ICAR PUBLICATIONS

D. K. M. A.

636 (05) CODEN : IJASA4 **68** (4) : 289-416 (1998) II ISSN 0367-8318

The Indian Journal of Animal Sciences

INDIAN COUNCIL OF AGRICULTURAL RESEARCH
NEW DELHI

EDITORIAL BOARD

	Inland	*Foreign*
Single copy	Rs 75	UK £ 5, US $ 7
Annual subscription	Rs 900	UK £ 60, US $ 84 (for Surface mail)
		UK £ 70, US $ 100 (for air mail)

Available from

The Business Manager
DIRECTORATE OF INFORMATION AND PUBLICATIONS ON AGRICULTURE
INDIAN COUNCIL OF AGRICULTURAL RESEARCH
KRISHI ANUSANDHAN BHAVAN, PUSA, NEW DELHI 110 012

THE INDIAN JOURNAL OF
ANIMAL SCIENCES

Vol. 68, No. 4 April 1998

CONTENTS

ANIMAL HEALTH

Short Communications

Indian Journal of Animal Sciences 68 (4) : 289-296, April 1998

Comparative immunogenicity of urea and EDTA extracted cell surface components from *Salmonella typhimurium* in mice

G C CHATURVEDI[1], MINAKSHI[2], Y K KAURA[3] and A K PRUTHI[4]

CCS Haryana Agricultural University, Haryana, Hisar 125004

Received : 6 January 1997; Accepted 15 September 1997

ABSTRACT

Swiss albino mice (group 1a to 4g) immunized with urea extracted cell surface components (UECSCs), recycled UECSCs, peak I (P I), recycled UECSCs pooled peaks (PI+PII+PIII) and EDTA extracted CSCs of *S. typhimurium* 163, respectively, showed humoral (HIR) and cellular immune responses (CIR). The HIR was exhibited by increase in immunoglobulin (Ig) level at weekly intervals postimmunization (PI) and postchallenge (PC), and immediate type footpad thickness (ITFT) at 6 hr postinoculation of eliciting antigen (PEA) in the left hind footpad. However, the mice in group 4f immunized with EDTA extracted CSC showed comparatively higher level of Ig at 4 weeks PI and 3 weeks PC. In immunized mice treated with carrageenan (group 1b, 2d, 4g), the Ig level decreased abruptly at 1, 2 and 3 weeks PC. The CIR was exhibited by delayed type footpad thickness (DTFT) at 72 hr PEA, nitroblue tetrazolium (NBT) reduction test at weekly intervals PI and PC. Postchallenge heavy mortality was observed in mice treated with carrageenan (macrophage depleted).

The difference between the per cent protection of immunized groups (1a, 2c, 3e) as well as immunized and carrageenan treated groups (1b, 2d) and mock vaccinated group (5h) was statistically significant. The corresponding values in groups i.e. 4f, 4g and 5h, were highly significant. The liver and spleen of the sacrificed mice surviving challenge in the immunized groups showed more immunological, less immunopathological and pathological alterations on microscopic examination, while mice in group 4f showed only immunological alterations. The EDTA extracted CSCs were more immunogenic and protective compared to UECSCs in mice.

Key words: Carrageenan treated (macrophage depleted) mice, EDTA extracted CSCs, Humoral and cellular immune responses, Immunogenicity, Mice, Nitroblue tetrazolium reduction test, Per cent protection, *Salmonella typhimurium*, Urea extracted cell surface components (UCSCs),

The merits and demerits of live/killed vaccines as well as superiority of subcomponent vaccine have already been described by Chaturvedi *et al.* (1994). The predominant role of cell-mediated immunity (CMI) in comparison to humoral immunity (HI) has been opined in protection of mice against *Salmonella typhimurium* and S. *enteritidis* (Collins and Mackaness 1968, Collins 1969) of chickens and guineapigs against *S. gallinarum* and *S. dublin* (Cameron 1976). Chaturvedi and Sharma (1981a, b) reported the major role of CMI in protection of calves against virulent challenge with *S. dublin*. The central role of macrophages in resistance to intracellular infection has been well emphasized by Mackaness (1962) and Collins (1974) in mouse typhoid. However, no information is available regarding the role of macrophages in mice immunized with *Salmonella* vaccine as well as treated with carrageenan in protection against virulent challenge with *S. typhimurium*.

The significance of protein as protective immunogen has been reported in mice by Bhatnagar *et al.* (1982) and Udhayakumar and Muthukkaruppan (1989). The protein-lipopolysaccharide (PLPS) complex isolated from *S. typhimurium* and *S. dublin* was able to induce protective immunity in mice (Plant *et al.* 1982) and rabbits (Chaturvedi *et al.* 1994) respectively. Jorbeck *et al.* (1981) reported that mice significantly cleared challenge dose of *S. typhimurium* immunized passively with rabbit anti-O-antibodies. Further, Killian and Morrison (1986) reported that LPS-lipid A-associated protein complex protected mice against lethal infection with *Salmonella*, whereas the LPS alone did not. Therefore in the light of aforesaid facts, the present study was undertaken to study the immune response of mice immunized with cell surface components (CSCs) of *S. typhimurium* extracted by urea and EDTA treatments and role of macrophage in protective immunity.

Present address: [1] Senior Scientist, [2]Assistant Scientist, Department of Veterinary Microbiology; [3] Associate Professor, Department of Veterinary Pathology.

[3] Professor and National Fellow (Retd.) H.No. 10-C, Friends Colony, Hisar, Haryana.

MATERIALS AND METHODS

Mice

Swiss albino mice were procured from Disease Free Small Animal House (DFSAH), CCS Haryana Agricultural University, Hisar.

Bacterial strain

The culture *S. typhimurium* 163 (phage type 166) was isolated from diarrhoeic dead cow-calf of organized dairy farm (Kaura 1988). The culture was passaged twice in mice for increasing its virulence.

Isolation of cell surface components (CSCs)

The cell surface components (CSCs) of *S. typhimurium* were isolated by urea method (Bouzoubaa *et al.* 1987). The urea extracted cell surface components (UECSCs) were fractionated on ultrogel AcA-34 column (1.6 × 100 cm). The peaks (PI, PII, PIII) (Fig. 1) obtained were concentrated on Amicon dialyzer PM 30 and dialyzed against Tris - HCl buffer 0.05 M, pH 7.5 containing 1 mM EDTA and 0.02% sodium azide. Each of the 3 peaks was recycled on ultrogel AcA-34 column (1 × 40 cm). The CSCs of *S. typhimurium* were also extracted by the cold EDTA (ethylene diamine tetra-acetic acid) extraction method of Kabir (1987).

Immunochemical characterization of various preparations of S. typhimurium 163

Biochemical analysis: The protein and carbohydrate in the UECSCs, its recycled peaks and cold EDTA extracted CSCs were determined as per Lowry *et al.* (1951) and Dubois *et al.* (1956) respectively.

Immunological reaction : The UECSCs, its recycled peaks and EDTA extracted CSCs of *S. typhimurium* were tested for their immunological activities against antiporin of *E. coli* K12 and antiserum prepared against sonicated antigen of *S. typhimurium* 163 by immunodiffusion test. The immunodiffusion test was performed as per Hudson and Hay (1980) with slight modifications.

Immunization of mice

The mice were divided into 5 groups comprising 47, 40, 30, 24 and 28 mice in groups 1, 2, 3, 4 and 5 respectively. The mice in groups 1, 2, 3 and 4 were immunized with UECSCs, recycled CSCs on ultrogel AcA-34 column peak (PI), recycled pooled peaks 1, 2, 3 (P1, 2, 3) and EDTA extracted CSCs of *S. typhimurium* 163, respectively, as per Udhayakumar and Muthukkaruppan (1987). The mice in each of the 4 groups were immunized with 50 µg protein./ mouse, respectively, by subcutaneous route twice at 2 weeks intervals. After separating 20, 10 and 10 mice from groups 1a, 2c and 4f for treating with carrageenan 24 hr before challenge, were named as 20I[b], 10II[d] and 10 IV[g] and in control group as V[h].

Table 1. The protein and carbohydrate contents in the various preparations of *S. typhimurium* 163

Preparations	Protein (mg/ml)	Carbohydrate (mg/ml)
UECSCs	304.0	14.59
Recycled PI of UECSCs	5.3	1.90
Recycled PI[a] of UECSCs	4.0	1.20
Recycled PII[a] of UECSCs	2.0	0.95
Recycled PII[b] of UECSCs	3.8	1.60
Recycled PII[c] of UECSCs	3.5	0.20
Recycled PIII of UECSCs	1.5	Nil
EDTA extracted CSCs	12.0	10.5

CSCs , Cell surface components of *S. typhimurium* 163: PI[a] , recycled subpeak from PI; PII[a], PII[b], PII[c] ,recycled subpeaks of PII.

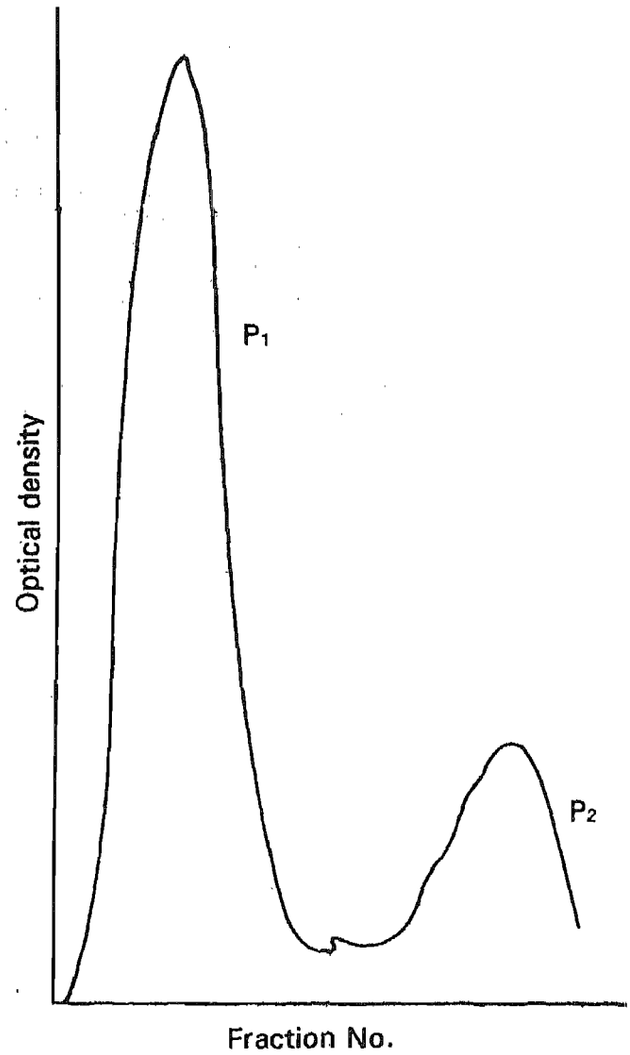


Fig. 1. Recycled peak-I of urea extracted outer membrane proteins (porins) of *S. typimurium* 163 on ultragel AcA-34 column (1×40 cm).

Monitoring of humoral immune response (HIR)

Collection of sera: Immunized and control mice were bled retro-orbitally with the capillary tube. The sera were separated from the clot by centrifugation and stored at –20°C before use.

Quantitation of total immunoglobulin (Ig): The Ig levels in the serum samples of immunized and control mice collected at 0 day and at weekly intervals postimmunization (PI) and postchallenge (PC) were determined as per Pfeiffer *et al.* (1977) as adopted by Chaturvedi and Kaura (1987).

Detection of antibodies in the serum samples of immunized and challenged mice: The serum samples collected at 0 day, weekly intervals PI and PC were used for the detection of antibodies with the immunodiffusion test.

Immediate type footpad hypersensitivity reaction (ITFHR): The ITFHR was performed as per Collins and Mackaness (1968). The footpad biopsy was collected randomly in 10% formol-saline from each group of mice showing peak reaction at 6 hr from antigen and saline inoculated sites for the histopathological examination.

Cell-mediated immune response (CMIR)

Nitroblue tetrazolium (NBT) reduction test : NBT reduction test was performed at 0 day, weekly intervals PI and PC (Frymus *et al.* 1985).

Delayed type of footpad hypersensitivity reaction (DTFHR): The DTFHR was recorded at 12, 24, 48, 72 hr on the sites at which ITFR was recorded PEA. The skin biopsy at peak reaction of 72 hr was collected in formol-saline for the histopathological examination.

Determination of role of macrophage in immunity: Twenty, ten and ten mice immunized with UECSCs (group 1b), recycled UECSCs PI (group 2d) and EDTA extracted CSCs (group 4g) were separated from the respective groups 24 hr before challenge and treated with type V Iota carrageenan following the method of Ishizaka *et al.* (1977) with slight modifications.

Challenge experiment: Immunized and control groups of the mice were challenged at 2 weeks post second vaccination by I/P route with 2 LD_{50} culture of *S. typhimurium* 163 (twice mice passaged containing 1.16×10^5 cells). The surface viable count of the culture was done by spreading method (Cruickshank *et al.* 1965).

PC mortality pattern and microscopic examination of tissue alterations: The mortality pattern of the mice immunized with various preparations of UECSCs, its recycled peaks and EDTA extracted CSCs was recorded up to 3 weeks PC. The mice died PC and sacrificed at 3 weeks surviving challenge were examined for the presence/absence of lesions and bacterial load/ reisolation (Chaturvedi *et al.* 1994) of challenge organism in the liver and spleen. The liver and spleen of the surviving mice sacrificed at 3 weeks PC were preserved in 10% formol-saline and examined microscopically for the tissue alterations.

Percentage protection

The percentage protection of the PC surviving mice was calculated at 3 weeks PC in immunized and control groups.

RESULTS

Isolation, purification of CSCs from S. typhimurium

The UECSCs of *S. typhimurium* 163 were fractionated on ultrogel AcA-34 column (1.6 × 100 cm) and 3 peaks were

Table 2. Reisolation of challenge organism of *S. typhimurium* 163 from the liver and spleen of surviving mice sacrificed at 3 weeks postchallenge

Group No.	Total No.	Immunizing agent	Total No. mice surviving challenge and sacrificed	Total No. of mice positive for challenge organism (04,12 antisera)*	Total No. of mice negative for challenge organism (04,12 antisera)*
Vaccinated					
1a	27	UECSCs	12	11	1
1b	20**	UECSCs	2	2	nil
2c	30	Recycled UECSCs PI	12	5	7
2d	10**	Recycled UECSC PI	nil	nil	nil
3e	30	Recycled UECSCs PI+PII+PIII	11	8	3
4f	14	EDTA extracted CSCs	8	7	1
4g	10**	EDTA extracted CSCs	nil	nil	nil
Mock-vaccinated					
5hr	28	NSS	6	2	4

*, Colonies reisolated reacted with 0-4, 12 antisera by slide agglutination test; **, each mouse injected 3 mg carrageenan by I/P route 24 hr. before challenges; the mice were separated 24 hr before challenge for treating with carrageenan; CSCs, cell surface components.

Table 3. Immunopathological alterations in the liver and spleen of immunized mice sacrificed at 3 weeks surviving challenge with *S. typhimurium* 163

Organs Alterations	Group 1a	Group 2c	Group 3e	Group 4f	Group 5h
Liver immunological					
Infiltration of lymphocytes around bile duct and central vein	+	+	+	+	+
Intravascular aggregation of monocytes in the central vein	+	+	+	+	-
Congestion of sinusoids	+	-	-	-	-
Immunopathological					
Formation of granuloma inside the central vein	+	+	+	-	-
Small granuloma at some places	+	+	+	-	-
Pathological					
Cloudy swelling	+	-	-	-	-
Necrosis/necrosis coagulative type	++	+	-	-	-
Spleen immunological					
R.E. cells hyperplasia	++	++	+	-	ND
Formation of secondary follicles	+	+	-	+	ND
Immunopathological					
Granuloma formation	+	-	-	-	ND
Pathological					
Congestion	-	+	-	-	ND
Macrophage infiltration	-	+	-	-	ND
Neutrophil infiltration	-	+	-	-	ND

Group 1a, Immunized with UECSCs; group 2c, immunized with recycled PI of UECSCs; group 3e, immunized with recycled pooled PI+PII+PIII of extracted UECSCs; group 4f, immunized with EDTA extracted CSCs; group 5h, mock vaccinated with NSS.

obtained in the elution profile. These 3 main peaks were recycled on ultrogel AcA-34 column (1 × 40 cm) and eluted in large and some small peaks (Fig. 1).

Immunochemical characterization of various preparations of S. typhimurium 163

Biochemical analysis: The protein and carbohydrate contents are shown in Table 1.

Immunological reaction: The UECSCs of *S. typhimurium* formed 2 precipitin bands with antiserum of sonicated antigen of the *S. typhimurium* and a single precipitin band with antiporin of *E. coli* K 12. The recycled PI of UECSCs and CSCs of EDTA extracted and UECSCs of *S. typhimurium*

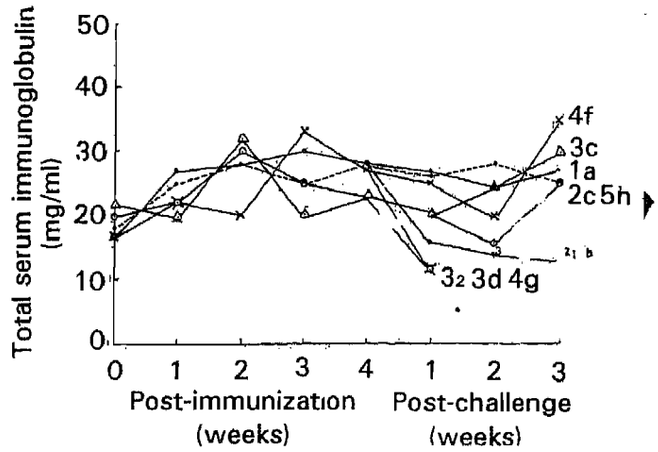


Fig. 2. Immunoglobulin level in immunized mice with and without carrageenan treatment.

●------● $^{a}_{Ig}$ of 5IMM Mice Gr 1c
●------● $^{b}_{Ig}$ of 5 IMM and Carr treated Mice Gr 1b
●------● $^{c}_{Ig}$ of 5 IMM mice Gr 2c
●------● $^{d}_{Ig}$ of 5 IMM and Carr treated Mice Gr 2d
Δ------Δ $^{e}_{Ig}$ of 5 IMM Mice Gr 3e
×------× $^{f}_{Ig}$ of 5 IMM Mice Gr 4v
×------× $^{g}_{Ig}$ of 5 IMM and Carr treated Mice Gr 4g
●------● $^{h}_{Ig}$ of 5 control mice Gr 5h

IMM Imunized, Carr-Carrageenan. Superscript-surviving mice less than 5; Gr, group.

showed precipitin line with antiporin of *E. coli* K 12, while recycled PII^{a}, II^{b}, II^{c} and PIII did not show any precipitin line. Thus, the positive reactivity of these preparations with antiporin of *E. coli* K12 confirms the presence of porin therein.

Monitoring of immune response

HIR: The serum Ig levels in the groups 1a, 2c, 3e and 4f showed increasing pattern in the levels of serum Ig (Fig. 2). While at 1 week PC, the mice showed fall in the level of Ig and reached at the level of prechallenge at 3 weeks PC. However, the levels of the Ig in the immunized and carrageenan treated mice (groups 1b, 2d, 4g) was comparatively very low after challenge. On the other hand, in mock vaccinated mice (5 h), the level of Ig was almost constant with slight fluctuation. The mice in group 4 immunized with EDTA extracted CSCs showed higher level of Ig and higher percentage of survival compared to other groups. However, in groups of mice immunized and treated with carrageenan, there was abrupt fall in the level of Ig along with their heavy mortality.

Detection of antibodies in the serum samples of immunized and challenged mice: The serum samples of mice collected at weekly intervals PI and PC showed precipitin line with UECSCs, recycled PI and EDTA extracted CSCs of *S. typhimurium*.

ITFHR: The ITFHR in immunized mice in response to

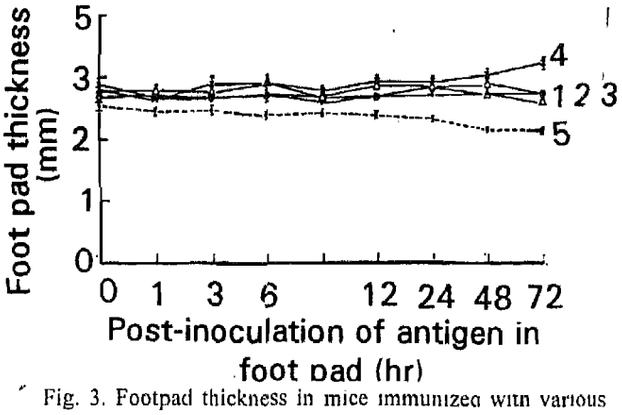


Fig. 3. Footpad thickness in mice immunized with various preparations of CSC (s) *S. typhmurium* 163

------●---- Footpad thickness of 3 IMM Mice Gr. 1
------O---- Footpad thickness of 3 IMM Mice Gr. 2
------Δ---- Footpad thickness of 3 IMM Mice Gr. 3
------×---- Footpad thickness of 3 IMM Mice Gr. 4
------●---- Footpad thickness of 3 control Mice Gr. 5

eliciting antigens is shown in Fig.3. The ITFHR in response to various preparations of UECSCs and EDTA extracted CSCs at 6 hr was 2.73 ± 0.12, 2.70 ± 0.02, 2.83 ± 0.03, 2.83 ± 0.03 mm in groups 1 to 4, respectively, while at the control sites reaction ranged from 2.37 ± 0.22 to 2.57 ±0.12 mm inoculated with NSS. However, ITFHR in mock vaccinated mice inoculated with NSS was 2.40 ± 0.03 mm.

The microscopic examination of the footpad biospy collected at 6 hr PEA revealed mild oedema, congestion of blood vessels, infiltration of neutrophils and few mononuclear cells in the dermis.

CMIR

NBT reduction test: The mice immunized in groups 1a to 4g (before separation and carrageenan treatment) showed more OD of the dye (formazan) released by the lysis of the phagocytic cells, which was 0.25-0.75, 0.26-0.75, 0.25-0.80 and 0.29-0.70, respectively, at 1 to 4 weeks PI. In mice in the control group 5h, the OD of the dye ranged between 0.19 - 0.70 at weeks postmock immunization. In groups 1 to 4 the OD of the dye was 0.24 - 0. 64, 0.31 - 0.60, 0.25 - 0.48 and 0.21 - 0.52 at weeks PC respectively. In control group 5h the OD of the dye ranged between 0.24 - 0.64 at 1 to 3 weeks PC. However, groups of mice (1b, 2d, 4g) immunized and treated with carrageenan showed less OD of the dye than the mice immunized but not treated with carrageenan. On comparative analysis, it was clear that mice treated with various preparations of CSCs showed activation of phagocytic cells. In groups 1a, 2c and 4f, there was comparatively more OD than the other groups. However, in groups 1b, 2c and 4g treated with carrageenan the OD was less in comparison to untreated groups.

DTFHR: The DTFHR recorded at the antigen inoculated sites at 72 hr ranged from 2.65 ±0.04 mm, while reactions, recorded at control sites inoculated with NSS was in the range of 2.10± 0.00 to 2.20 ± 0.00 mm. On the other hand, mock vaccinated mice showed 2.12 ±0.04 mm thickness at 72 hr postinoculation of NSS. However, footpad thickness was comparatively more in group of the mice immunized with EDTA extracted CSCs than in other groups, but in control mice inoculated with NSS, the thickness was almost negligible (Fig. 3). On histopathological examination, infiltration of mononuclear cells and proliferation of histocytes in the dermis were observed in the footpad biopsy.

