Atlantic salmon to bacterial kidney diseases (BKD) under specific environmental conditions. Supplementation of the diets with certain trace elements (Cu, Co, Mn, Zn) showed lower incidence of BKD (Halver 1989). The macro- and micro-mineral composition (Ca, P, Cu, Co, Mn and Zn) of different species of fish is shown in Table 2. The respective elements varies from 5.1 to 12.1 g/100 g DM, 2.2 to 3.5 g/100 g DM, 0.35 to 1.58, 1.09 to 2.05, 1.2 to 7.58 and 8.35 to 25.69 mg/100 g DM in different varieties of fish. The values reported appeared to be normal for maintaining the growth performance of fish (Halver 1989). From the present study it is inferred that the fish reared in village ponds under different managerial conditions, if consumed in adequate quantities may help in ameliorating the nutritional deficiencies disorders prevelant amongst the people of the region.

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Indian Journal of Animal Sciences **70** (4): 436, April 2000

Book-Review

BREEDING FOR DISEASE-RESISTANCE IN FARM ANIMALS. (2nd edn). 1999. (Eds), Axford R F E, Bishop S C, Nicholas F W, and Owen J B. CABI Publishing, CAB International, Wallingford Oxon. OX 10 8 DE, UK. 418 pp. Illustrations 31. £ 75.000 (U.S. $ 1140.00). H.B. ISBN-085-199-325.

This book has full coverage of important areas of resistance development, and is categorized in 5 parts consisting of 19 chapters. First part is **'Principles and Methods'** which consists of 5 chapters on DNA Markers, Genetics Maps and Identification of QTL: General Principles; Modelling Farm Animal Diseases; The Immune System; The Major Histocompatibility Complex and its Role in Disease-Resistance and Immune Responsiveness; and Rodent Models of Genetic Resistance to Parasitic Infections. Second part is **'Parasites and Vector'** in which Genetics of Helminth-Resistance; Host-Resistance to Ticks and Tick-borne Diseases-its Role in Integrated Control; Genetic Aspects of Resistance to Ovine Cutaneous Myiasis; and Exploitation of Resistance to Trypanosomes and Trypanosomosis. Third Part is **'Bacteria'** which carries 3 chapters as Genetic Aspects of Resistance to ovine Foot-rot, Mastitis in Dairy Cattle and *Escherichiacoli* and *Salmonella* Diarrhoea in figs. Fourth Part is **'Viruses and Subviruses'** in which 3 chapters are covered. These are Viral Diseases; Diseases caused by Maedi-Visna and Other Ovine Lentiviruses; and transmissible spongiform Encephalopathies. Fifth part **'Production Diseases'** has 4 chapters, viz. Genetics of Susceptibility in Cattle in Sheep; Breeding for Disease-Resistance to Production Diseases in Poultry; Genetics Aspects of Health and Disease-Resistance in Pigs; and Lameness.

This first edition, brought out in 1991, was only based on conferences, while present, second edition provides a more comprehensive literature and to the point review of the subject. As the subject of breeding for disease-resistance rather than controlling diseases using expensive chemical continues to be topical, this book will attract wide interest to students and researches in animal breeding and genetics and in veterinary medicine.

Salient features of the books also include attractive photographs, short and crisp sentences with easy language which can be understood by all especially students.

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