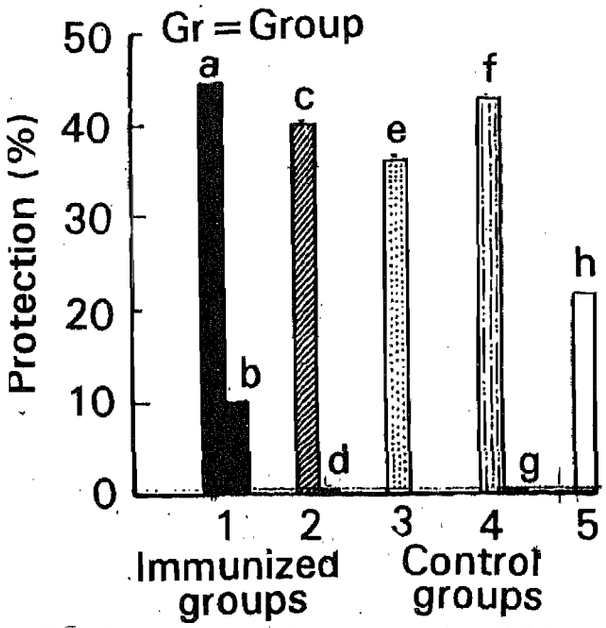
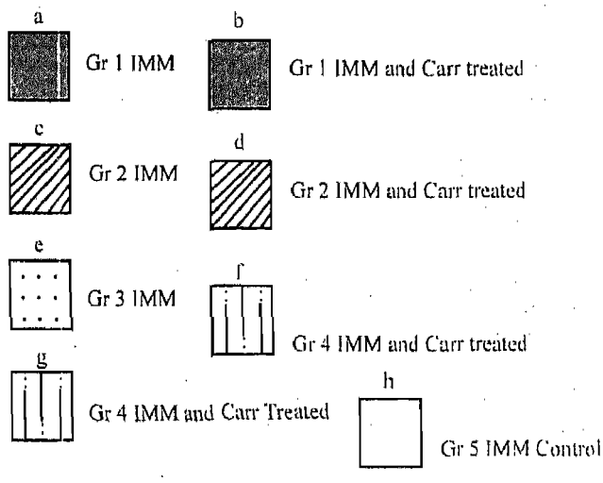


Fig. 4. Immunizing ability of various preparations of CSC (s) of *S. typhimurium* 163 in mice against challenge with *S. typhimurium* 163



IMM : Immunized: Carr. Carrageenan, Gr. Group.

Challenge experiment and percentage protection

The mice in each group were challenge with 2 LD_{50} by I/P route and mortality pattern was recorded up to 3 weeks PC. The mice in groups 1a, 2c, 3e and 4f survived 44.44, 40.0, 36.66 and 42.86%, respectively, while in the control group 5h the survival was 21.43% (Fig.4). Further, mice immunized and treated with carrageenan in groups 1b, 2d and 4g showed 10.00, 0.00 and 0.00% survival respectively. It shows that the treatment of mice with carrageenan made the mice more susceptible for PC infection by destroying macrophages.

On statistical analysis by applying Chi-square test, the effect of the vaccine in each group was tested and difference between percentage protection of immunized versus immunized and carrageenan treated mice as well as difference between immunized and control groups were significant. In group 4, the difference between 4f and 4g was highly significant. The mice immunized with UECSCs (group 1a) and EDTA extracted CSCs (group 4f) showed higher percentage protection than the mice immunized with UE recycled PI and recycled pooled peaks PI to PIII. However, the percentage protection in control mice (group 5h) was comparatively poor. Further, the mice immunized with UECSCs and treated with carrageenan (group 1b), mice immunized with recycled PI and treated with carrageenan (group 2d) and mice immunized with EDTA extracted CSCs and treated with carrageenan (group 4g) showed abrupt increase in the mortality pattern than the untreated groups (1a, 2c, 3e and 4f; (Fig. 4).

The viable count of the challenge organism dropped in the liver and spleen counted at weekly intervals of the immunized mice died PC. The decrease in the count ranged from 5.52 to 483.33 fold in the liver and from 8.72 to 5.8×10^4 in the spleen over the original challenge inoculum which was 1.16×10^5/ml. In contrast, there was approximately 5×10^5 fold increase in the viable count in the spleen of the control group over the challenge inoculum. The viable count of the challenge organism in the spleen of the immunized and carrageenan treated PC died mice was more than immunized mice and almost equal to the control mice.

Reisolation of the challenge organism from the post-challenged surviving sacrificed mice

The surviving mice in groups 1a and 2c (12 mice in each group), 11 and 5 sacrificed surviving challenge were positive for the challenge organism respectively. On the other hand, in immunized and carrageenan treated groups 1b, 2 of the surviving mice were positive for the challenge organism. In groups, 3e and 4f, 8 out of 11 and 7 out of 8, the surviving mice were positive for the challenge organism respectively. However, immunized and carrageenan treated mice in groups 2d and 4g died cent percent PC. In the control group (5h) 2 out of 6 mice were positive for the challenge organism (Table 2).

Microscopic alterations in the liver and spleen of immunized mice sacrificed surviving challenge

In groups 1a, 2c, 3e, there was immunological and immunopathological changes in the liver, while in group 4f, there was immunological reaction and no pathological changes were observed (Table 3). Further, in spleen of immunized groups 1a, 2c, 3e, there was more immunological reaction and no pathological changes except in groups 1a and 2c. However, in group 4f, some immunological reaction and no pathological reaction were recorded on the microscopic examination.

Mice died 1 week PC examined randomly in the immunized groups showed reticuloendothelial cell hyperplasia, congestion of sinusoids, while mice died in the control group, showed moderate infiltration of neutrophils around the central vein and bile duct, mild granuloma formation, severe perihepatitis, thickening of liver capsule and chronic infection.

DISCUSSION

Mice immunized with UECSCs, recycled peaks and EDTA extracted CSCs of *S. typhimurium* 163 showed both humoral and cellular immune responses. These immunological responses were also reported by Udhayakumar and Muthukkaruppan (1987), Muthukkumar and Muthukkaruppan (1993) and Villamil *et al.* (1995). The UECSCs and EDTA extracted CSCs of *S. typhimurium* were immunogenic and protective in mice. These findings are consistent with the findings in poultry (Bouzoubaa *et al.* 1987) and rabbits (Kabir 1987).

In this investigation, the UECSCs and EDTA extracted CSCs are OMP-LPS-complex of *S. typhimurium* comparatively contained more LPS and provided better protection PI in mice of groups 1a and 4f, respectively, than that of the purified, recycled UECSCs - PI and recycled UECSCs pooled peaks (PI+PII+PIII) containing less LPS, used for the immunization of mice of groups 2c and 3e respectively. The current evidence supports our findings that protective immunogens in the killed vaccine are associated with LPS (Muthukkumar and Muthukkaruppan 1993), while purified oligosaccharides derived from the O-specific antigens of *S. typhimurium* are not potent protective immunogens (Jorbeck *et al.* 1981, Svenson and Lindberg 1981) and small size of oligosaccharides in these vaccines of mouse typhoid might be responsible for the loss of immunogenicity (Hsu *et al.* 1985).

In this investigation, CMI studied by DTFHR, NBT reduction test, heavy mortality in macrophage depleted mice (treated with carrageenan), indicated that CMI might be playing a major role in the clearance of challenge organism in the light of intracellular nature of organism, thus helping in the survival of the challenged mice PI. This finding is corroborated by the findings of Cameron *et al.* (1974) and Collins (1974), who also emphasized the significance of CMI

in the survival of poultry and mice challenged PI with antigens of *Salmonella* species. The role of macrophages in PI immunity was demonstrated by NBT reduction test and carrageenan treatment of mice. The abrupt increase in OD of NBT reduction test was observed at 1 week PI and PC, indicating the phagocytic activity of the activated macrophages in the immunized mice. Further, immunized mice treated with carrageenan (macrophage depleted mice) died 90.0% in group 1b, 100.0% in group 2d and group 4g, PC with *S. typhimurium* indicated the significant role of macrophages in protection against intracellular infection. Selective toxicity of carrageenan for macrophages, but not to T and B-cells was proved by *in vitro* lymphocyte transformation studies in human and guinea pig (Lake *et al.* 1971), *in vivo* antibody production by B-cells in response to T-independent and dependent antigens in mice (Ishizaka *et al.* 1977). The immunized mice treated with carrageenan showed 90.0 to 100.0% PC mortality in comparison to the other groups. The death of immunized and carrageenan treated mice might be possibly due to unhindered growth of the challenge organism in the liver and spleen, which showed more viable count of the challenge organism than the other immunized groups. Our findings are consistent with the findings in mice treated with silica (macrophage depleted mice) and infected with *S. typhimurium* (O'Brien *et al.* 1979) and infected with *C. neoformans* (Monga 1981).

Histopathological evidence of protection provided by immunological reaction (cellular infiltration) in the visceral organs (liver, spleen) of immunized mice sacrificed surviving challenge, was absent in the control mice. However, there was only immunological reaction in the liver and spleen of mice immunized with EDTA extracted CSCs and this preparation was comparatively more immunogenic. These findings were supported from the results by Teuscher *et al.* (1988) and Chaturvedi *et al.* (1994). Further in the light of immune responses, it would be worth, if cell surface components could be used with the suitable immunomodulator for the immunization trial in the laboratory animals.

ACKNOWLEDGEMENTS

This investigation was supported by a grant No. 27-5/83/Edn.IV, dated 30.4.84 from the Indian Council of Agricultural Research, New Delhi to Professor Y K Kaura. Authors thank Dr P H Makela, National Public Health Institute, Helsinki, Finland, for a gift of antiporin of *E. coli* K 12.

REFERENCES

Bhatnagar N, Muller W and Schlecht S. 1982. Proteins from *Salmonella* R mutants mediating protection against *Salmonella typhimurium* infection in mice.1 .Preparation of protein free from lipopolysaccharide using various chromatographic methods. *Zentralblatt fur Bakteriologie Hygiene* I. *Abstract Orig.* A. **253**: 88-101.

Bouzoubaa K, Nagaraja K V , Newman J A and Pomeroy B S. 1987. Use of membrane proteins from *Salmonella gallinarum* for prevention of fowl typhoid infection in chickens. *Avian Diseases* **31**: 699-704.

Cameron C M. 1976. Immunity to *Salmonella* infections. *Journal of South African Veterinary Association* **47**: 89-91.

Cameron C M , Bratt O L and Fuls W J P. 1974. The effect of immunosuppression on the development of immunity to fowl typhoid. *Onderstepoort Journal of Veterinary Research* **41**: 15-22. *(fide* Bouzoubaa K *et al.* 1987. *Avian Diseases* **31**: 699-704.)

Chaturvedi G C and Sharma V K. 1981a. Cellular and humoral immune response in calves experimentally infected with *Salmonella dublin*. *Zentralblatt fur Bakteriologie Microbiologie and Hygiene* I. *Abstract. Orig.* A. **249**: 494-503.

Chaturvedi G C and Sharma V K. 1981b. Cell mediated immunoprotection in calves immunized with rough *Salmonella dublin*. *British Veterinary Journal* **137**: 421-30.

Chaturvedi G C and Kaura Y K. 1987. Estimation of serum immunoglobulin levels by sodium sulphite and zinc sulphate turbidity tests. *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases* **8**: 60-64.

Chaturvedi G C, Kaura Y K and Minakshi. 1994. Immune response of rabbits immunized with outer membrane proteins (porins) of *Salmonella dublin* 51. *Indian Journal of Animal Sciences* **64** : 1308- 15.

Collins F M.1969. Effect of specific immune mouse serum on the growth of *Salmonella enteritidis* in unvaccinated mice challenged by various routes. *Journal of Bacteriology* **97**: 667-75.

Collins F M. 1974. Vaccines and cell mediated immunity. *Bacteriological Review* **38**: 371-402.

Collins F M and Mackaness G B. 1968. Delayed hypersensitivity and Arthus reactivity in relation to host resistance in *Salmonella* infected mice. *Journal of Immunology* **101** : 830-45.

Cruickshank R, Duguid J P and Swain R H A. 1965. *Medical Microbiology.* 11th edn, pp 870. ELBS. Great Britain.

Dubois M, Gilles K A, Hamilton J K, Roberts P A and Smith F. 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* **28**: 350-56.

Frymus T and Decorski A. 1985. Nitroblue tetrazolium reduction test and serum lxsozyme assay in new born Arabian foals and mares. *Zentralblatt Veterinary Medicine* **B-32** : 280-86.

Hsu H S, Nakoneszne I and Guo U. 1985. Hisopathological evidence of protective immunity induced by sonicated *Salmonella* vaccine. *Canadian Journal of Microbiology* **31** : 54-61.

Hudson L and Hay F C. 1980. *Practical Immunology.* 2nd edn, pp. 117-20. Blackwell Scientific Publications, Oxford, London.

Ishizaka S, Otani S and Morisawa S. 1977. Effect of carrageenan of immune responses.1.Studies on the macrophage dependency of various antigens after treatment with carrageenan. *Journal of Immunology* **118** : 1213-18.

Jorbeck H J A, Svenson S B and Lindberg A A. 1981. Artificial *Salmonella* vaccines: *Salmonella typhimurium* O and antigenspecific oligosaccharide - protein conjugates elicit opsonizing antibodies that enhance phagocytosis. *Infection and Immunity* **32**: 497-502.

Kabir S. 1987. Preparation and immunogenicity of a bivalent cell-surface protein polysaccharide conjugate of *Vibrio cholerae*. *Journal of Medical Microbiology* **23**: 9-18.

Kaura Y K. 1988. A two-year surveillance (1984-86) on the incidence of *Salmonella* serotype in diarrhoeic calves at some of

the organized animal farms. *Indian Journal of Animal Sciences* **58**: 1361-68.

Killian J W and Morrison D C. 1986. Protection of C3H He J mice from lethal *Salmonella typhimurium* LT2 infection by immunization with lipopolysaccharide-lipid A associated protein complexes. *Infection and Immunity* **54**: 1-8.

Kuusi N, Nurminen M, Saxen H and Makela P H. 1981. Immunization with major outer membrane proteins (Porins) preparations in experimental salmonalosis of mice: effect of lipopolysaccharide. *Infection and Immunity* **34**: 328-32.

Lake W W, Bice D, Schwartz H J and Salvagglo J. 1971. Suppression of *in vitro* antigen induced lymphocyte transformation by carrageenan, a macrophage toxic agent. *Journal of Immunology* **107**: 1745-51.

Lindberg A A, Rosenberg L T, Liunggren A, Ganegg P J, Svenssen J and Wallin N H. 1974. Effect of synthetic disaccharide-protein conjugate as an immunogen in *Salmonella* infection in mice. *Infection and Immunity* **10**: 541-45.

Lowry C H, Rosebrough N J, Farr A L and Randall R J. 1951. Protein measurement with folin phenol reagent. *Journal of Biological Chemistry* **193**: 265-75.

Mackaness G B. 1962. Cellular resistance to infection. *Journal of Experimental Medicine* **116**:381-406.*(fide* Monga D P. 1981. *Infection and Immunity* **32**: 975-78.)

Muthkumar S and Muthukkaruppan V R. 1993. Mechanism of protective immunity induced by porin-lipopolysaccharide against murine salmonalosis. *Infection and Immunity* **61**: 3017-25.

O'Brien A D, Scher Irwin and Formal S B. 1979. Effect of silica on the innate resistance of inbred mice to *Salmonella typhimurium* infection. *Infection and Immunity* **26**: 513-20.

Pfeiffer M E, McGuire T C, Bendel R B and Weikel J N. 1977. Quantitation of bovine immunoglobulin: comparison of single radial immunodiffusion, zinc sulphate turbidity, serum electrophoresis and refractometer methods. *American Journal of Veterinary Research* **38**: 693-98.

Plant J E, Wilson B M and Glynn A A. 1982. The protein-lipopolysaccharide complex extracted with trichloracetic acid from *Salmonella typhimurium* effective in protection of mice against *Salmonella typhimurium* infection. *Parasite Immunology* **4**: 259-71. *(fide* Muthukkumar S and Muthukkaruppan V R. 1993. *Infection and Immunity* **61**: 3017-25.)

Svemson S B and Lindberg A A. 1981. Artificial *Salmonella* vaccines: *Salmonella typhimurium* O antigen specific oligosaccharide- protein conjugates elicit protective antibodies in rabbits and mice. *Infection and Immunity* **32**: 490-96.

Teuschar E, Contrue Y and Matovelo J A. 1988. Observations on the pathology of experimental salmonalosis (*S. typhimurium*) in calves, with special consideration of the haematopoietic organs (thymus, mesenteric lymphnodes, spleen and bone marrow). *Schwizer Archio fur Tierneiliunde* **130**: 195-210.

Udhayakumar V and Muthukkaruppan V R. 1987. Protective immunity induced by outer membrane proteins of *Salmonella typhimurium* in mice. *Infection and Immunity* **55**: 816-21.

Udhayakumar V and Muthukkaruppan V R. 1989. Protective immunity induced by porin against *Salmonella* infection in mice. *Indian Journal of Medical Research* **89**: 121-27.

Villamil M, Rueda E, Gallego I, Marino O C and Gutierrez De Gerardino A. 1995.Immune humoral and cellular response in guinea pigs immunized with outer membrane proteins of *Brucella abortus* strains RB 51. *Archivos de Medicina Veterinaria* **27**: 77-84. *(fide Veterinary Bulletin* 1996 **66**: No. 12, 7834.)

Indian Journal of Animal Sciences 68 (4) : 297-299, April 1998

Comparative studies on efficacy of different vaccines against fowl typhoid in chickens

A K ARORA[1], K S SANDHU[2] and S S SODHI[3]

Punjab Agricultural University, Ludhiana, Punjab 141 004

Received :3 March 1997 ; Accepted: 28 October 1997

ABSTRACT

Studies were conducted to compare the efficacy of live avirulent 9R vaccine, protein extracts and formalin- killed bacterin for the protection of chickens against fowl typhoid. Vaccination of birds at 9 weeks of age using strain 9R with and without Freund's complete adjuvant resulted in 95 and 100% survivors, but the challenge strain persisted in the organs of 1 bird each. The use of crude protein extract + adjuvant resulted in 100% survival rate along with total clearance of the challenge strain of *Salmonella gallinarum* from all the survivor birds. Addition of Freund's complete adjuvant during primary vaccination resulted in higher antibody titres and survival rate. Killed bacterin without adjuvant gave minimum protection against fowl typhoid.

Key words: Fowl typhoid, Killed bacterin, Membrane proteins, *Salmonella gallinarum*, Vaccines live

The outbreak of fowl typhoid caused by *Salmonella gallinarum*, result in devastating losses in adult fowls and in young chicks. Efforts have been made to control the disease by vaccination of birds with different vaccines (Bouzoubaa *et al.* 1987, 1989, Barrow 1990). The present study was undertaken to compare the efficacy of different live or killed vaccines for protection of birds against fowl typhoid.

MATERIALS AND METHODS

Live vaccine

The 9R strain of *S. gallinarum* was used as a live vaccine. Growth of 9R organisms on Mueller Hinton agar was harvested with sterile normal saline solution. This suspension was used in aqueous or adjuvanted form after mixing it with equal volumes of normal saline or with Freund's complete adjuvant (FCA) so that the finished product contained 40×10^7 organisms/ml.

Killed vaccine

A strain of *S. gallinarum* isolated from a case of fowl typhoid was used for the preparation of formalin- killed bacterin. The overnight growth of organisms in Roux flasks containing Mueller Hinton agar was harvested with normal saline solution and given 3 centrifugal washing. After adjusting the concentration of the suspension to 10^{11} organisms/ml, the organisms were killed with 0.3% formalin. This suspension was mixed with equal volume of FCA or used as an aqueous suspension.

Table 1. Per cent reactors detected by rapid plate agglutination test after vaccination with various vaccines against *S. gallinarum*

Group	Vaccine	Adju-	Days post-vaccination						
		vant*	10	20	30	40	50	60	70
I	9R	-	5	20	15	30	25	100	100
		+	15	60	50	75	65	100	100
II	Killed-	-	85	100	100	100	100	100	100
	bacterin	+	95	100	100	100	100	100	100
III	Crude	-	90	100	100	100	100	100	100
	protein extract	+	95	100	100	100	100	100	100
IV	Purified	-	80	90	85	100	100	100	100
	protein extract	+	90	95	95	100	100	100	100
V	Control		0	0	0	0	0	100	100

*Incorporated during primary vaccination only.

Membrane protein extract

The strain of *S. gallinarum* used for preparation of bacterin, was used for the isolation of outer membrane proteins by the method described by Bouzoubaa *et al.* (1987) with slight modifications. The pooled supernates after extraction were concentrated with the help of lyophilizer. This extract was purified by gel filtration through a column of 2.5 cm × 50 cm containing Sepharose-6B. The purified protein extract was used @ 2 mg/bird. The protein extract

Present address: [1]Assistant Bacteriologist, Department of Gynaecology and Obstetrics; [2]Assistant Professor, Department of Bacteriology and Virology; [3]Professor, Department of Immunology.

Table 2. Geometric mean (log 2 × 10) antibody titre for various vaccines against *S. gallinarum*

Group	Vaccine	Adju-	Days post-vaccination						
		vant*	10	20	30	40	50	60	70
I	9R	-	1.0	1.2	1.1	1.2	1.1	3.8	3.4
		+	1.1	1.7	1.6	2.1	1.8	4.2	3.7
II	Killed-	-	3.0	4.1	3.9	4.3	4.1	5.1	4.6
	bacterin	+	3.5	4.7	4.5	5.1	4.9	5.5	5.3
III	Crude	-	3.2	4.3	4.0	4.7	4.4	5.0	4.8
	protein extract	+	3.7	4.9	5.1	5.3	4.9	5.7	5.0
IV	Purified	-	2.3	3.0	2.8	3.6	3.4	4.2	4.0
	protein extract	+	3.3	4.0	4.0	4.9	4.3	4.9	4.7
V	Control		0.0	0.0	0.0	0.0	0.0	3.2	4.2

* Incorporated during primary vaccination only.

was also used in its crude form (without gel filtration) @ 3 mg/bird. Both the extracts were used in aqueous form or after blending with FCA.

Experimental design

The birds were distributed into 4 groups of 40 birds each. Each group was further divided into subgroups of 20 birds each. One subgroup within each group was given the respective vaccine as aqueous suspension and the other with FCA at 9 weeks of age. The booster dose of respective vaccine in the form of aqueous suspension only, was given to all the birds at 13 weeks of age. One group of 20 birds served as unvaccinated controls. Blood samples were collected from all the birds at 10 day intervals. The birds were subjected to serological testing by rapid plate agglutination test using *S. pullorum* stained antigen (IVRI). Sera samples were also subjected to microagglutination test as described by Williams and Whittemore (1971) with minor modifications. The working dilution of antigen was determined by checker board titration.

The birds in all the groups including controls were challenged 3 weeks after the second vaccination with 1 ml of diluted 18 hr broth culture of *S. gallinarum* mixed with 0.3 g of alkali powder (Smith 1955). Daily mortality was recorded for 28 days and the dead birds were subjected to bacteriological examination for the isolation of the challenge strain. After 28 days, all the birds were killed and the samples from liver, spleen, and ileocecal junction were cultured to see the clearance of systemic infection.

RESULTS AND DISCUSSION

In the group vaccinated with 9R vaccine without adjuvant, a maximum of 30% reactors were detected on 40th day post-primary vaccination, whereas 75% of the birds injected 9R + adjuvant showed antibody production (Table 1). In the other vaccinated groups most of the birds gave positive reaction. In general, addition of adjuvant resulted in more

Table 3. Protection rate afforded by different vaccines against fowl typhoid

Group	Vaccine	Adju-	Total mortality out of 20		No. of survivors positive for challenge strain/total survivors	
		vant				
I	9R	-	0	(0)	1/20	(5.0)
		+	1	(5)	1/19	(5.26)
II	Killed-	-	7	(35)	4/13	(30.76)
	Bacterin	+	3	(15)	2/17	(11.76)
III	Crude	-	2	(10)	0/18	(0.0)
	protein extract	+	0	(0)	0/20	(0.0)
IV	Purified	-	4	(20)	1/16	(6.25)
	protein extract	+	2	(10)	1/18	(5.55)
V	Control		13	(65)	5/7	(71.42)

Figures in parentheses indicate percentage.

reactors. The development of antibody following vaccination with 9R and protein extract has been reported (Bouzoubaa *et al.* 1989). As regards the microagglutination test (Table 2), geometric mean antibody titres were lowest in the birds vaccinated with live avirulent (9R) vaccine. Highest titre (before challenge) were observed 40 days post-primary vaccination in the birds vaccinated with crude protein extract + adjuvant followed by birds vaccinated with bacterin + adjuvant. The serological titres in the group vaccinated with purified protein extract + adjuvant were slightly less than those vaccinated with crude protein extract. Antibody levels were higher in the birds injected with vaccines containing adjuvant. Booster dose of different vaccines resulted in higher antibody titres. Silva *et al.* (1981) have reported that vaccination with killed *S. gallinarum* + adjuvant stimulated the highest and most uniform antibody response and that addition of mineral oil adjuvant to 9R enhances the antibody response. The mortality pattern in the vaccinated birds following oral challenge with *S. gallinarum* (Table 3) reveals that 9R vaccine resulted in 95 and 100% survivors when used with or without adjuvant respectively. The challenge strain was isolated from the ileocecal junction of 1 bird each in 9R and 9R + adjuvant vaccinated birds that survived the 28 day observation period postchallenge. Therefore, it seemed that some of the birds were not totally cleared of infection. The results agree with the work of other investigators who have reported that 9R vaccine gave variable protection against natural or experimental fowl typhoid (Silva *et al.* 1981). The membrane protein extract when used in crude form along with adjuvant resulted in 100% protection. But gel-filtered protein extract afforded 90% protection when used along with adjuvant and 80% when used without adjuvant. Systemic infection seemed to be totally cleared from the birds vaccinated with crude protein extract, but 1 bird cultured positive out of 16 and 18 surviving birds administered purified protein extract without and with adjuvant respectively.

Bouzoubaa *et al.* (1987) have reported that serological titres and protection rate increased with the increase in amount of protein injected. In this study, comparatively lower protection by purified protein extract might have been because of the presence of lesser amount of immunogenic components compared to that present in the crude extract.

The birds vaccinated with formalin-killed bacterin with and without adjuvant suffered 15 and 35% mortality on challenge. The challenge strain could be isolated from 11.76 and 30.76% of the survivors respectively. So killed bacterin did not seem to be effective in reducing the losses from fowl typhoid. These observations are similar to those of Silva *et al.* (1981). Within a group mortality was less in the birds immunized with adjuvant incorporated vaccines except in the birds vaccinated with '9R + adjuvant' in which 1 bird died out of 20. Silva *et al.* (1981) reported that addition of oil adjuvant to 9R vaccine interfered with the protection and gave even less protection than inactivated oil adjuvanted vaccine.

In this present study, there was no direct relationship between the antibody titres and the level of protection afforded by different vaccines thereby indicating a little role of humoral factors in the protection of birds against fowl typhoid (Padmanaban *et al.* 1981, Bouzoubaa *et al.* 1987).

REFERENCES

Barrow P A. 1990. Immunity to experimental fowl typhoid in chickens induced by virulence plasmid-cured derivative of *Salmonella gallinarum*. *Infection and Immunity* **58** : 2283-88.

Bouzoubaa K, Nagaraja K V, Newman J A and Pomeroy B S. 1987. Use of membrane proteins from *Salmonella gallinarum* for prevention of fowl typhoid infection in chickens. *Avian Diseases* **31** : 699-704.

Bouzoubaa K, Nagaraja K V, Kabbaj F Z, Newman J A and Pomeroy B S. 1989. Feasibility of using proteins from *S. gallinarum* vs. 9R live vaccine for prevention of fowl typhoid in chickens. *Avian Diseases* **33** : 385-91.

Padmanaban V D, Mittal K R and Gupta B R. 1981. Cross-protection against fowl typhoid. Immunization trials and humoral immune response. *Developmental and Comparative Immunology* **5** : 301-12.

Silva E N, Snoeyenbos G H, Weinack O M and Smyser C F. 1981. Studies on the use of 9R strain of *Salmonella gallinarum* as a vaccine in chickens. *Avian Diseases* **25** : 38-52.

Smith H W. 1955. Observations on experimental fowl typhoid. *Journal of Comparative Pathology* **65** : 37-54.

Williams J E and Whittemore A D. 1971. Serological diagnosis of pullorum disease with microagglutination system. *Applied Microbiology* **21** : 394-99.

Indian Journal of Animal Sciences 68 (4) : 300-304, April 1998

Clinicopathological studies on inclusion body hepatitis in bursectomized, thymectomized and normal chicks

RANA DILBAG SINGH[1] and BALWANT SINGH[2]

Punjab Agricultural University, Ludhiana, Punjab 141004

Received: 12 April 1997; Accepted : 28 October 1997

ABSTRACT

Clinicopathological studies on IBH was carried out in bursectomized and thymectomized chicks and findings were compared with normal chicks. After bursectomy and thymectomy, chicks free from avian adeno virus detected by dot EIA method were grouped into thymectomized, bursectomized and normal. Each group was further divided into 2 subgroups. One subgroup from each group was inoculated by IBH virus @ $10^{7.5}$ $TCID_{50}$. The other subgroup of each group was kept as control. Haematological studies revealed fall of haemoglobin (Hb) and total erythrocyte count (TEC) values at 3 and 5 days post- inoculation (DPI) followed by slight increase which indicated anaemia in first stage followed by haemoconcentration due to diarrhoea. Anaemia was more severe in thymectomized infected and bursectomized infected groups. The absolute lymphocyte count as also T cell population increased considerably. Among biochemical parameters, uric acid, lactic dehydrogenase, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, alkaline phosphatase increased in all the infected groups as compared to the non-infected groups. The increase was more marked in thymectomized infected and bursectomized infected groups. Gross lesions were seen only in liver at 5 and 7 DPI which comprised enlargement, mottling and congestion. Kidneys were slightly nephrotic. Histopathologically, liver has vacuolar degeneration with congestion, haemorrhage and sometimes necrosis, intranuclear inclusion bodies in hepatocytes were seen between 5 and 7 DPI. In T_1 group , the lesions were more severe and inclusions appeared 3 DPI onwards followed by BI group. Studies revealed that thymectomized group was most susceptible than other groups.

Key words : Bursectomized, Chicks, Inclusion body hepatitis, Thymectomized

Inclusion body hepatitis, a disease of young chicks caused by adenovirus result in heavy mortality in association with stress of any kind or immunosuppressive factors. It has, therefore, been envisaged to study the clinicopathology of the disease in thymectomized and bursectomized chicks as compared to the disease in chicks with intact thymus and bursa.

MATERIALS AND METHODS

Day-old broiler chicks (200) were procured from Punjab Agricultural University hatchery. Chicks were divided in 3 groups. In the first group, bursectomy was conducted (Eslami 1967) using halothane as general anaesthesia at day-old stage. Using the same anaesthesia, thymectomy was performed in the second group (Eslami 1967). On second day, cloacal swabs were subjected to indirect-dot EIA (Oberoi *et al.* 1993). Birds positive to viral antigen were discarded, and only negative birds were used for experimental studies. Chick embryo passaged IBH virus was obtained from Department of Bacteriology and Virology, PAU, Ludhiana,

Present address: [1] I/C Veterinary Hospital Ghanauli Distt. Patiala.
[2]Professor, Veterinary Pathology, College of Veterinary Sciences.

Table 1. Procedure followed for experimental studies in various groups

Groups	No. of birds in each groups	Dose of virus inoculated at 21 days of age intraperitonally per chick.	No. of chicks sacrificed DPI*			
			3	5	7	9
Normal control (NC)	30	-	5	5	5	4
Normal infected (NI)	30	log $10^{7.5}$ $TCID_{50}$	6	6	6	6
Bursectomized control (BC)	30	-	5	5	5	4
Bursectomized infected (BI)	30	log $10^{7.5}$ $TCID_{50}$	6	6	6	5
Thymectomized control (TC)	30	-	5	5	5	4
Thymectomized infected (TI)	30	log $10^{7.5}$ $TCID_{50}$	6	6	6	5

*Materials collected at the time of sacrificing; 1, blood in heparin for haematology; 2, blood for serum for biochemical studies; 3, tissues in 10% formalin saline.

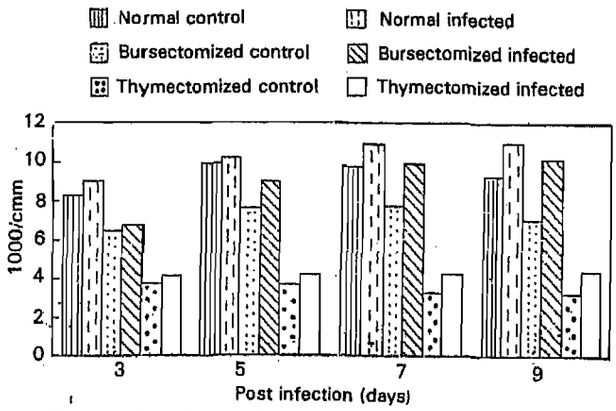


Fig. 1. Alterations in haemoglobin concentration in various groups of the experiment.

which was isolated from the liver of naturally infected birds.

The chicks found free from adenovirus were divided in different groups and treated (Table 1).

The chicks were closely observed for any clinical symptoms and mortality daily to ascertain the effect of IBH virus on chicks. Blood was collected from each bird in 2 clean autoclaved vials before sacrificing the birds (Table 1). One vial contained 0.1 ml heparin for collecting blood for haematological studies. Blood was collected in the second vial without any anticoagulant for serum separation for biochemical studies. Haemoglobin (Hb) was determined by haemoglobinometer. Total erythrocyte count (TEC) and total leukocyte count (TLC) were made (Wiseman 1931). Differential leukocyte count (DLC) was done by staining the blood smears with Wright's stain and counting at least 100 cells. T and B cell status has been ascertained on the basis of demonstration of esterases in peripheral blood leukocytes (Osbaldiston *et al.* 1978). Alpha naphthyl acetate esterase activity was demonstrated as reddish brown staining granules in T lymphocytes. Cells showing no such activity were considered as ß cells and negligible population of natural killer cells.

Biochemical parameters, viz. protein, albumin, alkaline phosphatase (AKPase), lactic dehydrogenase (LDH), uric acid, serum glutamic pyruvic transaminase (SGPT), serum glutamic oxalate transaminase (SGOT), were analyzed on auto analyzer.

Representative tissue pieces of kidneys, spleen, bursa of Fabricius, thymus, heart, lungs, and brain were collected in 10 % formalin. Sections were cut at 5 µ thickness by paraffin embedding technique and stained with H&E stain.

RESULTS AND DISCUSSION

After inoculating IBH virus intraperitonally at 21 days of age, the chicks were observed for 9 days. No clinical signs were observed in the control group. The infected groups did not show any clinical signs up to 5 DPI after which reduced feed intake and ruffled feathers were observed up to 8 DPI, when some of the chicks passed sticky faeces. After 8 DPI the chicks showed signs of recovery.

The Hb and TEC values were comparable in NC, BC and TC groups (Figs 1-3). In NI group, Hb and TEC values decreased at 3 DPI and further decreased at 5 DPI, slightly increased at 7 DPI and almost came to normal level at 9 DPI. The fall in Hb and TEC values indicate anaemia. The rise in values of Hb and TEC at 7 and 9 DPI appears to be due to haemoconcentration as a result of diarrhoea. Anaemia has been recorded earlier by Mishra (1988). In BI group, the Hb and TEC values were significantly lower compared to BC but higher than that of NI group up to 9 DPI which may be because of more severe diarrhoea and thus haemoconcentration. In T_1 group, Hb and TEC values were significantly lower than TC group. These values decreased at 3, 5 and 7 DPI but increased at 9 DPI. This indicate anaemia and later haemoconcentration due to diarrhoea.

The TLC and ALC values in different groups are given in Table 2. The values in NC group remained almost stable. In BC group, these values decreased due to the absence of B cells. Since circulating lymphocytes are T cells, thus TLC and ALC values were more low in TC group. The NI group revealed increased TLC and ALC up to 7 DPI with slight

Table 2. Total leukocyte and absolute lymphocyte values in different groups

Name of groups	Days post-inoculation	Total leukocyte count $\times 10^3$	Absolute lymphocyte values $\times 10^3$
Normal control	3	8.30±1.32	6.2±0.27
	5	9.93±1.06	7.30±0.17
	7	9.84±1.28	7.34±0.04
	9	9.32±1.52	6.99±0.06
Normal infected	3	9.05±0.75	7.05±0.06
	5	10.21±1.29	8.21±0.18
	7	10.98±0.78	9.03±0.16
	9	10.98±1.26	8.48±0.23
Bursectomized control	3	6.48±1.26	4.66±0.2
	5	7.68±1.32	5.58±0.06
	7	7.72±1.88	5.65±0.18
	9	7.09±1.75	5.12±0.07
Bursectomized infected	3	6.78±1.06	6.60±0.23
	5	9.05±0.95	6.90±0.11
	7	9.95±1.14	7.36±0.36
	9	10.20±1.25	7.59±0.35
Thymectomized control	3	3.75±0.62	2.97±0.04
	5	3.68±0.21	2.99±0.07
	7	3.29±0.62	2.63±0.06
	9	3.28±0.72	2.64±0.07
Thymectomized infected	3	4.16±1.09	3.00±0.06
	5	4.17±1.12	2.88±0.82
	7	4.28±1.06	2.93±0.07
	9	4.38±1.06	2.95±0.02

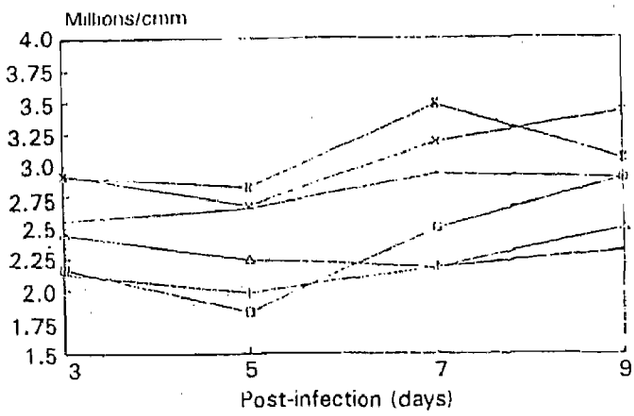


Fig. 2. Alterations in total erythrocyte count (TEC) in various groups of experiment.

fall at 9 DPI. In BI group, significant lymphocytosis (significant at 0.01 % level) was observed up to 9 DPI in comparison to BC and NC group but not beyond NI group. However, in TI group, significant lymphopaenia was seen as compared to the NC and NI groups. However, there was slight and nonsignificant increase in lymphocytes as compared to the TC group. This indicated that ß cell population increased in the defense process. The role of the T cells can be expressed by the significant increase in the population of T cells in NI and BI groups.

The biochemical parameters were studied at 3,5,7,9 DPI in all the experimental groups.

There was nonsignificant fluctuation of total serum proteins at different stages of experiment in each group. However, total proteins varied significantly between infected groups. Total proteins increased at 3 DPI in NI group which decreased at 7 DPI but again slightly increased at 9 DPI. In BI group, the total protein values were lower compared to the BC group throughout the experiment with progressive decrease up to 7 DPI in BI but with slight increase at 9 DPI. Similar trend of decrease in total protein was seen in TI group compared to the TC group up to 7 DPI with slight upward trend at 9 DPI. The protein values can be correlated to the extent of degenerative changes in liver.

Serum albumin concentration varied nonsignificantly. The serum albumin values were comparable in all the control groups. In the NI group, the values decreased at 5 DPI which further decreased at 7 DPI but increased at 9 DPI. In BI group, the values decreased up to 7 DPI but increased at 9 DPI. In the TI group, the value decreased throughout the experiment and were lower than those found in other infected groups. The nonsignificant variations indicate that albumin part is not affected much and only globulin part of total serum protein has been depressed by the virus infection in bursectomized and thymectomized groups.

Uric acid concentration was comparable in all the control groups. In NI group, constant increase was observed as also recorded by Mishra (1988). In BI and TI groups, the increase was more marked up to 7 DPI after which the values decreased but remained higher compared to NI group. This trend appears to be correlated with the severity of the lesions particularly in kidneys.

The LDH values varied significantly in control and infected groups. Values were highest in NI group. In other infected groups there was nonsignificant increase in values. Thus no specific conclusion can be drawn.

SGOT levels varied nonsignificantly in control groups during different stages of the experiment. In NI group, the level was significantly higher than control groups. In BI and TI groups, the levels initially increased followed by decrease. The increase in all the infected groups indicate soft tissue damage. The present results are similar to those of Mishra (1988).

The control groups showed almost identical picture of SGPT levels. All the infected groups showed significant increase. The SGPT values are indicative of liver damage which is required for the leakage of enzyme into blood stream (Kaneko 1989). The necrosis was more in the liver of BI group which explains the highest level of the enzyme in this group.

AKpase values rose steeply in all the infected groups. The increase was highest in TI group followed by BI and NI groups (Kohlar and Hromatka 1974).

The chicks of the control groups ie, normal control (NC), bursectomized control (BC), thymectomized control (TC) sacrificed at different intervals did not reveal any gross lesions. Grossly the liver of chicks belonging to normal infected (NI) were comparable to control groups at 3 DPI, however, at 5 DPI slight enlargement was observed. At 7 DPI, liver showed rounding of the borders, enlargement and slight congestion and mottling. At 9 DPI, the extent of the lesions decreased. Kidneys were enlarged and nephrotic at 5 DPI and 7 DPI. The severity of the lesions decreased at 9 DPI . No other significant changes were observed in other organs. In bursectomized and thymectomized infected (BI,TI) groups, the chicks showed gross lesions identical to NI group except the presence of petechial haemorrhages in the thymus of BI group.

Histopathologically, the control groups did not reveal any changes except cloudy swelling in the hepatocytes of some of the cases.

In NI group chicks, liver had varying degrees of vacuolar degeneration with or without mild lymphoid cell infiltration. These lesions became more severe on 5 DPI (Fig.4). At 7 DPI, congestion and haemorrhages and sometimes the necrosis were the additional changes. At 9 DPI, the intensity of the lesions decreased. However, the mononuclear cell infiltration was seen . The intranuclear inclusion bodies in hepatocytes (INIB) seen at 5 DPI, were more frequent at 7 DPI but rare at 9 DPI.

Kidneys had lesions of nephrosis at 3 and 5 DPI

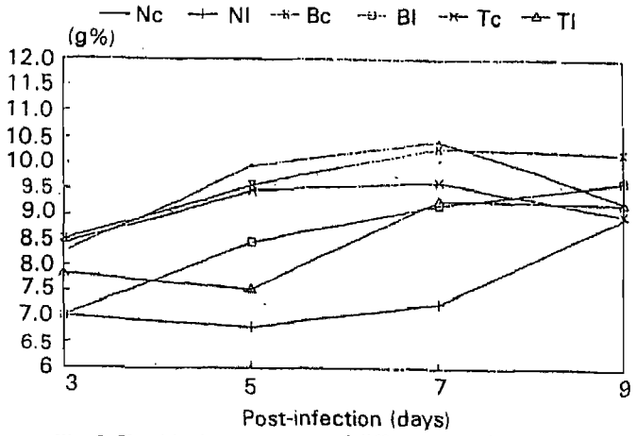


Fig. 3. Total leukocyte counts of different experimental groups of broiler chicks.

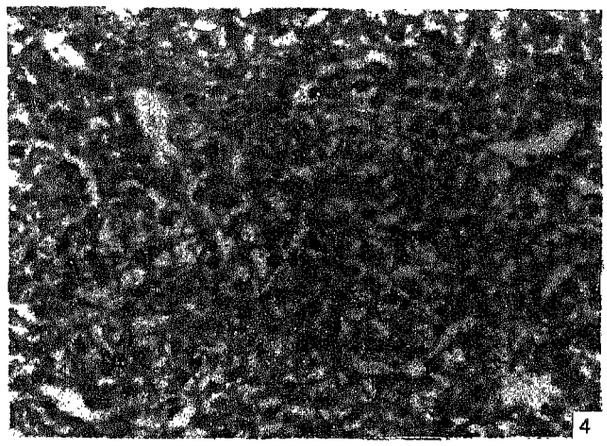


Fig. 4. Normal infected group at 5 DPI: Hepatocytes having extensive vacuolar degeneration. H & E. 125.

comprising granular and vacuolar degeneration and even coaggulative necrosis of the tubular epithelial cells. At 7 and 9 DPI, the degenerative changes persisted with additional lesions of congestion and haemorrhages and attempts at tubular epithelial cell proliferation .

Myocardium had congestion and mild mononuclear cell infiltration at 3 DPI ,lymphoid cell aggregates at 5, 7 and 9 DPI. At 9 DPI, the intensity of the lesions decreased. Bursa of Fabricius showed rarefaction of lymphoid cells particularly at 7 DPI. Other organs did not reveal any significant changes except mild congestion and haemorrhages.

In BI group chicks, the lesions in liver and kidneys were similar to those of NI group except that the lesions at 5 and 7 DPI were relatively more severe, when the congestion and haemorrhages were prominent changes. INIB were more frequent at these intervals. Myocardium had granular degeneration at 3 DPI, mild haemorrhages and extensive mononuclear cell infiltration along with proliferation of mesothelial cells at 5 DPI. At 7 DPI, lesions were similar to those of NI group.

In TI group, the lesions were more severe compared to NI and BI groups. Liver had congestion , haemorrhages and degenerative changes and INIB from 3 DPI onwards which were maximum at 7 DPI. Kidney lesions were identical to NI group at 3 and 9 DPI . At 5 and 7 DPI subcapsular haemorrhages and degeneration were more marked. Myocardial lesions were more severe than other groups. Bursa had mild fibrosis and heterophilic infiltration. Rest of the organs had only mild congestion.

The gross lesions in the NI group were similar to those recorded by Christensen and Saifuddin (1989) and Sandhu *et al.* (1995). Gross lesions in BI and TI groups were similar to NI group except petechiae on the thymus of BI group. Thymus haemorrhage was reported by Hoffman *et al.* (1978).

Histopathological lesions in liver and kidneys in NI group were similar to those recorded by Nayak *et al.* (1990) and Sandhu *et al.*(1995). Lungs and bursa lesions were similar to those recorded by Mishra (1988) and Sandhu *et al.*(1995). Lesions in BI and TI group were more severe compared to NI group possibly because of immunosuppression. Bursectomy has also been reported to induce outbreaks of IBH (Hoffmann *et al.*1978). The TI group had more severe lesions than NI and BI chicks, thus indicating that the cell-mediated immunity play the main defense role. Sandhu *et al.* (1995) studied the effect of immunosuppression on the course of IBH. They inoculated chicks with IBH virus and fed with aflatoxin or ochratoxin or both simultaneously and recorded the most severe lesions in the combined treatment groups than individual treatment groups.

It can, therefore, be concluded that the severity of the disease increase in thymectomized and bursectomized groups compared to the normal group.

REFERENCES

Christensen N H and Saifuddin M D. 1989. A primary epidemic of inclusion body hepatitis in broilers. *Avian Diseases* **33**(4) : 622-30.

Eslami M B. 1967. Neonatal bursectomy and thymectomy of day-old chicks. Laboratory animal techniques for immunology. *Handbook of Experimental Immunology.* (Ed.) Weir D M. :A 3.20-3.25.

Hoffmann R, Fiedler H, Losch U and Wessling E. 1978. Spontaneous occurrence of infectious hepatomyelopoietic disease (Inclusion body hepatitis) in bursectomized chicks. *Zentrablatt Veterinary Medicine B.* **25** : 478-84.

Kaneko J J. 1989. *Clinical Biochemistry of Domestic Animals.* 4th edn. Academic Press Inc., San Diego, California 92101.

Kohlar H and Hromatka Vesicek L. 1974. Inclusion body hepatitis in broilers in Austria (Preliminary communication). *Weiner Tierarztliche Monatsschrift* **61** : 90-94

Mishra S K. 1988. 'Studies on the prevalence and pathology of inclusion body hepatitis in broiler chicks.' Ph. D. Thesis. Punjab Agricultural University, Ludhiana.

Nayak M C, Chakrabarti K, Chakrabarti S and Chakrabarti A.

1990. An outbreak of inclusion body hepatitis in broiler chickens in West Bengal. *Indian Veterinary Journal* 67: 7-9.

Oberoi M S, Ramneek, Maiti N K and Sharma S N. 1993. A rapid indirect dot enzyme immunoassay for detection of avian viruses. *Journal of Comparative Microbiology Immunology and Infectious Diseases* 14:1-3.

Osbaldiston G W, Sullivan and Fox A.1978. Cytochemical demonstration of esterases in peripheral blood leukocytes. *American Journal of Veterinary Research* 39 : 683-85.

Sandhu B S, Singh H and Singh B. 1995. Pathological studies in broiler chicks fed aflatoxin or ochratoxin and inoculated with inclusion body hepatitis virus singly and in concurrence. *Veterinary Research Communications* 19:1611.

Wiseman B K. 1931. An improved method for obtaining white cell count in avian blood. *Proceedings of Experimental Biology Methods* 28: 1030-33.

Indian Journal of Animal Sciences 67 (4) : 305-307, April 1998

Zoonotic importance of cryptosporidiosis among some animals at Gharbia Province in Egypt

A H EL-HOHARY[1] and A M ABDEL-LATIF[2]

Mansoura University, Egypt, P B 35516

Received : 10 April 1997; Accepted : 27 October 1997

ABSTRACT

A survey of cryptosporidiosis was carried out in some animals and human beings in contacts. Faecal specimens (180) from patients suffering with gastroenteritis at Gharbia province in Egypt were screened for cryptosporidium oocysts. The oocysts were detected in 9 patients (5.0%). Faecal specimens (44) were collected from the households living with the patients who showed cryptosporidiosis by laboratory investigation. The oocysts were detected in 2 of these households. Faecal samples (223) were collected from different animal species (calves 45, dogs 25, cats 16, sheep 52, goats 35, pigeon's and chicken's droppings 35) living in contact with patients who showed cryptosporidiosis. 15 of these samples were soil contaminated with dog and cat excreta, the oocysts were detected in one of these samples.

Key words : Cryptosporidiosis, *Cryptosporidiosis*

Cryptosporidiosis is among the zoonotic diseases that can be transmitted by animal wastes. The causative agent, *Cryptosporidiosis* is a well recognized gastrointestinal coccidian protozoan parasite of the class Sporzoasida, subclass Coccidiasina, the order Eucoccidiorida, the suborder Eimeriina, Family Cryptosporidiidae, genus Cryptosporidium, that inhabits the intestinal epithelium and develop intracellularly in the microvillous border of the small intestine (Anderson 1982). This agent has emerged as a cause of chronic diarrhoea primarily in persons with various immunologic disorders, particularly in patients with AIDS (Acquired Immunodeficiency Syndrome) (Tzipori 1983, Soave and Armstrong 1986).

The role of cryptosporidium species as a cause of enteritis in man and farm animals has been the subject of much recent interest (Pitlick *et al.* 1983, Schultz 1983). It was reported to be transmitted from infected kittens to their owners (Current *et al.* 1983, Kock *et al.* 1983) and from infected calves to healthy animal-handlers (Angus 1983, Current *et al.* 1983, Pitlick *et al.* 1983). Most recently, it has been shown that zoonotic infection occurs with transmission of the disease from diarrhoic calves to man (Amira 1985, Magda and El-Ahraf 1988).

Therefore on account of the importance of cryptosporidiosis in man and animals, this work has been undertaken to investigate the incidence of cryptosporidiosis in animals and human-beings in contact and to investigate the role of soil contaminated with infected excreta as a reservoir of the disease.

Present address : [1] Department of Animal Hygiene, Husbandry and Zoonosis, Faculty of Veterinary, Mansour University , Egypt P.O. Box. 35516.

MATERIALS AND METHODS

Collection of samples

Stool specimens (Human patients) : In this study, 180 swabs and stool specimens (100 adults, 80 children) were collected in snap-capped vials containing 10% formalin (HCOH), from patients of all ages suffering from severe gastrointestinal symptoms such as abdominal cramps, vomiting and diarrhoea; most of these patients were otherwise healthy, at the Public Health Laboratory of El-Mahalla General Hospital-Gharbia province and submitted for routine bacteriological and parasitological examination. Meanwhile , 44 stool samples were also collected from the house of holds of these patients.

Faecal samples (in-contact animals)

Fresh faecal samples (208) were collected from different animal species living in contact with human cases who showed cryptosporidiosis after laboratory investigations. Faecal samples were collected either by direct collection of the faecal samples from the soil contaminated with fresh under the animal and transferring them to snapcapped vials containing 10 ml of 10% fromalin solution, or by using rectal swabs, which utilizes sterile cotton-tipped wooden sticks and placing them in the collection vials.

Soil samples

Soil samples (15) contaminated with fresh dogs and cats

Table 1. Frequency distribution of cryptosporidium oocysts detected from parasitological examination of stool specimens collected from human-beings in contact with infected animals

Cases	Collected specimens	Cryp. oocysts	(%)	condition		Overall (%)
Adults	100	4	4.00	35H	1	2.86
				65 D	3	4.62
Children	80	5	6.25	18 H	1	5.66
				62D	4	6.45
Total	180	9	5.00	180	9	5.00

H, Healthy ; D, diseased (had a severe gastrointestinal disturbance.

excreta were collected and placed in snap-capped vials containing 10 ml of 10% formalin solution and were labelled and transferred with a minimum of delay to the laboratory for parasitological examination.

Procedures of preparing of samples

Crytosporidum oocyst were detected as per Garcia *et al.* (1983) and California Department of Health Service (1984).

Staining of faecal samples

Stool specimens and faecal samples were stained for the detection of *Cryptosporidium* oocysts.

Modified Ziehl-Neelsen Stain Technique (MZN) (Garcia *et al.* 1983), one of the 3 previously prepared slides was fixed in absolute methanol for 10 min, then immersed in concentrated cold fuchsin (1.0 g fuchsin, 10 ml ethanol and 90 ml of 5% phenol) for 5 min. The slides were then rinsed with tap water for 2 min, decolourized with 10% H_2SO_4 for 30 sec and rinsed again in tap water for 2 min. Counter staining with 5% malachite green (5.0 g of malachite green, 100 ml of 10% ethanol) was done for 1 min. The smears were rinsed with tap water, air-dried and examined microscopically for about 10 min under the oil immersion lens at a magnification of ×1000 to detect *Cryptosporidium* oocysts. Oocysts were identified as spherical red bodies 3-6 mm in diameter, with evidence of more deeply stained internal structure.

Auramine-Rhodamin staining technique (Sobieh et al. 1987)

The second slide, previously prepared was air dried, fixed by passing through a flame 3 times or / with methanol and stained by the Auramine - Rhodamine method (Vestal 1975). Smears were scanned for Cryptosporidium oocysts by fluorescence microscope at × 250 and × 400. All positive findings were confirmed with additional direct smears prepared directly and stained by the Kinyoun acid - fast method (Vestal 1975) with 10% H_2SO_4 for decolourization.

Safranin - methylene blue staining technique (SMB)

It is used as a confirmatory technique after Baxby *et al.* (1984). The third slide was fixed by passing, the slide was then examined under the microscope at × 400, as well as × 1000.

RESULTS AND DISCUSSION

This survey indicated the occurrence of *Cryptosporidum* species in stool specimens submitted for routine bacteriological and parasitological examinations from patients with with gastrointestinal symptoms in Gharbia province, Egypt, and confirmed recent reports from elsewhere that *Cryptosporidium* species may be an unrecognized cause of diarrhoea and other gastrointestinal symptoms in patients who are otherwise healthy (Hunt *et al.* 1984, Mata *et al.* 1984). Whether cryptosporidial infections occur primarily as sporadic cases or in outbreaks is not known (Hunt *et al.* 1984) nor can we speculate on whether our prevalence data represent endemic or epidemic rates.

Cryptosporidium oocysts were identified in 9 faecal specimens (5.0%) out of 180 patients admitted to the El-Mahalla General Hospital, suffering from severe gastrointestinal symptoms. Of these *Cryptosporidium* oocysts positive cases included 5 young children under 3-years-old (2 were < 1-year-old and, 3 were 3-year-old) were expecting *Cryptosporidia* (6.25%), while the other 4 positive cases (40%) out of these 9 were adult persons (Table 1). We also found that *Cryptosporidium* species to be one of the common intestinal prastitic pathogens in the patient population screened during this survey. This is in agreement with recent studies which have shown *Cryptosporidium* to be one of the leading enteric pathogens currently recognized (Jokipii *et al.* 1983, Mata *et al.* 1984).

The opportunistic behaviours of *Cryptosporidium* species so clearly established in patients with Acuqired Immune Deficiency Syndrome may well be applicable to patients to patients at extremes of age. Crytosporidial infections have been reported to be more common in children (6.25%) than in adults (4.0%) (Table 1, Tzipori *et al.* 1983, Hunt *et al.* 1984). Although there was an indication of this trend,

Table 2. Frequency distribtion of cryptosporidiosis among the house holds and different animal species in contact with patients suffering from severe gastrointestinal disturbances

Sources of samples	Total no. examined samples	+ve samples Cryptosporidium oocysts	Overall (%)
Faecal specimens from house holds	44	2	4.55
Soil contaminated with different animal excreta	15	1	6.67
Faecal samples from calves	45	13	28.89
Faecal samples from dogs	25	3	12.00
Faecal samples from cats	16	1	6.25
Faecal samples from sheep	52	4	7.69
Faecal samples from goats	35	2	5.71
Pigeon's droppings	15	-	0.0
chicken's droppings	20	-	0.0
Total	267	26	71.76

Table 3. Frequency of *Cryptosporidium* species and other parasitic pathogens in diarrhoeic stools

Parasites	No. of +ve cases	Overall (%)
Giardia lamblia	10	5.6
Cryptosporidium species	9	5.0
Ascaris lumbricoides	5	2.8
Trichuris trichiura	4	2.2
Entamoeba histolytica	4	2.2
Dientamoeba fragilis	2	1.1
Hookworms	-	0.0

* Based on 180 stool specimens.

cryptosporidium oocysts were equally detected in older children and adults (Current *et al.* 1983). This result agrees with Tzipori *et al.* (1983) and Hunt *et al.* (1984).

It cannot be said with certainty that finding of *Cryptosporidium* oocysts in faecal specimens implied an etiologic role in every case was that observed, but a cryptosporidial etiology was strongly suggested in most instances. Follow-up stool examination could be carried out, there were other parasitic pathogens in diarrhoeic stool specimens including : *Giardia lamblia* 5.6%, *Ascaris lumbricoides* 2.8%, *Trichuris trichuria* 2.2%, *Entamoeba histolytica* 2.2% and *Dientamoeba fragilis* 1.1%. In terms of frequency , *Cryptosporidium* species was the second most common parasitic agent of known pathogenicity after *Giardia* species (Table 3)

Mean while , this survey indicated also the occurrence of *Crypotosporidium* oocysts in faecal samples which were taken from their households (4.55%), samples were also taken from in contact animals and the soil contaminated with their excreta (6.67% ; Table 2). The animals in contact were of different species being ; calves (28.89%) , dogs (12.00%), cats (6.25%), sheep (7.69%) and goats (5.71%), while in pigeons and chicken droppings showed that all these samples were negative to *Cryptosporidium* oocysts. Our detection findings may not be considered exclusive. Further investigation which needs more longer time and a greater number of samples may give a satisfactory answer on the prevalence of the disease at Gharbia province in Egypt. From the hygienic point of view, the detection of *Cryptosporidium* oocysts in soil contaminated with infested animal excreta, throw some light on the role of soil as reservoir of the disease.

REFERENCES

Amira R Iskander. 1985. A case report of crypotosporidia infection among calves in Egyptign. *Veterinary Medical Journal* **33** No 2.

Anderson B C. 1982. Cryptosporidia : a review . *Journal of American Veterinary Medical Association* **180** : 1455-57.

Angus K W. 1983. Cryptosporidiosis in man, domestic animals and birds : a review. *Journal of Society of Medicine* **76** : 62-70.

Baxby D, Blundell N and Tlart C A. 1984. Staining of Cryptospoidum Oocysts. *Journal of Hygene* **9** (13) : 317-20.

California Department of Health Services.1984. Cryptosporidiosis: newly recognized cause of diarrhoela illness in day care centers. *Calif. Morb. Whly Report*, 41 : 1

Current W L, Reese N C, Ernst J V, Bailey W S, Heyman M B and Weinstein W M. 1983. Human Crytosporidiosis in immunocompetent and immunodeficient persons. *New England Journal of Medicine* **308** : 1252-57.

Garcia L S, Bruckner D A, Brewer T B and Shimizn R Y. 1983. Techniques for the recovery and identification of Cryptosporidium oocysts from stool specimens. *Journal of Clinical Microbiology* **18** : 185-90

Hunt D A, Shannon R, Palmer S R and Jephcott A E. 1984. Cryptosporidiosis in an urban community. *British Medical Journal* **289** : 814-16.

Jokipii L, Pohjola S and Jokipii A M M. 1983. Cryptosporidium : a frequent finding in patients with gastrointestinal symptoms. *Lancet* **ii** : 358-61.

Kock K L, Shankey v, Weinstein G S, Dye R E, Abi A B, Current W L and Eyster M E. 1983. *Annals of Internal Medicine* **99** : 3347. (*fide Bennett et al.* 1985.)

Mata L, Bolanos H, Pizarro D and Vives M. 1984. Crytosporidiosis in children from some highland Costa Rican rural and urban areas. *American Journal of Tropical Medicine and Hygiene* **33** : 24-29.

Pitlick S D, Fainstein V, Garza D, Guarda L, Boliver R, Rios A, Hopfer R L and Mansell P A. 1983. Human Cryptosporidiosis: Spectrum of disease. Report of six cases and review of the literature. *Archives International Medicine.* **143** : 2269-75.

Schultz M G. 1983. *New England Journal of Medicine* **308** : 1283. (*fide* Bennett *et al.* 1985.)

Soave R, and Armstrong D. 1986. Cryptosporidium and Cryptosporidiosis. *Review of Infectious Diseases* **8** : 1012-23.

Sobieh M, Tacal J V, Wilcke B W, Lawrence W, and El-Ahraf A. 1987. Investigation of cryptosporidial infection in calves in San Bernardino Country, California. *Journal of American Veterinary Medical Association* **191** (7) : 826-18.

Tzipori S. 1983. Cryptosporidiosis in animals and humans. *Microbiology. Review.* **47** : 84-96.

Tzipori S, Smith M, Birch C, Barnes G and Bishop R. 1983. Crptosporidiosis in hospital patients with gastrorenteritis. *American Journal of Tropical Medicine Mygiene.* **32** : 931-34.

Vestal A L. 1975. *Procedures for the Isolation and identification of Mycobacteria.* Center for Disease Control Atlanta, GA.

Wolfson J S, Richer J M, Waldron M A, Weber D J, McCarthy D M and Hopkins C C. 1985. Cryptosporidiosis in immunocompetent patients. *New England Journal of Medicine* **312** : 1278-82.

Indian Journal of Animal Sciences **68** (4) : 308-312, April 1998

Haematopathology of mice in acute experimental edwardsiellosis

B R SINGH[1] and K P SINGH[2]

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122

Received : 13 December 1996; Accepted : 27 October 1997

ABSTRACT

In experimentally induced edwardsiellosis in mice pathogenesis study was conducted. *Edwardsiella tarda* infection in mice after inoculation of LD_{50} dose intraperitoneally, caused increase in clotting time (20-25 min) and haemoconcentration. Total erythrocyte count and leucocyte count dropped down within first 12 hr of infection then rose steadly. Differential leucocyte count depicted specific pattern. Gross as well as histopathological lesions corresponding to acute vasculitis were observed apart from desquamative gastroenteritis. Focal mononuclear cell infiltration were evident in hepatic lobules, renal medulla, interalveolar septa and serosa-submucosa of glandular stomach and upper small intestine.

Key words : Cytotoxin, *Edwardsiella tarda*, Mice, Pathology, Vasculitis

Edwardsiella tarda has frequently been isolated from snakes, turtles, frogs, alligators, sea lions, aquatic birds, fish and domestic animals, viz. cattle and pigs (Farmer and McWhorter 1984, Holmes and Gross 1990). It causes diarrhoea in human beings. (Hadely 1969, Clarridge *et al.* 1980, Holmes and Gross 1990). Edwardsiellosis is a lethal disease of pond-fish and causes considerable economic loss (Salati *et al.* 1991). Edwardsiellosis has been established as zoonotic disease (Darrow *et al.* 1993) but very little is known about its pathogenesis in warm blooded animals. This study was conducted for understanding the pathogenesis of *E. tarda* in mice.

MATERIALS AND METHODS

Bacterial strains

Two strains of *E. tarda* (4E1 and 6E6) isolated from fish were obtained from food-borne infection at Infection and Intoxication Laboratory, Indian Veterinary Research Institute, Izatnagar. Both the strains were maintained by regular subculturing on brain heart infusion agar (Cruickshank *et al.* 1968) slants at 90 days interval.

LD_{50}

Fifty per cent lethal dose for mice was determined as per Cruickshank *et al.* (1968) by inoculating 6-hr-old serially diluted (in PBS pH 7.2) broth culture of *E. tarda,* through intraperitoneal (IP) route, and observed for 21 days for lethality.

Table 1. Mortality pattern in mice injected (IP) with $2 \times LD_{50}$ dose of *E. tarda* strains

Strain No.	Total No. of mice inoculated	Cummulative No. of mice died within 6hr	12hr	18hr	24hr	48hr	72hr	96hr
4E1	42	0	4	22	24	30	36	36
6E6	42	0	6	28	32	32	35	37

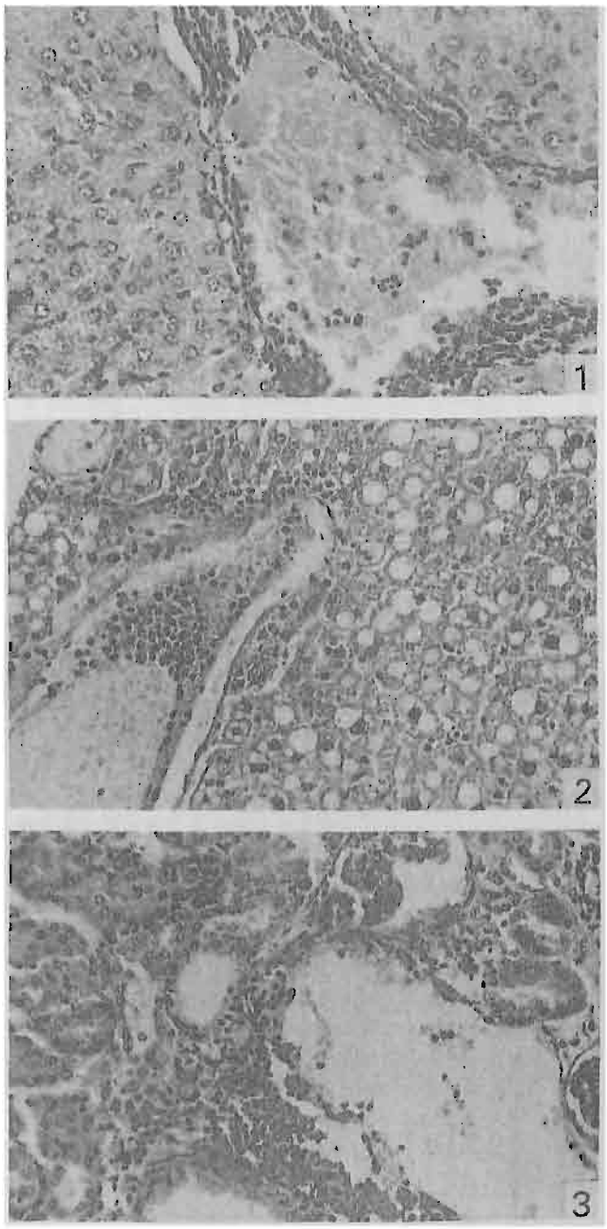
hr, Hours after inoculation ; remaining 11 mice did not die within 21 days of observation.

Experimental design

Swiss albino mice (105),30-day-old, obtained from disease free stock of the institute were divided into groups A, B and C. Groups A and B consisted of 42 mice each and were injected (IP) with $2 \times LD_{50}$ (in 0.1 ml) dose of 4E1 and 6E6 strains of *E. tarda* respectively. Mice in group C (21) were injected (IP) with 0.1 ml PBS (pH 7.2) each. Blood samples from each group were collected by bleeding (from inner canthus) 3 mice from each group, randomly picked at 0, 6, 12, 18, 24, 36, 48, 60, 72, 84, 96 hr and conclusion (21 day). Blood samples were analysed for total erythrocyte count (TEC), haemoglobin concentration (HbC), packed-cell volume (PCV), total leucocyte count (TLC), differential leucocyte count (DLC) and clotting time (capillary method) according to Fox *et al.* (1984).

Mice that died during experimentation were examined on postmortem and selected pieces of various organs were collected in formal saline for routine histopathology. Isolation

Present address : [1]Scientist, Division of Veterinary Public Health. [2]Scientist, Centre for Animal Disease Research and Diagnosis.



Figs 1-3. **1.** *Monocytic and neturophilic cell reaction in mucosa* and submucosa of glandular stomach of a mouse. (× 250, H and E stained). **2.** Villus necrosis in mouse intestine, infiltration of inflammatory cells of lamina propria. (× 250, H and E stained). **3.** Mouse kidney showing visually (× 250, H and E stained).

of *E. tarda* was attempted from intestinal contents, spleen and heart-blood (Farmer and McWhorter 1984).

RESULTS

LD_{50}-lethal dose (50%) of 4E1 and 6E6 strains of *E. tarda* for 30-day-old mice (Swiss albino) was determined to be equal

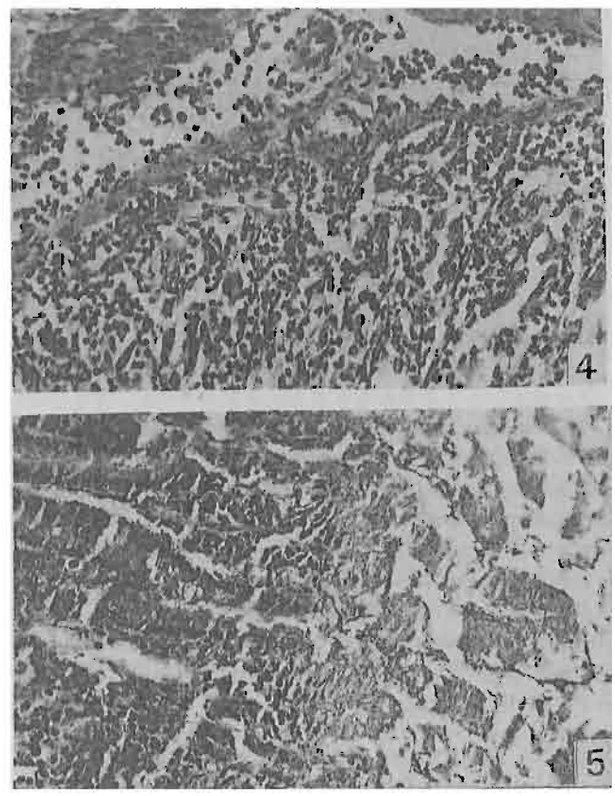
Table 2. Isolation of *E. tarda* from experimentally infected mice after death from various sites

Time lapse between death and infection	No. of mice examined for *E. tarda* isolation	Isolation of *E. t.* (No. of mice) from B	S	I	B+S	B+I	S+I	B+S+I
12 hr	10	1	0	0	5	0	0	4
18 hr	40	0	0	0	0	1	2	37
24 hr	6	0	0	0	0	0	5	1
48 hr	6	0	0	3	0	0	2	1
72 hr	9	0	0	7	0	0	2	0
96 hr	2	0	0	1	0	0	1	0
*21 days	11	0	0	2	0	0	0	0

*Mice were sacrificed : B. blood from heart ; S. spleen ; I. intestinal contents : B+S . heart blood and spleen ; B+I . heart blood and intestinal contents : S+I. spleen and intestinal contents, B+S+I. heart blood. spleen and intestinal contents.

to 1.2×10^4 and 0.8×10^4 live organisms intraperitoneally respectively. Most of the deaths occurred within first 5 days and rarely after seventh day.

The mice in groups A and B that were injected with $2 \times LD_{50}$ dose of either of the strain, died within 4 days of inoculation



Figs 4-5 : **4.** Mouse liver with vasculitics. (× 250, H and E stained). **5.** Mouse liver with vasculities and fatty changes. (× 250. H and E stained).

Table 3. Various haematological values of mice after *E. tarda* infection

Time (hr) lapse after inoculaiton	Haematological values											
	Group A				Group B				Group C			
	CT min	TEC $\times 10^6$/mm^3	PCV %	Hb g/dl	CT min	TEC $\times 10^6$/mm^3	PCV %	Hb g/dl	CT min	TEC $\times 10^6$/mm^3	PCV %	Hb g/dl
6	7.0 (0.4)	14.25	36	16.2	06.0 (1.4)	5.62	32	17.0	5.0 (0.6)	20.06	320	15.0
12	6.0 (0.6)	21.00	33	18.0	9.0 (1.3)	5.97	34	15.8	6.0 (0.4)	26.04	32	14.8
18	9.0 (1.4)	9.20	33	16.6	12.0 (1.4)	22.64	25	13.8	6.0 (1.4)	21.30	28	12.7
24	11.0 (1.3)	5.80	26	14.2	16.0 (0.6)	20.20	23	11.7	4.0 (1.4)	24.70	30	14.2
36	14.0 (1.4)	18.00	24	12.2	17.0 (0.4)	21.20	22	10.2	5.5 (1.3)	24.90	33	15.2
48	18.5 (0.4)	20.01	22	10.4	25.0 (0.6)	20.10	20	9.6	3.5 (0.4)	26.01	30	15.0
60	19.0 (0.6)	21.18	22	9.2	24.0 (1.4)	21.20	22	9.4	4.0 (0.4)	25.11	30	15.2
72	18.0 (1.4)	20.20	21	9.0	23.0 (1.3)	20.07	20	9.2	4.5 (0.4)	24.20	31	14.5
84	20.0 (1.3)	20.02	22	9.1	25.0 (0.6)	22.00	21	8.7	6.0 (1.3)	25.55	33	15.1
96	22.0 (1.4)	21.14	23	9.8	24.0 (0.4)	21.80	23	9.2	5.5 (1.4)	25.02	32	15.0
* 21 days	5.5 (0.4)	22.80	33	15.0	6.0 (0.6)	22.10	28	13.2	5.0 (1.3)	22.30	32	14.7

CT. clotting time in min; figures in parenthesis are standard deviations; TEC, total erythrocyte count; PCV, packed - cell volume ; Hb, haemoglobin in g/100 ml (dl); group A , mice injected (IP) with $2\times LD_{50}$ dose of 4 E1 strain ; group B, mice injected (IP) with $2\times LD_{50}$ dose of 6E6 strain ; group C, mice injected (IP) with 0.1 ml PBS (7.2 pH).

(Table 1) while in group C all survived. *E. tarda* was isolated either from heart or spleen or intestine or from all of the organs of dead mice of groups A and B (Table 2).

E. tarda localized mostly in intestine after an initial stage of bacteraemia.

Haematological findings

Clotting time shows steep rise (Table 3) within first 48 hr. Characteristic transient rise in neutrophils (Table 4) immediately followed by neutropenia which persisted only for first 18 hr of infection ; 24 hr onward marked neutrophilia was observed. Lymphocyte count revealed opposite trend i.e. transient drop followed by slight recovery and thereafter persistent lymphopenia. Only short-term monocytosis (18hr) was evident (Table 4). Total erythrocyte counts as well as leucocyte counts were dropped initially and then continued slightly below the normal levels. Packed-cell volume and haemoglobin concentration remained at a lowered level similar to erythrocyte count (Table 3).

Clinical observations

Mice in groups A and B showed signs of lethargicit, diarrhoea and prostration within 18 hr of inoculation. In all the infected mice appetite decreased but they consumed more water, compared to group C mice.

Postmortem lesions

Postmortem examination of mice died during experimentation revealed congested liver, duodenum, jejunum and lungs. Stomach was empty while, duodenum and jejunum were full of yellow watery content which was pus like in those died after 36 hr of inoculation and had blood tinges at several places. Brain and meninges were congested in several cases, mostly in group B. In group C none of the mice died and had no apparent gross lesion in any of the organ.

Histopathology

Liver in mice of groups A and B had engorged central and portal blood vessels with mononuclear and neutrophilic cell infiltration in vessel walls and perivascular spaces (Fig. 4). Focal mononuclear cell aggregation was noticed in mice that died after 24 hr of inoculation. Hepatocytes revealed condensation and margination of chromatin material with marked swelling. Liver acinia had varying degree of degeneration, cytoplasmolysis and necrosis. Kuffer cells were prominent. Hepatocytes had fat vacuoles replacing the nucleus aside (Fig. 5), specially in mice that died at 18 hr post-inoculation with 6E6 strain. At few places pink homogenous mass was

Table 4. Total and differential leucocyte count in negative control and *E. tarda* infected mice

Group of mice	Haematolgoical parameter		Time (hr) lapse after inoculation										
			6	12	18	24	36	48	60	72	84	96	21st day
A	TLC × 10^3/mm^3		6.00	4.96	4.30	5.25	5.85	5.90	6.46	7.8	9.85	9.95	6.78
	DLC	N%	71	50	48	75	72	77	78	77	76	78	39
		L%	23	42	42	25	24	23	22	23	24	22	61
		M%	6	8	10	0	4	0	0	0	0	0	0
B	TLC × 10^3/mm^3		5.98	3.30	2.95	3.90	4.30	5.66	5.92	6.07	6.70	6.85	6.25
	DLC	N%	64	46	26	62	69	72	72	68	66	66	33
		L%	29	46	63	34	30	28	27	30	31	30	66
		M%	7	8	11	4	1	0	1	2	3	4	1
C	TLC × 10^3/mm^3		6.95	6.80	7.70	7.46	6.90	6.76	7.21	7.11	6.87	6.99	6.98
	DLC	N%	34	28	30	31	32	34	30	28	31	30	32
		L%	64	68	70	68	66	65	67	69	67	70	67
		M%	2	4	0	1	2	1	3	3	2	0	1

N. neutrophils ; L. lymphocytes ; M. monocytes. TLC. total leucocyte count ; DLC. differential leucocyte count ; group A. mice injected (IP) with 2×LD_{50} dose of 4E1 strain ; group B. mice injected (IP) with 2 × LD_{50} dose of 6E6 strain; group C. mice injected (IP) with 0.1 ml PBS (7.2 pH).

deposited in thickened blood vessel walls.

In lungs engorged interalveolar capillaries and peribronchiolar blood vessels had mononuclear and neutrophilic cell reaction in vessel walls. Interalveolar septa were thickened due to mononuclear cell infiltration. At various places alveolar spaces were filled with haemorrhagic and cellular exudate. Bronchi and bronchioles also revealed the presence of mucous in lumen. Lesions were identical in both A and B groups in contrast to C with normal histology.

Glomerular tufts and medullary capillaries in kidneys were engorged. Perivascular mononuclear and neutrophlic cell reaction (Fig. 3) was evident in medulla. Cortical tubules revealed swelling of epithelial cell lining with degeneration and occlusion of tubular lumen.

Both glandular and nonglandular part of stomach had engorged blood vessels in lamina propria. Necrosis and denudation of lining epithelium, neutrophilic and mononuclear cell infiltration in submucosa and serosa (Fig. 1). Perivascular mononuclear cell reaction was evident in serosa. Focal vacuolation in stratum malpighii of nonglandular stomach was usual.

In small intestine necrosis and denudation of the tips of villi was evident (Fig. 2). Mononuclear cell reaction and engorged blood vessels were noticed in lamina propria.

In spleen focal rarefication of malpighian corpuscles, congestion of red pulp and infiltration of haemosiderin ladden macrophages along with large population of megacaryocytes were observed in several mice died in groups A and B.

Heart, brain and pancrease revealed vascular congestion with mild mononuclear cell reaction in blood vessels.

DISCUSSION

Reisolation of *E. trada* suggested that after an initial bacteralmia the bacteria start to localize in small intestine which appears to be the predilecation site similar to other enteropathogens. However, spleen remains another common place for *E. tarda* yet it did not appear to be multiplication site because none of the mice sacrificed on 21 day of experiment yielded *E. tarda* from spleen even after enrichment method of isolation (Farmer and McWhorter 1984). Accumulation of fluid in intestine may be due to release of enterotoxin and cytotoxin by the *E. tarda* (Bockemuhal *et al.* 1983). The other factors responsible for fluid accumulation may be infiltration of inflammatory cells in gastrointestinal mucosa (Wallis *et al.* 1990) but enterotoxin of *E. tarda* might be a factor in inducing infiltration of leucocytes in intestinal walls and around blood vessels as *E. coli* and *Klebsiella* (Singh and Kulshreshtha 1992, Singh *et al.* 1992). For vasculitis lesions in edwardsiellosis *E. tarda* cytotoxin could be responsible for endothelial cell damage, similar to shiga like toxin IIv of *E. coli* (MacLeod *et al.* 1991).

Accumulation of mononuclear cells and neutrophils in hepatic parenchyma, necrosis of intestinal villi, septicemia, lesions in kidney and spleen noted in experimental edwardsiellosis here were reported earlier in salmonellosis in mice (Singh 1995).

Similar gross and histopathological lesions as observed in mice has also been reported from natural infections of *E. tarda* in sea-lions, alligators (Wallace *et al.* 1966) and piglets (Owens *et al.* 1974). This study proves the suitability of Swiss albino mice as comparable model for edwardsiellosis in warm

blooded animals.

Most of the haematological changes and histopathological lesions observed are common in bacterial diseases like salmonellosis and colibacillosis (Singh 1995) and can not be considered specific to edwardsiellosis except extensive vasculitis for which cytotoxin of *E. tarda* (Singh and Tiwari 1996) may be the cause. Other important observation was increase in clotting time for which no explanation is valid unless further studies on conducted are this aspect. Isolation of *E. tarda* from intestinal contents of 2 of the 11 mice sacrificed on 21st day of the study (Table 2) revealed the role of mice as reservoir / carriers of *E. tarda* similar to pigs reported earlier (Ownes *et al.* 1994) apart from other widely known reservoirs like fish and other cold-blooded animals (Farmer and McWhorter 1984).

To determine the details of pathogenesis more exhaustive long-term studies are essential because with increased inland fisheries *E. tarda* may become a more important infection due to its zoonotic nature.

REFERENCES

Bockemuhal J, Aleksic V, Wokatsch R and Aleksic S. 1983. Pathogenicity tests with strains of *Edwardsiella tarda* : Detection of heat stable enterotowin. *Zentralblatt fur Backteriologie Hygienie I Abstract Original A.* **255** : 464-71.

Clarridge J E, Musher D M, Fainstein V and Wallace (Jr) R J. 1980. Extraintestinal human infections caused by *Edwardsiella tarda. Journal of Clinical Microbiology* **11** : 511-14.

Cruickshank R, Duguid J P and Swain R H A. 1968. *Medical Microbiology.* 11th edn. ELBS and E&S Living stone Ltd. Edinburgh.

Darrow M, Foulkes G and Ayoub M . 1993. Zoonotic transmission of turtleborn *Edwardsiella tarda. Complications in Surgery* **12** : 33-35.

Farmer J J and McWhorter A C. 1984. *Edwardsiella. Bergey's Manual of Systematic Bacteriology.* Vol. 1, pp. 486-91. (Eds) Krieg N R and Holt J G, Williams and Wilkins, London.

Fox J G, Cohen B J and Loew F M. 1984. *Laboratory Animal Medicine.* Academic Press Inc. New York.

Holmes B and Gross R J. 1990. Colifrom bacteria. *Topley and Wilson's Principles of Bacteriology, Virology and Immunity.* 8th (edn.) Parker M T and Buerden B I. Edwards Arnolds. London.

MacLeod D L, Gyles G L, Validivieso-Garchia A and Clarke R C. 1991. Physico-chemical and biological properties of purified *Escherichia coli* shigalike toxin II Varient. *Infection and Immunity* **59** : 1300-06.

Owens D R, Nelson S L and Addison J B. 1994. Isolation of *Edwardsiella tarda* from swine. *Applied Microbiology* **27** : 703-05.

Salati F, Ono K and Kusuda R. 1991. Oral vaccination of glasseel (*Anguilla japonica*) against *Edwardsiella tarda* infection. *Fish and Shell-fish Immunology* **1** : 309-10.

Singh B R and Kulshreshtha S B. 1992. Preliminary examination on the enterotoxigenicity of isolates of *Klebsiella pneumoniae* from seafoods. *International Journal of Food Microbiology* **16** : 349-52.

Singh B R, Kulshreshtha S B, Paliwal O P and Kapoor K N. 1992. Pathology of mouse foot pad test for detection of *Escherichia coli* enterotoxin. *Proceedings of 9th Annual confernce of of the Indian Association of Veterinary Pathologists,* pp. 47. Madras Veterinary College, Madras India.

Singh Y. 1995. Purification and characterization of *Salmonella cytotoxin* . Ph.D. Thesis. Govind Ballabh Pant University of Agriculture and Technology, Pantnagar.

Wallace L J, White F H and Gore H L. 1966. Isolation of *Edwardsiella tarda* from a sealion and two alligators. *Journal of American Veterinary Medical Association* **149** : 881-83.

Wallis T S, Vaughan A T M, Clark G J and Starkey W G. 1990. Role of Leucocytes in the induction of fluid secretion by *Salmonella typhimurium. Journal of Medical Microbiology* **31** : 27-35.

Indian Journal of Animal Sciences 68 (4) : 313-316, April 1998

Histochemical and histoenzymic studies on caprine mammary gland following induced *Mycoplasma* F38 mastitis

M M DARZI[1], K S ROY[2], N SOOD[3] and P P GUPTA[4]

Punjab Agricultural University, Ludhiana, Punjab 141 004

Received : 31 March 1997 ; Accepted : 27 January 1998

ABSTRACT

The histochemical and histoenzymic studies were conducted on mammary glands of 14 lactating goats. The right mammary halves of 12 goats were intracisternally inoculated with 1 ml of *Mycoplasma* F38 culture containing approximately 10^9 cfu, whereas, their left halves received 1 ml of sterile PPLO broth only. The remaining 2 goats served as uninfected controls. All the infected goats developed acute mastitis in their right mammary halves which subsequently turned chronic characterized by marked decrease in their size and secretions. Histopathologically there was acute purulent mastitis initially followed by fibroplasia in the infected mammary halves. The histochemical (lipids) and histoenzymic (acid phosphatase, alkaline phosphatase, succinic dehydrogenase, lactate dehydrogenase, glucose-6-phosphate dehydrogenase and glutanic dehydrogenase) changes were suggestive of altered fat, carbohydrate and protein metabolism in the infected mammary halves.

Key words : Caprine, Mammary gland, Mastitis, *Mycoplasma* F 38

Mycoplasma F38 considered to be the causal agent of classical caprine pleuropneumonia (MaC Owan and Minnette 1976 and Litmoi *et al.* 1990), was isolated from the milk of mastitic cows and its pathogenicity was tested in hamsters and rats (Kumar *et al.* 1992a). However, the mastitogenic potential of the organism and histochemical and histoenzymic alterations induced by it had not been studied in dairy animals so far. This communication deals with the histochemical and histoenzymic alterations in the caprine mammary gland experimentally infected with *Mycoplasma* F38.

MATERIALS AND METHODS

Apparently healthy mastitis-free lactating goats (14) of non-descript breed, in the age group of 2 to 3 years were procured and kept under close supervision for 7 days before starting the experiment. They were found free of any bacterial, mycoplasmal or fungal infection. The right mammary halves of each of 12 goats were intracisternally inoculated with 1 ml of 48-hr-old culture of *Mycoplasma* F38 (CM-193, obtained from the Department of Veterinary Public Health and Epidemiology, C C S Haryana Agricultural University, Hisar, India) containing approximately 10^9 cfu/ml. The left mammary halves of these goats were inoculated with 1 ml of sterile PPLO broth only. Two goats served as uninfected controls. All the goats were observed daily for physical changes in mammary halves and their secretions. Their milk samples were also subjected to mastitis tests, somatic cell count and differential leukocyte count. Reisolation of *Mycoplasma* F38 was attempted regularly.

The experiment was continued for 24 days. The sequential pathological changes in mammary glands were studied after randomly sacrificing 2 animals each on 4th, 8th, 12th, 16th, 20th and 24th day post infection (DPI). The 2 controls were also killed on day 24.

Representative tissues from mammary glands, supramammary lymphnodes and other visceral organs were fixed in 10% buffered formal saline for histopathological studies. For histochemical and histoenzymic studies representative samples of mammary tissue collected at 4 and 16 DPI were frozen at –20°C. Demonstration of neutral lipids was done after staining 6µm thick cryosections with Sudan Black (Luna 1968), whereas for histoenzymic studies, the methods used are summarized in Table 1.

RESULTS AND DISCUSSION

The appetite of all the infected goats remained unaffected, whereas, their temperature rose transitorily by 1° to 2°C within 24 hr post-infection (PI), thereafter it remained normal. The right mammary glands of the goats were slightly swollen, tender and painful within 24 hr of infection, but they de-

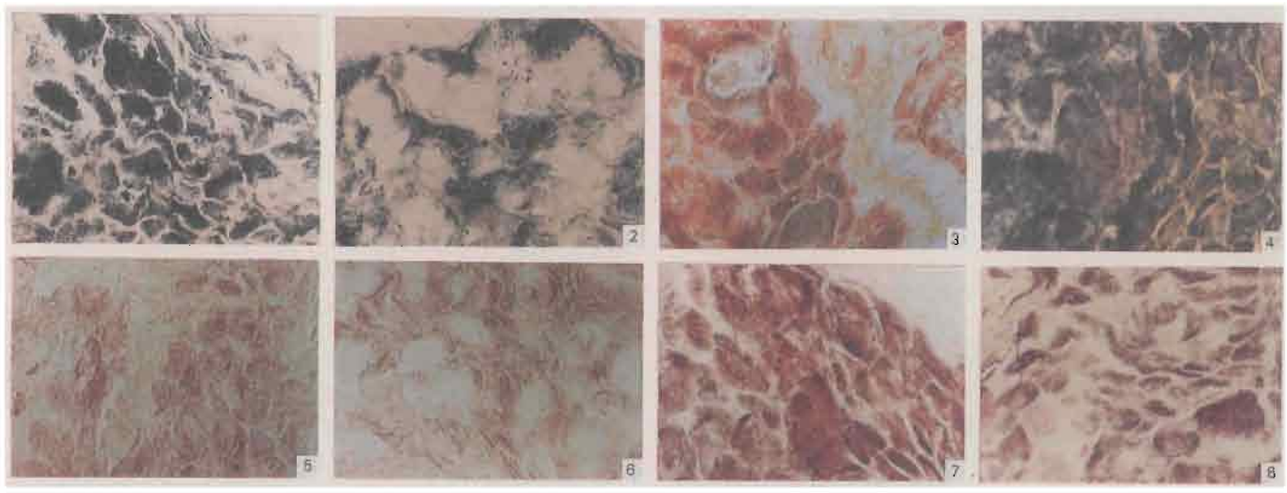
Present address : [1]Assistant Professor-cum-Junior Scientist, Department of Pathology, Faculty of Veterinary Sciences and Animal Husbandry, Shuhama, Aulusteng, Srinagar, Kashmir.

[2]Professor, Department of Anatomy and Histology, [3]Assistant Professor, [4] Professor, Department of Veterinary Pathology.

Table 1. Methods used in histoenzymic studies on udder tissues

Enzyme	Substrate	Method	Incubation time (min)	Reference
Acid phosphatase (ACPase)	Napthol AS T-R phosphate with hexazonium pararosanilin	Simultaneous coupling azodye method using substituted naphthol	60	Barka and Anderson (1963)
Alkaline phosphatase (AKPase)	Napthol A S-Mx phosphate disodium salt in combination with diazonium salt fast blue RR	Simultaneous coupling azodye method using substituted napthols	30	Barka and Anderson (1963)
Succinic dehydrogenase (SDH)	Di-Na succinate	Standard method for bound enzymes (Nitro BT method)	15	Pearse (1972)
Lactate dehydrogenase (LDH)	Na-DI lactate	Standard method for bound enzymes (Nitro BT method)	15	Pearse (1972)
Glucose - 6- phosphate dehydrogenase (G-6-PD)	Di-Na-glucose 6-phosphate	Standard method for bound enzymes (Nitro BT method)	15	Pearse (1972)
Glutamic dehydrogenase (GD)	Na-L-glutamate monohydrate	Standard method for bound enzymes (Nitro BT method)	15	Pearse (1972

creased in size from fourth day onwards and the trend continued throughout the experiment and by day 24 PI, they became markedly shrunken and smaller compared to the left udder halves due to fibrosis. The right supramammary lymphnodes of all the infected goats showed reactive hyperplasia from day 4 PI till the end of the experiment. The



Figs 1-8 : **1.** Cryosection of infected right udder half at **4** DPI., showing marked increase of coarse lipid droplets in alveoli. Sudan Black B method × 70. **2.** Cryosection of right infected udder half of a goat at DPI, showing increase in lipid droplets in focal areas. Sudan Black B method × 70. **3.** Cryosection of infected right udder half at 4 DPI, showing marked increase in ACPase activity. Azo Dye method × 70. **4.** Cryosection of infected right mammary gland of a goat at 4 DPI, showing intense AKPase activity in the secretory alveoli. Azo dye method × 300. **5.** Cryosection of infected right udder half of a goat at **4** DPI, showing increased SDH activity in the secretory alveoli. Some alveoli show focal loss of SDH activity. Nitro B.T. method × 70. **6.** Cryosection of infected right udder half of a goat at 16 DPI, showing less SDH activity. Stroma shows negative SDH activity. Nitro B.T. Method × 70. **7.** Cryosection of infected right udder half of a goat at 4 DPI, showing strong LDH activity in a secretory lobule. Nitro B.T. method × 70. **8.** Cryosection of infected right udder half of a goat at 4 DPI, showing marked increase in G-6-PD activity . Nitro B. T. method × 70.

milk yield from the infected udder halves decreased sharply from day 4 PI and continued to diminish till day 21, when these were agalactic. The milk secretions were initially pus like and later became thin and watery. The somatic cell counts in the milk from infected mammary halves increased significantly and different leukocyte count revealed that the neutrophils were the predominant cells initially and lymphocytes and macrophages were the main cell types later. *Mycoplasma* F38 was isolated from mastitic milk secretions up to 16th DPI, but not from the milk of left uninfected halves or both the mammary halves of the controls. Histopathologically there was diffused purulent mastitis up to day 4 PI, which became interstitial from 8 DPI with corresponding increase in lymphocytes and fibroblasts and later by 24 DPI, only remnants of glandular tissue were left in the fibrous tissue pool.

Histochemistry

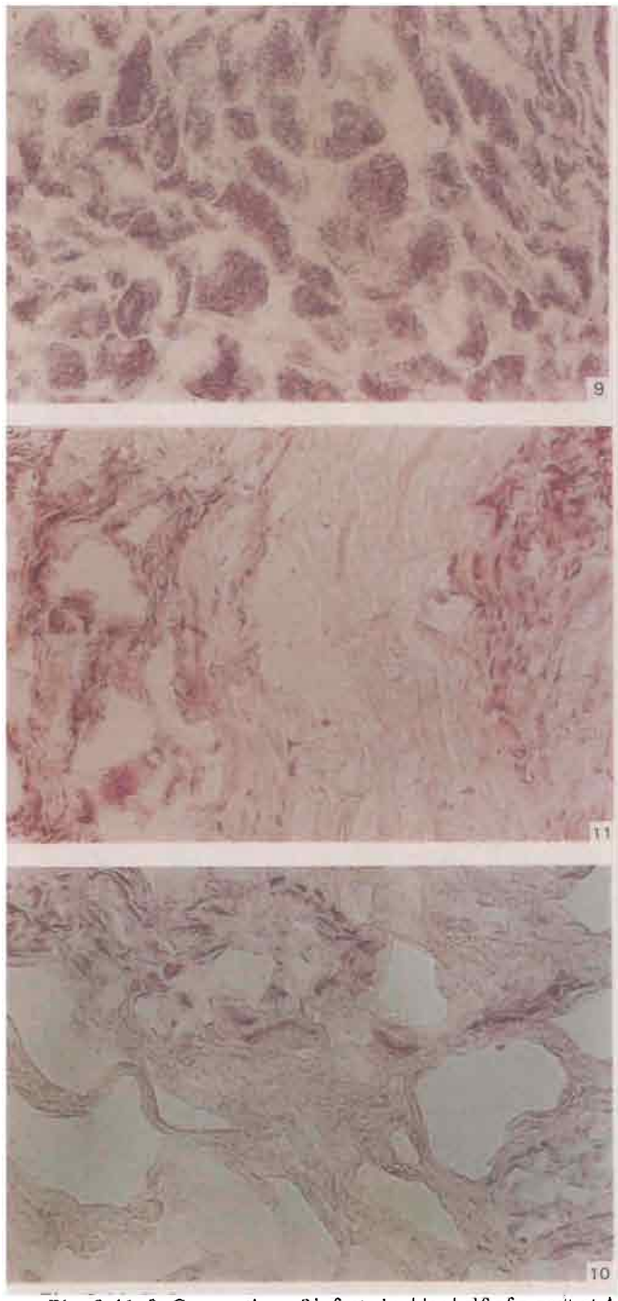
Lipids

Inoculation of *Mycoplasma* F38 into the right mammary halves resulted in increased lipid globules in the acinar epithelium at both acute (4th DPI) and chronic (16th DPI) stages of the mastitis (Figs 1, 2), which hinted at faulty lipid metabolism, leading to its accumulation. Injury by the organism could have deranged any part of the lipid metabolic pathway creating a massive backlog of triglycerides that dominate the cytopathic changes of fatty degeneration (Cheville 1989).

Histoenzymology

Phosphatase

ACPase and AKPase, showed diffuse but weak to moderate activities in acinar epithelium of control mammary glands, but markedly increased in acute mastitis (Figs 3, 4). The increased ACPase activity might by due to increased neutrophil infiltration. Further more, neutrophil lysosomal ACPases could have been released on immune complex phagocytosis, by dead neutrophil ,by leakage during formation of phagocytic vacuoles and for reverse by endocytosis (Tizard 1988, Kumar *et al.* 1992b). The ACPase activity is weak in acute and chronic tissue damage (Kotz *et al.* 1978). On the contrary Bogin *et al.* (1976) attributed increased ACPase activity to altered metabolic pathway in the udder tissue during inflammation and repair. However, findings in our study fell in line with those of Raekailio (1970). The AKPase on the other hand, catalyses dephosphorylation of adenosine triphosphate (ATP) and thereby plays a key role in the absorption and transport of ions across the membranes (Cope 1977). The enzyme is also present in the larger and more numerous specific (secondary) granules of neutrophils (Cheville 1989). Therefore degeneration of neutrophils could also have contributed to the increased AKPase activity in acute stage (4DPI). In mastitis increased AKPase due to *Corynebacterium pyogenes* (Jain and Sharma 1965), *Streptococcus agalactiae* (Mohamad *et al.* 1981), *Cryptococcus neoformans* (Verma *et al.* 1985, Singh



Figs 9-11. **9.** Cryosection of infected udder half of a goat at 4 DPI, showing increase in GDH activity in secretory lobule. Nitro BT method × 100. **10.** Cryosection of infected right udder half of goat at 16 DPI, showing decrease in G-6-PD activity. Stroma is devoid of any activity. Nitro BT method × 70. **11.** Cryosection of infected right udder half of a goat at 16 DPI, showing decreased GDH activity in secretory lobule and no activity in stroma. Nitro B.T. method × 100.

et al. 1995) and chlamydial mastitis (Koul 1987).

SDH generally increased on day 4PI (Fig. 5), but showed focal losses. The loss in SDH activity was more prominent on day 16 PI (Figs. 6). LDH markedly increased by day 4 PI (Fig.

7) but was less so by day 16. SDH , an iron metalloenzyme, acts as a catalyst in TCA cycle, while LDH activity suggested that Embeden Meyerhoff pathway was operative. Increased activities of these mitochondrial enzymes indicated glycolysis (Zugibe 1970, Leninger 1984) due to high metabolic activity in the mastitic organ. On the other hand, focal losses of SDH activity suggested cellular injury especially, due to degeneration of membranes, thereby affecting normal metabolic pathway of TCA cycle in injured cells (Patterson 1973). Comparative decrease of both the enzymes during chronic stages hinted at reduced metabolic state. Bogin *et al.* (1976) observed slight increase in LDH activity in mastitic udders. Sonneck and Sommer (1979) found that LDH activity correlated with cell counts and CMT, and therefore considered it a sensitive indicator of mammary gland infections. Similarly, Koul (1987) recorded elevated LDH and SDH activities in experimental chlamydial mastitis in goats.

G-6-PD and GDH were markedly increased by day 4 PI (Figs 8, 9) but decreased considerably by 16 DPI (Figs 10, 11). G-6-PD is key enzyme in the pentose phosphate pathway of glucose metabolism resulting in promotion of NADPH, fructose - 6- phosphate and D-ribose - 5 phosphate. The latter acts as a precursor for nucleotide synthesis, necessary for the formation of protein components (Leninger 1984). Therefore the increased G-6-PD, suggested increased glucose utilization and/or increased protein synthesis in acute mastitis. The latter was further substantiated by the rise in GDH, which was of fundamental importance in the biosynthesis of amino acids (Leninger 1984). From the histochemical and histoenzymic studies it could therefore be safely concluded that in F38 mastitis, fat, carbohydrate and protein metabolism was increased during acute mastitis , whereas it was depressed during chronic mastitis.

ACKNOWLEDGEMENTS

The first author received senior research fellowship from the Indian Council of Agricultural Research. The authors are thankful to Mr G K Sharma for meticulous typing of the article.

REFERENCES

Barka T and Anderson P J. 1963. *Histochemistry - Theory, Practice and Bibliography.* 2nd edn. Harper and Row Publishers Inc., New York.

Bogin E, Ziv G and Avidar J. 1976. Enzyme activities in normal and inflammed bovine udder tissues. *Zentralblatt fur Veterinarinermedizin* (B) **6** : 460-66.

Cheville N S. 1989. *Cell Pathology.* 2nd edn. Iowa State University Press, USA.

Cope G H. 1977. *Archives of Oral Biology* **22** : 715. (*fide* Pinkstaff CA 1980. The Cytology of Salivary Glands. (Eds) Bourne G H and Danielli J F. *International Review of Cytology* **63** : 142-250.)

Jain N C and Sharma G C. 1965. Alkaline phosphatase and glycogen in the udder and liver of goats with mastitis due to *Corynebacterium pyognes*. *Cornell Veterinarian* **55** : 164-71.

Kotz J, Basmadji K and Madej J A. 1978. Activity of lysozyme and acid phosphatase in bovine mastitis. *Medycyna Veterinariya* **34** : 89-91.

Koul S. 1987. 'Studies on experimental chlamydial mastitis in goats.' M. V. Sc. Thesis, Punjab Agricultural University, Ludhiana.

Kumar A, Garg D N and Mashajan S K. 1992a. Experimental pathogenicity of Mollicutes of bovine udder origin in hamster tracheal ring organ culture. *Indian Journal of Experimental Biology* **7**: 607-10.

Kumar V, Cotran R S and Robbins S L. 1992b. *Basis Pathology.* 5th edn. W B Saunders Co, Hrcout Brace Jovanovich, Inc. A Prism Indian edition.

Leninger A L. 1984. *Biochemistry.* 2nd edn. Kalayni Publishers Ludhiana, New Delhi.

Litamoi J K, Wanyangu S W and Simam P K. 1990. Isolation of *Mycoplasma* biotype F38 from sheep in Kenya. *Tropical Animal Health Production* **22** : 260-62.

Luna L G. 1968. *Manual of Histogic Staining Methods of the Armed Forces Institute of Pathology.* 3rd edn. McGraw-Hill Book Co., New York.

MaCOwan K J and Minnette J E A. 1976. A mycoplasma from acute contagious caprine pleuropneumonia in Kenya. *Tropical Animal Health Production* **8** : 91-95.

Mohamad A H, Dessouky M I, Abdel -Ghgani M and Bakeer A M. 1981. Contribution of alkaline phosphatase in milk of mastitic goats. *Egyptian Journal of Dairy Science* **9** : 105-12.

Pearse A G E. 1972. *Histochemistry : Theoretical and Applied* . 3rd edn, Vol. 2, Churchil Livingstone, London.

Patterson D R S. 1973. Metabolism as a factor in determining the toxic action of the aflatoxin in different species. *Food and Cosmetic Toxicology.* Vol. 2. Pergamon Press, Great Britain.

Raekallio J. 1970. Enzyme histochemistry of wound healing. *Progress in Histochemistry and Cytochemistry.* Vol. I, No. 2, pp. 2-20 Gustav Fusher verlang stuttgard U S A.

Singh M, Roy K S, Rona J S and Sood N. 1995. Experimental cryptococcal mastitis in goats. Histochemical studies . *Indian Journal of Animal Sciences* **65** (7) : 742-45.

Sonneck R and Sommer H. 1979. The relationship between cell count and lactic dehydrogenase (LDH) activity in cow milk. *Abstracts of Proceedings of the 21st World Veterinary Congress.* Moscow USSR Vol. I. Moscow USSR Ministry of Agriculture Section 10, 121-22.

Tizard I R. 1988. *Immunology. An Introduction.* 2nd edn. W.B. Saunders Co., Philadelphia.

Verma P C, Kalra D S and Bhargava D N. 1985. Some aspects of histochemical studies in experimentally produced mycotic mastitis. *Haryana Veterinarian* **24** : 27-34.

Zugibe F T. 1970. *Diagnostic Histochemistry.* Saint Louis . The C.V. Mosby Co.,

Indian Journal of Animal Sciences 68 (4) : 317-319, April 1998

Plasma levels and pharmacokinetics of neomycin in buffaloes (*Bubalus bubalis*) following its single intrauterine infusion

PANKAJ SOOD[1], A S NANDA[2] and A K SRIVASTAVA[3]

Punjab Agricultural University, Ludhiana, Punjab 141 004

Received : 21 April 1997; Accepted : 28 October 1997

ABSTRACT

The plasma levels, pharmacokinetics and dosage regimen of neomycin were investigated in healthy and normal cycling buffaloes after its intrauterine administration (5 mg/kg). The drug was rapidly absorbed from the uterus. At 5 min 3.68 ± 1.01 μg.ml^{-1} of neomycin was detected in plasma. The absorption and elimination half-lives were calculated to be 0.51 ± 0.19 hr and 5.87 ± 0.47 hr respectively. The apparent volume of distribution, total body clearance and bioavailability were 0.51 ± 0.02 L.kg^{-1}, 86.7 ± 4.4 ml.kg^{-1}.hr^{-1} and 69 % respectively. Based on pharmacokinetic parameters, a satisfactory intrauterine dosage regimen of neomycin in buffaloes would be 9 mg.kg^{-1} followed by 7 mg.kg^{-1} at 12 hr intervals.

Key words : Aminoglycoside, Dosage regimen, Neomycin, Pharmacokinetics

Neomycin, a broad spectrum aminoglycoside antibiotic covers almost all common microorganisms responsible for uterine infections (Dobson and Noakes 1990) and is being effectively employed as intrauterine medication (Paufler 1969, Boyd and Allen 1988, Beggard *et al.* 1992). Majority of the reports available on intrauterine treatment with antibiotics are based on the *in vitro* antibiogram without the knowledge of their disposition kinetics (Murray *et al.* 1990). For a successful antibiotic therapy it is imperative that the dose administered should be such that concentration of drug in blood and target organs should not fall below minimum therapeutic level. The pharmacokinetics of neomycin have been studied after systemic administration in calves (Black and Claxton 1982) and horses (Baggot *et al.* 1985). But the kinetic studies of neomycin after intrauterine infusion are completely lacking in animals particularly in buffalo species. Keeping this in view, the pharmacokinetics of neomycin was investigated in healthy and normal cycling buffaloes following its single intrauterine infusion at the dose of 5 mg/kg body weight.

MATERIALS AND METHODS

Experimental animals and drug administration

Studies were conducted on 5 healthy and normal cycling female buffaloes weighing between 449 and 500 kg. The animals were maintained under standard conditions and feeding schedule with free access to water. The reproductive health status was confirmed on the basis of history and thorough clinico-gynaecological examination. Neomycin was

Table 1 .Pharmacokinetic parameters of neomycin in healthy and normal cycling buffaloes (n=5) after a single intrauterine infusion of 5 mg/kg body weight

Determinants[a]	Units	Mean ± SE
A	μg.ml^{-1}	7.06 ± 1.35
Ka	hr^{-1}	2.46 ± 0.85
$t_{1/2}$(Ka)	hr	0.51 ± 0.19
B	μg.ml^{-1}	6.99 ± 0.44
ß	hr^{-1}	0.12 ± 0.01
$t_{1/2}$(ß)	hr	5.87 ± 0.47
AUC	μg.ml^{-1}.L	55.09 ± 2.45
$V_{d(area)}$	L.kg^{-1}	0.51 ± 0.02
Cl_B	ml.kg^{-1}.hr^{-1}	86.70 ± 4.40
t_d	hr	4.25 ± 0.34
F	per cent	69
C_{max}	μg.ml^{-1}	5.28 ± 0.11
t_{max}	min	62.00±25.71

[a] Pharmacokinetic determinants are as described by Gibaldi and Perrier (1982). A and B , zero-time plasma drug concentration intercepts of the regression line of absorption and elimination phases, respectively; Ka and ß are absorption and elimination rate constants, respectively; $t_{1/2}$(Ka) , absorption half-life; $t_{1/2}$(ß), elimination half-life; AUC ,area under plasma concentration-time curve; $V_{d(area)}$, apparent volume of distribution; Cl_B , total body clearance; t_d , duration of therapeutic plasma concentration; F , bioavailability; C_{max} , maximum concentration of drug; t_{max} , time of peak plasma concentration of drug.

Present address : [1]Assistant Professor, Department of Gynaecology and Obstetrics, College of Veterinary Science, HPKVV, Palampur 176012.

[2]Professor of Gynaecology and Obstetrics,[3]Professor and Head, Department of Pharmacology and Toxicology, College of Veterinary Science.

commercially obtained as neomycin sulphate (unimycin) and infused into the uterus of buffaloes during estrus at the dose of 5 mg.kg^{-1} body weight.

Blood sampling and neomycin assay

Blood samples were withdrawn from the cannulated jugular vein before and at 1, 2.5, 5, 7.5, 10, 15, 30, 60 min and 2, 3, 4, 6, 8 and 12 hr after administration of drug in heparinized glass tubes. Plasma was separated at room temperature after centrifugation at 3 000 rpm for 15 min and stored at –20°C till analysis, usually within 8-10 days of collection.

At the time of assay the plasma was suitably diluted with 0.1M phosphate buffer (pH 7.9). Neomycin levels in plasma were determined by employing the standard cylinder-plate bioassay technique using *Staphylococcus aureus* (ATCC 6538 P) as test organism (Arret *et al.* 1971, Rampal *et al.* 1992). A standard curve was prepared with different concentrations of neomycin to check the accuracy of the method. This method permitted the detection of 1.6 μg.ml^{-1} of neomycin in plasma.

Pharmacokinetic calculations

Various pharmacokinetic parameters were determined according to the method of computed least square technique (Gibaldi and Perrier 1982). Based on these data a suitable dosage regimen of neomycin in buffaloes was calculated (Baggot 1977).

RESULTS AND DISCUSSION

The plasma concentration of neomycin following its single intrauterine administration are shown in Fig. 1. Appreciable amount of neomycin (3.68 ± 1.01 μg.ml^{-1}) was detected at 5 min. This was followed by a gradual increase and the peak plasma level of 5.28 ± 0.11μg.ml^{-1} was recorded at 120 min. At 720 min the mean neomycin level was 3.47 ± 0.32 μg.ml^{-1}. The peak plasma level of neomycin in buffalo species, as established in present study was quite high than that reported in mares. In healthy mares after its intrauterine infusion during estrus the peak plasma level was 1.68 μg.ml^{-1} at 240 min (Boyd and Allen 1988).

Table 2.Calculated dosage regimens of neomycin in healthy and normal cycling buffaloes following intrauterine administration to maintain specific plasma concentrations

Desired plasma concentration (μg/ml)	Dosage interval (hr)	Initial dose (mg/kg)	Maintenance dose (mg/kg)
2	8	2.8	1.7
	12	4.6	3.5
3	8	4.2	2.6
	12	6.8	5.3
4	8	5.6	3.5
	12	9.1	7.0
5	8	7.0	4.4
	12	11.4	8.8
6	8	8.4	5.2
	12	13.6	10.6

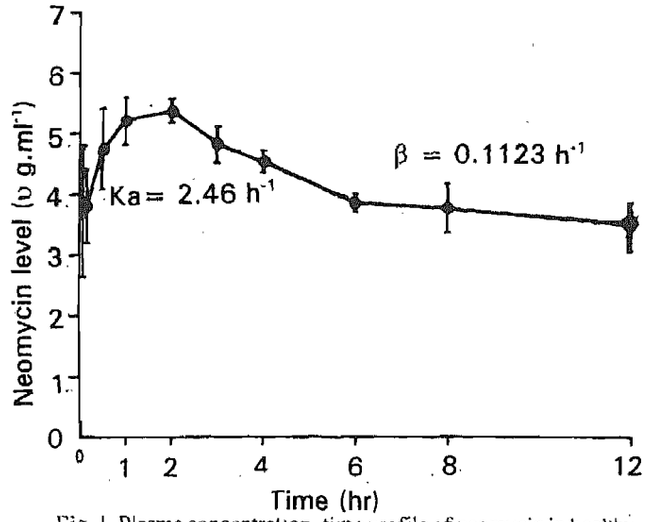


Fig. 1. Plasma concentration- time profile of neomycin in healthy buffaloes following a single intrauterine dose of 5 mg/kg body weight. Values are presented as mean ± SE of 5 animals. Data were analyzed according to a one-compartment open model.

The evaluation of the results on observed plasma levels of neomycin revealed a monophasic decline. The data were best fitted to one-compartment open model and were described by the equation

$$Cp = Be^{-\beta t} - Ae^{-Kat}$$

where Cp is the neomycin concentration at time t, A and B are the zero time intercepts of absorption and elimination phases respectively, Ka and ß are the absorption and elimination rate constants, respectively, and e represent the base of natural logarithm.

Table I presents the kinetic parameters of neomycin calculated on the basis of plasma concentration. High value of absorption rate constant (2.46 ± 0.85/hr) revealed that the drug was rapidly absorbed into systemic circulation after intrauterine administration. The absorption half-life of neomycin in calves of varying age groups was 11.8 - 24.9 min (Black and Claxton 1982) as compared to 30.6 ± 11.4 min in buffaloes. The value of elimination half-life ($t_{1/2\beta}$) was 5.87 ± 0.47 hr which was considerably longer than that reported in calves (Burrows *et al.* 1987) and horses (Baggot *et al.* 1985). The elimination half- lives of neomycin in calves and horses were reported to be 1.5 - 2.7 hr and 2.1 hr respectively.

High values of $V_{d\ (area)}$ (0.51 ± 0.01 L.kg^{-1}) in present investigation indicated that neomycin has extensive penetration into various body fluids and tissues of buffaloes after intrauterine administration. Relatively low values of $V_{d(area)}$ have been reported in calves (Black *et al.* 1983) and equines (Baggot *et al.* 1985). The values of $V_{d\ (area)}$ in calves and

equines were 0.39 and 0.23 $L.kg^{-1}$ respectively. The bioavailability (F) of neomycin which refers to the percentage of drug entering into systemic circulation after intrauterine administration was also calculated. For bioavailability, neomycin was administered in the same animals after a gap of 15 days by intravenous route, at the same dose rate and various kinetic parameters were obtained. The bioavailability of neomycin was good in buffalo species as it was 69 %. The bioavailability of neomycin in mares was reported to be 5.6% (Boyd and Allen 1988), whereas it was 75-85 % in sheep after intramuscular, subcutaneous and intratracheal administration (Errecalde *et al.* 1990).

The prime aim of kinetic disposition study of a drug is to determine a satisfactory dosage regimen. It may be computed by employing the kinetic data established in the present investigation. The maintenance dose (D') is based on minimum therapeutic concentration of neomycin and is obtained by the following equation.

$$D' = Cp(min).V_{d(area)}.(e^{\beta t}-1)$$

Priming dose (D) is calculated by omitting -1 from the above equation.

$$D = Cp(min).V_{d(area)}.e^{\beta t}$$

The primary and maintenance doses of neomycin at different dosage intervals to maintain various therapeutic plasma concentration were calculated and presented in Table 2. For neomycin a concentration of 4 $\mu g.ml^{-1}$ (2-6 $\mu g.ml^{-1}$) is considered to be adequate as minimum therapeutic concentration (Weinstein 1975). Taking 12 hr as suitable dosage interval with an adequate minimum therapeutic concentration of 4 $\mu g.ml^{-1}$, the intrauterine dosage regimen of neomycin in buffalo under field conditions would be 9 $mg.kg^{-1}$ followed by 7 $mg.kg^{-1}$.

REFERENCES

Arret B, Johnson D P and Krishbaum A. 1971. Outline of details for microbiological assay of antibiotics : Second Revision. *Journal of Pharmaceutical Sciences* **60** : 1689-94.

Baggot J D. 1977. Principles of drug disposition in domestic animals. *The Basis of Veterinary Clinical Pharmacology*. pp. 33-189. W.B. Saunders Publishing Co., Philadelphia.

Baggot J D, Love D N, Rose R J and Raus R. 1985. Selection of an aminoglycoside antibiotic for administration to horses. *Equine Veterinary Journal* **19** : 195-97.

Beggard A F J M, Vanden-Hafen M J and Kriele C P M A. 1992. Rationale for treatment of retained placenta in cows with neomycin metronidazole. *Veterinary Record* **130** : 349-50.

Black W D and Claxton M J. 1982. Distribution of neomycin in bull calves after intramuscular administration. *Canadian Veterinary Journal* **23** : 276-78.

Black W D, Holt J D and Gentry R D. 1983. Pharmacokinetic study of neomycin in calves following intravenous and intramuscular administration. *Journal of Comparative Medicine* **47** : 433-35.

Boyd E H and Allen W A. 1988. Absorption of neomycin from equine uterus. Effect of bacterial and chemical endometritis. *Veterinary Record* **122** : 37-39.

Burrows G E, Barto P B and Martin B. 1987. Comparative pharmacokinetics of gentamicin, neomycin and oxytetracycline in new born calves. *Journal of Veterinary Pharmacology and Therapeutics* **10** : 54-63.

Dobson D P and Noakes D E. 1990. Use of uterine pessary to prevent infection of uterus of the cow after parturition. *Veterinary Record* **127** : 128-31.

Errecalde J O, Lanvsse C E and Londoni M F. 1990. Pharmacokinetics of neomycin in sheep after intravenous, intramuscular, subcut and intratracheal administration. *Journal of Veterinary Medicine* **37** : 163-69.

Gibaldi M and Perrier D. 1982. *Pharmacokinetics*. pp. 443-44. Marcel Dekker Incorporated, New York.

Murray R D, Allison R P and Gard R P. 1990. Bovine endometritis: Comparative efficacy of alfaprostol and intrauterine therapies and other factors influencing clinical success. *Veterinary Record* **127** : 86-90.

Paufler S. 1969. Intrauterine application of Bykocillin-M (neomycin plus penicillin) suspension at the time of insemination. *Tierarzt Umschung* **24** : 588-91.

Rampal S, Srivastava A K and Chaudhary R K. 1992. Disposition kinetics, urinary excretion and dosage regimen of kanamycin in buffalo calves following single intravenous administration. *Veterinary Research Communications* **15** : 219-25.

Weinstein L. 1975. Antimicrobial agents. *Goodman and Gilman's The Pharmacological Basis of Therapeutics*. pp. 1167-82. (Eds) Gilman A G, Goodman L S, Rall T W and Murad F. Macmillan Publishing Company Incorporated, New York.

Indian Journal of Animal Sciences 68 (4) : 320-323, April 1998

Clinicopathological profile of induced chronic arsenic toxicity in goats

U BISWAS[1], S SARKAR[2], M K BHOWMIK[3] and S ROY[4]

Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal 741 252

Received : 2 April 1997 ; Accepted : 29 October 1997

ABSTRACT

Chronic arsenic toxicity was induced in goats by oral administration of sodium arsenite @ 25.0 mg/kg b.wt. daily for 12 weeks. Clinically ,there were development of gastrointestinal and nephritic signs with 100% mortality. Intoxicated goats had leucopaenia, anaemia and increased erythrocyte fragility rate. Rumen pH became acidic with complete absence of its protozoan activity. An increased BSP clearance time was observed in the intoxicated goats. Urinanalysis revealed low specific gravity and pH alongwith marked presence of protein, sugar, cast, pus cells and arsenic (>70% mg%). Significantly decreased concentrations of urea nitrogen and creatinine with increase of alkaline phosphates were observed in urine. The elimination of arsenic mainly occurred through the faeces and skin.

Key words : Arsenic, Clinicopathology, Goats, Toxicity

Arsenic (As) in ground water in 8 districts of West Bengal, is affecting the quality of 4.4 million human life (Anonymous 1996). Being close to some ecological system, the farm animals are also likely the victims of the catastrophes arising from the As pollution. However, information are limited on the biological effects of an in farm animals, excepting a report on accidental arsenical poisoning in cattle (Robertson *et al.* 1984). This study reports a clinico-pathological features in induced As toxicity in goats.

MATERIALS AND METHODS

Healthy female Black Bengal goats (12) approximately 1-year-old, and weighing 11.0 kg, were divided into 2 equal groups, viz. 1 and 2. The animals were acclimatized to the laboratory conditions for 15 days. They were fed on 0.5 kg goat ration; 1 kg hybrid Napier grass, supplemented with feed additives twice a day, and were given deionized water *ad lib.* in stainless steel troughs. Animals were housed in the groups in indoor concrete floored pens of laboratory animal sheds. Reagent grade powdered sodium arsenite was weighed and acute lethal dose 50 (LD 50) was determined in healthy goats (Biswas 1993). About 1/5th of acute LD 50 (i.e. 25.0 mg/kg b.wt.) was dissolved in 300 ml deionized water and fed in a feeding bottle to each goat of group 2 daily for 12 weeks or until death. The deionized water as the sole source of drinking water was available *ad lib.* The remaining 6 goats received the deionized water containing sucrose, serving as control (group 1) . All goats were examined atleast twice a day, and progressive development of clinical signs was recorded.

Blood, ruminal fluid, urine and faecal samples were collected on 0 day, and thereafter at 3 week intervals. EDTA admixed blood samples were used for haemoglobin (Hb), packed-cell volume (PCV), total erythrocyte and leukocyte counts (TEC, TLC) and the erythrocyte fragility test (EFT) (Dacie and Lewis 1975). Serum separated from a part of blood samples was used for bromosulfapthalein clearance test (BSP) to determine functional standard activity of liver (Kaneko 1987). Ruminal fluid samples were utilized to determine pH, protozoa count and cellulose digestion test (Slyter 1976). The routine examination of urine for specific gravity, pH, protein, sugar and caste (Banjamin 1985) and biochemical analysis for urea nitrogen (UN), creatine (Cr) and alkaline phosphatase (ALP) (Frankel *et al.* 1970) were also performed.

Blood, urine, faeces, ruminal fluid and hair samples were collected at 3 weekly intervals from the animals of 2 groups. A known volume of all the samples was digested in triple acid mixture (HNO_3 - 10 parts, $HClO_4$ - 3 parts and H_2SO_4 - 1 part) and dilution to a known concentration was finally made with deionized water (Sandle 1950). Arsenic contents in the samples were determined by molybdenum blue method (Stewart 1989).

The differences between the groups in the mean values of all parameters were analysed using student's t test (Snedecor and Cochran 1968).

Present address : [1]Veterinary Surgeon, Additional Block Animal Health Centre, Joyrambati, Bankura, West Bengal.

[2]Senior Lecturer, Department of Veterinary Medicine and Public Health; [3]Professor, Department of Veterinary Pathology ; [4]Reader, Department of Animal Products Technology and Marketing , West Bengal University of Animal and Fishery Sciences, Calcutta 700 037.

Table 1. Clinico-haematological and urinary biochemical profiles in control and As-induced goats (n = 6; mean ±SE)

Parameters	Groups	Weeks of observation				
		0(6)	3(6)	6(6)	9(6)	12(6)
Hb (g/dl)	C	10.32±0.35	10.40±0.05	1.20±0.06	10.24±0.07	10.20±0.09
	I	10.40±0.08	9.60±.10	6.90±0.018**	6.40±0.20**	5.70±0.11
TEC (×10⁶ µl)	C	12.57±0.08	12.38±0.11	11.56±0.14	11.34±0.26	11.20±0.22
	I	12.60±0.11	11.60±0.08	6.68±0.11	6.44±0.21	5.88±0.12
PCV (%)	C	3.12±0.48	31.46±0.32	31.22±0.33	31.02±0.20	30.90±0.26
	I	33.00±0.17	29.76±0.24	23.18±0.12**	19.57±0.28**	17.30±0.14**
EFT. 100% ;USOS	C	0.37±0.01	0.39±0.01	0.38±0.01	0.39±0.02	0.37±0.02
(% of NaCl)	I	0.37±0.02	0.54±0.02	0.67±0.01**	0.68±0.01**	0.70±0.01**
BSP (t½ life, min)	C	3.10±0.01	3.11±0.01	3.10±0.02	3.10±0.01	3.09±0.01
	I	3.11±0.02	3.25±0.02	3.69±0.01	4.77±0.02**	5.19±0.08**
Ruminal fluid pH	C	7.18±0.01	7.20±0.04	7.18±0.02	7.20±0.02	7.28±0.04
	I	7.16±0.01	6.88±0.02	6.28±0.04**	6.21±0.03**	6.10±0.02**
Protozoa count	C	9.04±0.12	8.90±0.11	8.96±0.08	9.00±0.12	9.10±0.08
(10^5/cm)	I	9.50±0.11	5.13±0.22	-	-	-
CDT (hr)	C	26.80±1.02	26.80±0.87	26.00±0.61	26.40±0.72	27.20±0.49
	I	27.20±0.40	34.0±1.08	-	-	-
Uninary urea N	C	1050.40±27.05	1020.70±19.86	989.50±23.11	1043.66±18.16	1010.71±27.08
(mg/dl)	I	1048.70±23.16	97.40±21.80	598.40±21.88**	389.10±11.70**	270.24±11.28**
Urinary creatinine	C	90.20±0.11	86.00±0.17	90.33±0.21	93.60±0.84	90.00±1.28
(mg/dl)	I	88.75±0.27	88.40±0.27	74.80±1.20**	60.10±0.89**	51.40±1.04
Urinary ALP/KA unit	C	1.60±0.11	1.70±0.31	1.66±0.08	1.68±0.20	1.72±0.12
	I	1.72±0.20	2.20±0.12	10.40±0.16**	12.40±0.75**	14.50±0.11

n = Numbers of animals; ** P < 0.01 ; C, control ; I, intoxicated ; 2 animals died on day 78 and the remaining 4 died on day 83.

RESULTS AND DISCUSSION

Arsenic (As) - intoxicated animals developed mild signs of toxicity from week 3, consisting of dullness and depression, with slightly reddish coloured urine. The As content in blood and hair of these animals were 0.51 and 5.52 mg% at this stage. In 4 to 5 weeks , there were partial loss of appetite, reddish coloured urine, oligouria and weakness. During the following weeks, brown pellet faeces with mucous, yellowish mucous membrane, rough body coat with erected hair, profound muscular weakness were observed. In 9 week and onward, there were polyuria, inability to get up and salivation. A significant (P<0.01) decrease in body weight gain (9.80±0.11 and 8.60±0.20 kg) and increase in respiratory rate (33.0±0.23 and 40.0 ± 0.13 / min) and heart rate (99.0±1.23 and 119.0±0.35/ min) were observed in 9 and 12 weeks respectively . Two animals died on day 78 and the rest on day 83 post intoxication. None of the control animals showed any clinical signs of toxicity.

Development of signs of toxicity appears primarily due to massive impairment of kidneys and gastrointestinal functions. Clinical signs of eliguria with reddish urine, followed by polyuria were the possible indications of renal failure in As toxicity in goats. The onset of gastrointestinal signs might be due to entry of As into the GI tract through mucous membrane. Appearance of yellowish mucous membrane clearly suggested jaundice which might be due to liver damage and formation of excessive bilirubin that occurred because of increased EFR. The progressive loss of body condition was possibly because of gradual onset of anorexia due to toxaemia.

Blood picture showed significant (P<0.01) decrease in Hb, PCV and TEC values on week 6 onwards in the As-induced goats than in controls (Table 1), suggesting anaemia which is possibly resulted either from toxic depression of bone marrow activity or reduction of erythropoietin secretion due to renal failure. EFT in intoxicated goats indicated 100% lysis of RBC in 0.70±0.01% NaCl solution, which was higher than the value (0.37±0.12% NaCl solution) in control animals. As-treated goats also had significant (P<0.01) leucopaenia from week 6 onwards indicating the possibility of depression in bone marrow activity and destruction in bone marrow activity and destruction of lymphoid tissues.

The 't' half-life for BSP clearance increased significantly (P<0.01) on week 2 onwards (Table 1), which suggested the damage of liver in the As-induced goats, possibly because of widespread necrosis of hepatic parenchyma and also the work load as a result of excessive bilirubin from increased rate of erythrocytic fragility.

The As-treated animals had a significantly low ruminal pH (P<0.01) on week 6 onward in spite of the alkaline nature of the As salt administrated (Table 1). The low rumen pH was

Table 2. Recovery of As in blood, ruminal fluid, urine, faeces and hair (n=6; mean ±SE)

Parameters	Groups	Weeks of observation				
		0(6)	3(6)	6(6)	9(6)	12(6)
Blood (mg%)	C	0.14±0.01	0.13±0.02	0.016±0.04	0.18±0.04	0.17±0.04
	I	0.12±0.00	0.51±0.04	2.05±0.10**	2.88±0.09**	3.17±0.08**
Ruminal fluid (mg%)	C	0.10±0.00	0.11±0.01	0.11±0.02	0.13±0.01	0.16±0.10
	I	0.09±0.00	0.53±0.01	2.33±0.06**	2.82±0.11**	3.87±0.04**
Urine (mg%)	C	0.08±0.00	0.09±0.02	0.10±0.000	0.07±0.01	0.011±0.02
	I	0.10±0.00	0.35±0.03	0.70±0.01**	0.58±0.04**	0.36±0.04**
Faeces (mg%)	C	0.12±0.02	0.14±0.01	0.30±0.18	0.40±0.10	0.71±0.11
	I	0.13±0.00	2.58±0.11	3.78±0.16**	4.88±0.71**	5.88±0.13**
Hair (mg %)	C	0.18±0.04	0.77±0.12	0.98±0.30	0.74±0.10	0.72±0.14
	I	0.22±0.02	5.52±1.10	7.88±1.00**	12.64±0.18**	18.19±0.17**

n , Number of animals ; ** P<0.01 ; C, control ; I, intoxicated.

perhaps attributed to reflex of chloride ion secreted as a result of delayed passage of clearance from the dilated abomasum as evident from gross pathological findings (Biswas 1993). The protozoan activity was also completely absent and the count was it from 6 week , suggesting the toxic effect of As salt on the ruminal flora. The complete loss of ability to cellulose digestion was possibly due to acidic nature of ruminal pH and the complete absence of protozoa in the fluid.

Results on urin analysis revealed marked decrease of pH (6.80±0.04 and 6.50±0.04), specific gravity (1.020 and 1.015) and increase in protein (2 to 3 + and 4+) and sugar (3 to 4+ and 5+), presence of granular / epithelia cast (4+ and 5+) and pus cells (5+ and 10 to 12+) in weeks 9 and 12 of As-induced animals. These changes clearly indicated extensive damage to kidney. Kumar and Pandey (1994) also reported similar observations in $HgCl_2$ induced goats. The lowered urinary pH is attributed to metabolic acidosis resulting from uraemia. A marked decrease in specific gravity of As-induced goats suggested lack of ability of the renal tubules to concentrate urine. Nephrosis caused by heavy metals usually results into proteinuria due to increased permeability of glomerular filtration (Parai and Pandey 1992). Renal glycosuria indicated a progressive distribution of nephrons characterized by tubular injury leading to impairment of tubular reabsorption. A significant presence of granular / epithelial casts and pus cells suggested the presence of massive tubular damage (Parai and Pandey 1992). A significant decrease (P<0.01) of Un and Cr of the As-intoxicated goats on week 6 onwards was anticipated in view of increase in their concerned blood values (Biswas 1993). ALP activity was 6.3, 7.2 and 8.4 times higher in As-induced goats on weeks 6 , 9 and 12, respectively, which could be attributed to damaged renal tubules releasing excess of enzyme in the urine. This finding is in agreement with earlier reports of $HgCl_2$ - induced renal dysfunction in sheep (Robinson and Hesketh 1976) and goats (Kumar and Pandey 1994).

The presence of higher concentration of As in the hair and faecal samples of treated animals clearly indicated that the element is mainly excreted through faeces and skin (Table 2). On the other hand ,the accumulation of As moderately in blood and ruminal fluid suggested its higher retention which might be responsible to induce cytotoxic effects in various organs as evident from the histopathological findings described elsewhere (Biswas 1993).

Results clearly suggested that inorganic arsenic is a nephrotoxic heavy metal. Further studies are warranted on tissue residues and cytomorphological changes to establish its cytotoxic effects in the animals since this metal is rapidly becoming an unavoidable environmental pollutant.

ACKNOWLEDGEMENT

We thank the Vice-Chancellor , Bidhan Chandra Krishi Vishwavidyalaya, for facilities provided for the study.

REFERENCES

Anonymous. 1996. *Investigation Report.* Department of Public Health Engineering ; Government of West Bengal.

Benjamin M M. 1985. *Outline of Veterinary Clinical Pathology.* Kalyani Publisher , New Delhi.

Biswas U. 1993. 'Studies on metabolism, toxicosis effect, immunoglobulin status and therapy of experimentally induced arsenic toxicity in goats.' M.V. Sc Thesis. Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, West Bengal.

Decie J V and Lewis S M. 1975. *Practical Haematology.* 5th edn. Churchill, Livingstone.

Frankel S Reitman S and Sonnenwrith A C. 1970. *Gradwohl's Clinical Laboratory Methods and Diagnosis.* 7th edn. The C.V. Mesby Company Saint Louis.

Keneke J J. 1989. *Clinical Biochemistry of Domestic Animals.* 4th edn. Academic Press. Inc., New York.

Kumar R and Pandey N N. 1994. Blood biochemical and urinary changes in mercuric chloride induced chronic nephrosis in goats. *Indian Journal of Animal Sciences* **64** : 239-43.

Parai T P and Pandey N N. 1992. Clinico-biochemical changes in

mercuric chloride induced nephrotoxicity in cattle. *Indian Journal of Animal Sciences* **62** : 924-27.

Pathak S K and Bhowmik M K. 1996. Chronic mercurial toxicity in goats : Haematological , biochemical and urinary changes . *Indian Journal of Veterinary Pathology* **20** : 638-41.

Robertson I D, Harms W E and Ketterer P J. 1984. Accidental arsenical toxicity of cattle. *Australian Veterinary Journal* **61** : 366-67.

Robinson M and Hisketh A. 1976. Effect of mercuric chloride on the structure and function of the kidney of sheep. *Journal of Comparative Pathology* **86** : 307-18.

Sandle E B. 1950. *Colorimetric Determination of Traces of Metals*. 2nd edn. Inter science Publishers, Inc. New York.

Slyter L L. 1976. Assessment of the activity of ruminal microflora. *Journal of Animal Science* **43** : 910-19.

Snedecor G W and Cochran W G. 1968. *Statistical Methods*. 7th edn. Iowa State University Press, Ames, Iowa.

Stewart E A. 1989. *Pollutants, Chemicals Analysis of Ecological Materials* .2nd edn, pp. 202-05. Blackwell Scientific Publications, Oxford , London and Melbourne.

Indian Journal of Animal Sciences 68 (4) : 324-327, April 1998

Congenital anomalies in swine male genitalia

R SINGH[1] and N S PARIHAR[2]

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122

Received : 14 June 1997; Accepted : 28 October 1997

ABSTRACT

Pathomorphological studies for finding out the occurrence of congenital lesions conducted on 418 males (82 entire and 336 castrated) revealed the occurrence of testicular hypoplasia in 12.19% (10/82), cystic epididymal appendix in 10.9% (9/82), segmental aplasia of epididymis in 1.22% (1/82), cryptorchidism in 13.41% (11/82), persistent penile frenulum in 3.11% (13/418) and deviation of penis in 0.48% (2/418) animals.

Key words : Anomalies, Genital system, Pigs

To understand the nature of various pathological conditions occurring in the male reproductive tracts affecting reproductive efficiency of the pigs, systematic studies were carried out to record and characterize various pathomorphological affections in 418 male reproductive tracts in animals at slaughter or during routine necropsy examination. The congenital anomalies, some hitherto undescribed, were encountered and have been described in this paper.

MATERIALS AND METHODS

For the present study the reproductive tracts from 336 castrated and 82 entire (210 and 18 *desi*, 2 and 14 Landrace, and 124 and 50 crosses) male pigs were collected, whether or not showing lesions, Bareilly, Aligarh Central Dairy Farm and the Division of Pathology, Indian Veterinary Research Institute, Izatnagar. The animals varied in age between 3 and 18 months. After complete evisceration of the carcasses, the entire genitalia were removed maintaining their anatomical continuity and carried to the laboratory under ice in polythene bags as quickly as possible. After clearing the fascia and fat, the genitalia were examined systematically from testicles to penis including prepuce and preputial diverticulum.

The representative pieces of tissues from constituent organs of genitalia, irrespective of the presence of lesions, were cut and preserved in ten times of 10% buffered neutral formalin. After proper fixation, the tissues were incised in thin pieces and washed overnight under running tap water. The tissues were then passed through ascending grades of ethyl alcohol, cleared in turpentine oil and embedded in paraffin wax (melting point 60°C). Four microns thick sections were obtained from these paraffin blocks. The sections were stained routinely with haematoxylin and eosin, duplicate sections, if necessary, were stained with special techniques after Culling (1974).

Present address: [1] Scientist (Senior Scale), [2] Principal Scientist, Centre for Animal Disease Research and Diagnosis.

RESULTS

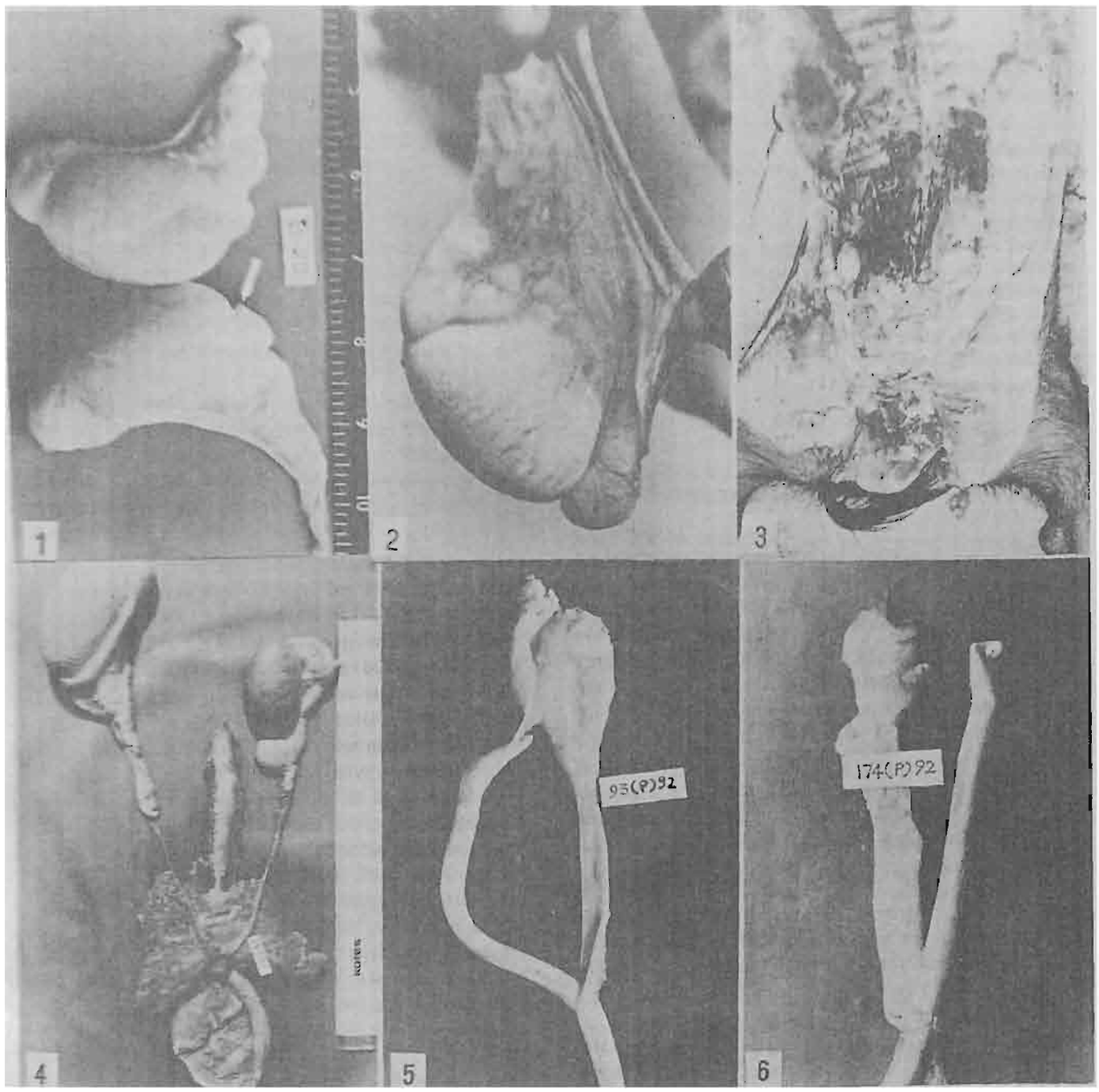
Testicular hypoplasia 12.19% (10 cases), cystic epididymal appendix 10.97% (9 cases), segmental aplasia of epididymis 1.22% (1 case), cryptorchidism 13.41% (11 cases), persistent penile frenulum 3.11% (13 cases) and deviation of the penis 0.48% (2 cases) were the congenital anomalies observed (Table 1).

Testicular hypoplasia

Intrascrotal testicles in 10 adult pigs were found to be abnormally small on one side in 6 cases (4 left, 2 right) and on both sides in 4 cases and each measured about 6 cm × 4 cm × 3 cm and weighed about 46 g. The epididymis in these cases were also smaller-each measuring about 10 cm in length, 1 cm in thickness and 4 g in weight. Microscopically some of the seminiferous tubules revealed thin tubular walls lined with variably thinner strata of germinal cells in which the sertoli cells revealed cytoplasmic vacuolations. Stem cells were comparatively less numerous among them. There were fewer spermatids, some degenerated, and sparse numbers of sperms in the lumina. At times the spermatids had coalesced into multinucleated structures. In severe hypoplasia the seminiferous tubules exhibited dominant presence of supporting (sertoli) cells with a few stem cells without showing mitotic activity. Generally the Leydig cell populations between the seminiferous tubules were larger.

Cystic epididymal appendix

Translucent cystic structures measuring 0.5 to 1.8 cm were encountered on one side in 8 cases (7 right side, 1 left side)



Figs 1-6. **1.** Cystic epididymal appendix at the junction of the epididymis and the testis. **2.** Segmental aplasia of the body of the left epididymis in an adult Landrace boar. **3.** Bilateral cryptorchidism. Both testes in abdomen. Epididymes loosely attached and located little away from testes proper **4.** Perceptibly small left cryptorchid testis. Epididymis loosely attached. Contralateral testis and epididymis normal. **5.** Thin frenulum joining prepuce with free end of the penis. **6.** Clock-wise twisting of glans penis in a cryptorchid pig.

and on both sides in 1 case. These structures were located facing the testis, near or at the junction of epididymal head or between epididymis and testis in 2, 5 and 2 cases respectively (Fig. 1). The epididymis, otherwise, were normal.

Segmental aplasia of left epididymis

An adult Landrace boar revealed absence of part of the left epididymis in its mid-region. The body of the left epididymis was thus missing (Fig. 2). The head and the tail, though present, were found underdeveloped i.e. smaller in size measuring 1.5 cm and 1.2 cm in lengths and weighing 10 and 8 g respectively. In the contralateral epididymis the head was about 3 cm long and 23 g in weight and the tail 2.2 cm long and 21 g in weight. The testes on both sides were

Table 1. Congenital anomalies in female pig genitalia

Lesion	Entire pigs (82)									Castrated pigs (336)			Freuency occurance (%)
	Desi (18)			Landrace (14)			Crossbred(50)			(210)	(02)	(124)	
	R	L	B	R	L	B	R	L	B	D	L	DXL	
Testicular hypoplasia	2	1	1	-	-	-	-	2	3	-	-	-	*9(10.97)
Cystic epididymal appendix	3	1	-	-	-	-	2	-	1	-	-	-	*7 (8.53)
Segmental aplasia of epididymis	-	-	-	-	1	-	-	-	-	-	-	-	*1 (1.22)
Cryptorchidism	1	1	1	-	1	-	2	1	1	-	-	-	*8 (9.75)
Persistent penile frenulum	-	-	-	-	-	-	-	-	-	2	-	10	12 (3.57)
Deviation of the penis	-	-	-	-	-	-	-	-	-	1	-	-	1 (0.29)
Testicular hypoplasia + cystic epidydymal appendix	-	-	-	-	-	-	1	-	-	-	-	-	* 1 (1.22)
Cyrptorchidism + cystic epididymal appendix	-	-	-	-	-	-	1	-	-	-	-	-	1 (1.22)
Cyrptorchidism + deviation of the penis	-	1	-	-	-	-	-	-	-	-	-	-	1 (1.22)
Cryptorchidism + persistent penile frenulum	-	-	-	-	-	-	-	1	-	-	-	-	1 (1.22)

Figure in parenthesis indicate the number of animals, *Out of 82 entire pig genitalia , D = desi, L = landrace. DXL = crossbred, R=right, L=left; B=bilateral

comparable and of normal size and shape and measured 5.5 cm × 4.0 cm × 3.2 cm and 62 g each. The seminiferous tubules were found affected in the left testicle with reduced numbers of germinal cells which were generally deficient in stages beyond the spermatocytes and were undergoing degeneration. The connective tissue septa revealed engorged blood vessels and sparse infiltration with mononuclear cells. The lumina of the ducts in the head of epididymis of the affected side revealed occurrence of small amounts of cellular debris consisting of degenerated spermatozoa, macrophages and detached epithelial cells. The ducts in tail of the epididymis on this side were completely empty i.e. devoid of contents.

Cryptorchidism

The testicles were observed in non-scrotal locations in 11 pigs including 6 adults (approx. 8 to 18 months), 4 piglets (4 to 5 months) and 1 foetus. The condition in 8 animals (5 adults, 2 piglets and 1 foetus) was unilateral (left side -5, right side -3) and in 3 cases (1 adult, 2 piglets) bilateral. The malpositioned testicles were found located behind the kidneys adhering to the abdominal wall in 10 cases (8 unilateral, 2 bilateral) and at the pelvic inlet in the remaining one (bilateral) case (Fig. 3). The scrotal sacs were quite small and tilting on one side in unilateral cryptorchidism. They were almost unrecognizable in bilateral retention of testes. The retained testicles were small, smooth, comparatively firm in texture and loosely attached with the epididymes (Fig. 4). The retained testes were smaller than normal testes in the adult pigs. The testes (10.87 cm × 4.15 cm × 3.41 cm and 51.12 g) and epididymes (7.25 cm × 0.28 cm and 4.55 g) in cryptorchid adults were almost double in size than testes (5.91 cm × 2.98 cm × 2.47 cm and 28.65 g) and epididymides (9.6 cm × 0.46 cm and 3.12 g) in cryptorchid piglets. The size of the epididymes in cryptorchid adults and piglets were inversely proportional : larger in piglets than in the adults. Microscopically the constituent seminiferous tubules in the affected testes were generally atrophied i.e. smaller in size and less numerous. They were lined mostly or entirely by supporting (sertoli) cells exhibiting vacuolated cytoplasm. The spermatogonial cells were few and far in between i.e. 2-3 cells/tubule. The lumina of a few tubules revealed little eosinophilic debris. The boundaries of all the tubules were prominent. The tunica albuginea were moderately thick and often revealed mild engorgement of blood vessels and mild infiltration with a few mononuclear cells and eosinophils. Similar leukocytic infiltrations were also observed in rete testis. The leydig cells in the connective tissue septa between the tubules were generally prominent, large and polyhedral with foamy eosinophilic cytoplasm. They were more numerous in the adult cryptorchids. The epididymal tubules were found devoid of spermatozoa in all the cases.

Persistent penile frenulum

The cranial portions (glans) of penises in 13 cases (12 castrated, 1 cryptorchid) were observed to have thin bands, 1 in each case, of tissue joining the area behind the urethral orifice with the proximal portion of prepuce on the ventral aspect (Fig. 5).

Deviation of the penis

The free portion of the penis i.e. glans in 2 cases (1 castrated and 1 cryptorchid) was twisted to an extent on its axis in clock-wise direction (Fig. 6).

DISCUSSION

During the examination of the pig genitalia from 82 entire

and 336 castrated adult males, the occurrence of congenital anomalies was rather common. The testicular hypoplasia of mild to severe degree in 10 (12.19%) adult pigs involved usually left testicles which were lighter in weight similar to those observed by Holst (1949) and Kojima *et al.* (1974). Besides inheritance testicular hypoplasia could be due to a number of factors in boars (Ladds 1993) which need elucidation.

The cysts of the appendix epididymis observed in 9 (10.97%) cases on single side have been considered to owe their origin to the remnants of mesonephric tubules (Ladds 1993) similar to parovarian cysts. Thomas and Raja (1974) in their studies found that 22 cases (little higher percentage) had these structures amongst 144 boars subjected to castration.

Segmental aplasia of the body of the epididymis, as encountered in a Landrace boar, has also been attributed by Ladds (1993) to anomalous mesonephric ducts. The condition has been encountered far rarely in the boars than in other domestic animals. Two cases in swine of ductus deferens aplasia were reported by Heath *et al.* (1982) in which tail of epididymis was continued into stumpy ductus deferens. The microscopic picture in the present case compared well with that described in rams by Ladds *et al.* (1990).

McEntee (1990) and Ladds (1993) stated that cryptorchidism is usually seen on one side and the same was the case in the present studies. Ten out of 11 (13.41%) cases were found unilaterally amongst 82 males studied which is on higher side than reported (0.24% to 0.41%) by Remes (1969), Schulman (1963), and Nador (1990) probably due to large number of cases studied by them. The descent of testes to the levels of kidneys in 10 cases and to the pelvic inlet in 1 case could have been due to gubernacular abnormalities as stated by Wensing (1968) and Levy and Husmann (1995). Microscopically occurrence of vacuolating supporting cells with few stem cells supported the contention of arrested spermatogenesis at abdominal temperature (Frankenhuis and Wensing 1979). The increase in Leydig cell population and its morphology as observed in these cases have been suggested by Wu and Munoro (1996) to be due to stimulation by sertoli cell-secreted mitogenic factor at increased temperature and same might hold true in the present study.

Persistent penile frenula were encountered in 13 cases. McEntee (1990) and Ladds (1993) stated that the conditions occurred in pigs subjected to early castration when separation of small penis from the prepuce got impaired due to deficiency of adequate androgen hormonal stimulation. Aamdal and Nes (1958) found the condition attributable to inheritance in Norwegian Landrace boars. The occurrence of cases of persistent penile frenulum indicated the need for further studies in the absence of any explanation available in the present cases.

The clockwise twisting of free portion of glans penis in 1 castrated and 1 cryptorchid pig could have been due to insufficiency of the dorsal ligament of the penis as observed by Walker (1971) in bulls.

ACKNOWLEDGEMENTS

We thank the Head, Division of Pathology, and Director of the Institute for the facilities provided

REFERENCES

Aamdal J and Nes N. 1958. Mating difficulties in boars caused by persistent frenulum of prepuce. *Nord Veterinaermedizin* **10** : 444-46.

Frankenhuis M T and Wensing C J W. 1979. Induction of spermatogenesis in the naturally cryptorchid pig. *Fertility and Sterility* **31** : 428-433.

Heath E, Kuba B and McEntee K. 1982. Segmental aplasia of the ductus deferens in the boars. *Veterinary Pathology* **19** : 202-204.

Holst S L. 1949. Sterility in boars. *Nord Veterinaermedizin* **1** : 87-120 (*fide* McEntee 1990.)

Kojima Y, Konoshita Y and Fukuda M. 1974. Multiple headed spermatozoa in the boar testes. *Japanese Journal of Veterinary Science* **36** : 363-74.

Ladds P W. 1993. The Male Genital System. In : *Pathology of Domestic Animals*, Vol. 3, 4th edn, pp. 471-528. Jubb K V F, Kennedy P C and Palmer N, (ed), Academic Press Inc., London.

Levy J B and Husmann D A. 1995. The hormonal control of testicular descent. *Journal of Andrology* **16** : 459-68.

McEntee K. 1990. *Reproductive Pathology of Domestic Mammals*. Academic Press, San Diego, California.

Nador A. 1990. Meat inspectional problems of the breeding boar production II. Cryptorchid boars. *Magyar Allatorvosok Lapja*, **45** : 274-276 (*fide Veterinary Bulletin* **60** : 8622).

Remes E. 1969. Statistical analysis of the most common anomalies in pigs. *Finsk Veterinartidskrift* **75** : 502-505 (*fide Veterinary Bulletin* **40** : 2478.)

Schulman A. 1963. Cryptorchidism in pigs in Finland. *Finsk Veterinartidskrift* **69** : 349-58. (*fide Veterinary Bulletin* **34** : 391.)

Thomas U P and Raja C K S V. 1974. Spermatic granuloma in boars. *Kerala Journal of Veterinary Science* **5** : 113-20.

Walker D F. 1971. Preputial disorders and deviation of the penis in the bull. *Proceedings of International Conference on Cattle Diseases*. 6th edn. Philadelphia (*fide* McEntee 1990.)

Wensing C J G. 1968. Testicular descent in some domestic animals. 1. Anatomical aspects of testicular descent. *Proceeding K Nederlandse akademie uan Wetenschappen Series C* 71 : 423-34 (*fide* McEntee 1990.)

Wu N and Munoro E P. 1996. Temperature and germ cell regulation of Leydig cell proliferation stimulated by Sertoli cell-secreted mitogenic factor : a possible role in cryptorchidism (Review). *Andrologis* **28** : 247-57.

Indian Journal of Animal Sciences 68 (4) : 328-329, April 1998

Accuracy of ultrasonic scanning for pregnancy diagnosis in ewes of five different breeds

R S MALIK[1], M A RAZZAQUE[2] and N M AL-KHOZAM[3]

Kuwait Institute for Scientific Research, Aridland Agriculture Department, Food Resources Division, Safat 13109 Kuwait

Received : 6 May 1997 ; Accepted : 28 October 1997

ABSTRACT

Linear-array real-time ultrasound scanning was used for the diagnosis of pregnancy in 167 ewes of 5 diverse breeds including fat-tailed Naeemi (N), thin-tailed Texel (T) and Border Leicester Merino (BLM), and semi-fat-tailed Chios (C) and N ' BLM. Pregnancy scanning was carried out at 45-60 days from the beginning of mating. The ewes detected as barren were rescanned after an interval of 45 days. The ewes diagnosed as pregnant at first scanning, however, were not rescanned. Accuracy of identification of pregnant ewes ranged from 66.7 to 95.5%. It was the highest for BLM and the lowest for N ewes. Early embryonic losses and undetected abortions may have reduced the accuracy of pregnancy diagnosis. For greater accuracy, rescanning of all the ewes at 85-100 days is recommended.

Key words : Ewes, Pregnancy diagnosis, Ultrasonic scanning

Diagnosis of pregnant ewes after breeding is an important management practice. The pregnant ewes can then receive more attention and better feed as they approach lambing. Nonpregnant ewes can be culled, slaughtered or kept on maintenance feed until the next breeding. Examination of udders and vulva of ewes or recto-abdominal palpation (Hulet and Shupe 1973) have been used routinely for pregnancy diagnosis with variable results. Attempts have been made over the years to diagnose pregnancy by testing blood progesterone levels. This method is expensive and time consuming, and lacks practical application (Russel and Foot 1971). Ultrasound scanning pioneered in Australia (Fowler and Wilkins 1980) has recently been used with a high degree of accuracy (White *et al.* 1984, Taverne *et al.* 1985, Logue *et al.* 1987). This paper presents the results of real-time ultrasound scanning in the diagnosis of pregnancy in ewes of 5 different breeds.

MATERIALS AND METHODS

Experimental animals

The sheep used for breeding and pregnancy diagnosis were Naeemi (N), Chios (C), Texel (T), Border Leicester Merino (BLM) and N ' BLM. All ewes were over 18 months of age and belonged to an experimental flock of the Kuwait Institute for Scientific Research. The animals were housed permanently in semi-closed sheds, ear tagged and joined with rams in October 1995. Their oestrous cycles were synchronized using progesterone- impregnated sponges, and ovulation was stimulated by intramuscular injection of 500 IU of pregnant mare serum gonadotrophin (PMSG). The rams were allowed to run with the ewes for 35 days. The feeding of ewes was withheld for 16 hr before ultrasonic scanning. The pregnancy diagnosis was carried out 45-60 days after the beginning of mating. Ewes diagnosed as pregnant were not rescanned, but those diagnosed as not pregnant were rescanned after an interval of 45 days. The exact stage of pregnancy at the scanning was determined from the lambing records. There was no culling of ewes before the end of the lambing season.

Equipment and scanning procedure

Ewes of the 5 breeds totaling 167 animals were scanned using a linear array ultrasound scanner with a dual pregnancy probe of 5.0/7.5 MHz. The probe covered depths and widths of 6-12 cm and 8-10 cm, respectively, from the skin surface.

The ewes were scanned in a standing position. Before scanning, a wool less region of the skin ventral and medial to the right precrural skin fold around the udder was cleaned with water. At the scanning, coupling gel was applied on the flat face of the transducer (probe) which was then gently pressed against the skin. The probe was turned along its longitudinal axis to scan the whole uterus. Diagnosis of pregnancy was made within a few seconds by viewing the images on the screen. A positive diagnosis (pregnant ewe) was made on the basis of a fluid-filled uterus, cotyledons or foetal structures such as head, thorax, neck, ribs, limbs etc., depending upon the stage of pregnancy. About 1 min of

Present address: [1,2,3]Kuwait Institute for Scientific Research, Aridland Agriculture Department, Food Resources Division.

Table 1. Results of pregnancy diagnosis by ultrasound imaging in ewes of 5 breeds

Pregnancy diagnosis	Breed*					
	N	C	T	BLM	N × BLM	Total
Number (%) of ewes correctly diagnosed as pregnant (a)	24 (66.7)	13 (81.3)	25 (83.3)	21 (95.5)	26 (83.9)	109
Number of ewes wrongly diagnosed as pregnant (b)	12 (33.3)	3 (18.7)	5 (16.7)	1 (4.5)	5 (16.1)	26
Number of ewes correctly diagnosed as barren (c)	26 (89.7)	0 (100.0)	0 (100.0)	1	2	29
Number of ewes wrongly diagnosed as barren (d)	3 (10.3)	0	0	0	0	3
Overall accuracy of scanning - No. (%)	65 (76.9)	16 (81.3)	30 (83.3)	23 (95.5)	33 (84.9)	167

*N, Naeemi; C, Chios; T, Texel; BLM, Border Leicester Merino; N ′ BLM, Naeemi ′ Border Leicester Merino.

scanning time was generally required to establish a negative diagnosis (barren ewe). The accuracy of pregnancy diagnosed was assessed on the basis of lambing results.

RESULTS AND DISCUSSION

Results of pregnancy diagnosis using ultrasound (Table 1). Of the 167 ewes scanned, 26 barren ewes (15.6%) were wrongly classified as pregnant and 3 pregnant ewes (1.8%) in group d were wrongly classified as barren. Accuracy of determination of pregnant ewes varied from 66.7 to 95.5% between breeds. The results of this study indicate that BLM ewes were much more often accurately diagnosed with scanning compared with N, C, T or N ′ BLM ewes. N is a fat-tailed sheep breed, C and N ′ BLM are semi-fat-tailed, whereas T and BLM are thin-tailed. Although different breeds within the experimental flock were diverse in origin and conformation, it is, however, unlikely that dissimilarity between breeds would have made any difference in the diagnosis as the quality of image was similar for ewes of the 5 breeds studied.

Maximum wrong diagnosis occurred in Naeemi (15/65) and minimum in BLM (1/23). A possible reason for incorrectly diagnosing 3 N ewes as barren, when in fact they were pregnant, could be the presence of rams with the ewes <30 days before scanning. As a result, these later conceiving ewes were scanned too early for the pregnancy to be detected. The errors arising from over estimation of ewes diagnosed as pregnant but not lambing could be due to foetal deaths, undetected abortions or resorption of foetuses. A relatively low accuracy in identifying pregnant ewes may be due to this problem, as lambing data were used to determine the accuracy of scanning. In a flock of ewes where rams were run with the ewes for 34 days, White *et al.* (1984) indicated that the optimum time to scan ewes was between 85 and 100 days after the beginning of the mating. In the present study, rams were run with the ewes for 35 days. Therefore, a second scanning at 85 to 100 days, of ewes judged as pregnant at 45-60 days but not lambing, could have improved the accuracy of scanning in our flock.

ACKNOWLEDGEMENTS

Thanks are due to the Kuwait Livestock Transport and Trading Company (KLTT) and Kuwait Institute for Scientific Research for providing facilities.

REFERENCES

Fowler D G and Wilkins. 1980. The identification of single and multiple bearing ewes by ultrasonic imaging. *Proceedings of the Australian Society of Animal Production* **13**: 492.

Hulet C V and Shupe W L. 1973. Predicting multiple births in sheep by rectal-abdominal palpation. *Proceedings of the Western Section of American Society of Animal Production* **24**: 237-39.

Logue D N, Hall J T, McRoberts S and Waterhouse A. 1987. Real-time ultrasonic scanning in sheep: The results of the first year of its application on farms in south-west Scotland. *Veterinary Record* **121**: 146-49.

Russel A J F and Foot J Z. 1971. The use of circulating progesterone concentrations in the indication of twin bearing ewes. *Animal Production* **13**: 377 (Abstract).

Taverne M A M, Lavoir M C, van Oord R and van der Weyden G C. 1985. Accuracy of pregnancy diagnosis and prediction of foetal numbers in sheep with linear-array real-time ultrasound scanning. *The Veterinary Quarterly* **7**: 256-63.

While I R, Russel A J and Fowler D G. 1984. Real-time ultrasound scanning in the diagnosis of pregnancy and determination of foetal numbers in sheep. *Veterinary Record* **115**: 140-43.

Indian Journal of Animal Sciences **68** (4) : 330-331, April 1998

Biochemical changes in trace elements in antral follicles of goat

R K SHARMA[1] and R VATS[2]

Kurukshetra University, Kurukshetra, Haryana136119

Received : 1 January 1997; Accepted : 28 October 1997

ABSTRACT

Biochemical analysis of 6 categories of normal developing antral follicles of goat (*Capra hircus*) was conducted to study the sequential pattern of changes in trace elements. Fe increased from 43.67 in category 1 to 54.59 µg/g in category 5 follicles. The variations in Cu and Mn did not reveal any specific trend but maxima for Mn was observed in medium antral follicles and for Cu in large antral follicles. These biochemical variations in trace elements have been discussed in relation to the physiology of the follicles.

Key words: Antral follicles, Biochemical analysis, Follicles, Follicular growth

Follicular growth and maturation in various mammalian species is characterized with increased cellular complexity associated with biochemistry of synthesis, accumulation and transport of different biomolecules (Sangha and Guraya 1989a,b, Sharma and Guraya 1990, Sharma *et al.* 1996). Very limited information is available on biochemical changes in macromolecules *vis-a-vis* follicle growth in domestic animals (Hafez 1987). Present investigation was conducted to study the pattern of changes in Mn, Fe and Cu, as will be very useful to determine their role in ovarian function and thus fertility. The details of biochemical and physiological inter-relationships will provide an index to examine deviation in situation wherein normal growth and development of antral follicles is threatened *in vitro* system.

MATERIALS AND METHODS

The goat (*Capra hircus*) ovaries, obtained from the abattoirs of Karnal and Chandigarh were brought to the laboratory at 0°C. The ovarian follicles at different stages of development and maturation were manually separated with the help of fine forceps and needles under the dissecting microscope. These follicles were then categorized into 6 categories on morphometric basis.

Each category of the manually separated follicles was washed first with deionized water. Thereafter, the weight and number of follicles were recorded. Each sample was weighing not less than 200 mg. The samples were digested in long-necked round-bottom flasks with triple acid (concentrated nitric acid : 70% perchloric acid : concentrated sulphuric acid, 10:3:1) in the ratio of 1:10 (w/v). This process was executed by heating the contents till most of the triple acid mixture evaporated from the flask. The contents of each flask were then washed with 2 ml of deionized water and were stored in plastic vials at 4° C.

Present address: [1]Principal Investigator, [2]Senior Research Fellow, Reproductive Biology Laboratory, Department of Zoology .

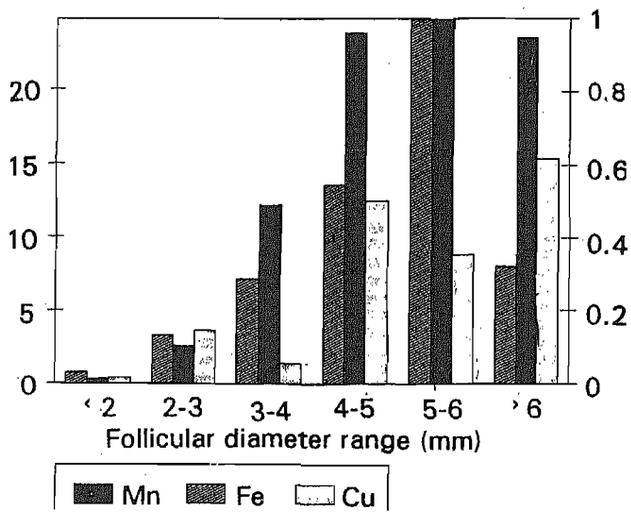


Fig. 1. Pattern of changes in different trace elements during folliculogenesis.

The concentration of trace elements was determined by atomic absorption spectrophotometer (Ludmilla 1976). Students 't' test was used to test the validity of results (Zar 1984).

RESULTS AND DISCUSSION

The concentration of Fe increased, and those of Cu and Mn varied minutely with the advancement in follicular size (Tables 1,2; Figs 1,2). These values are in agreement with the earlier findings on rat (Sangha *et al.* 1993). Iron content of developing follicles showed an increase up to category 5,

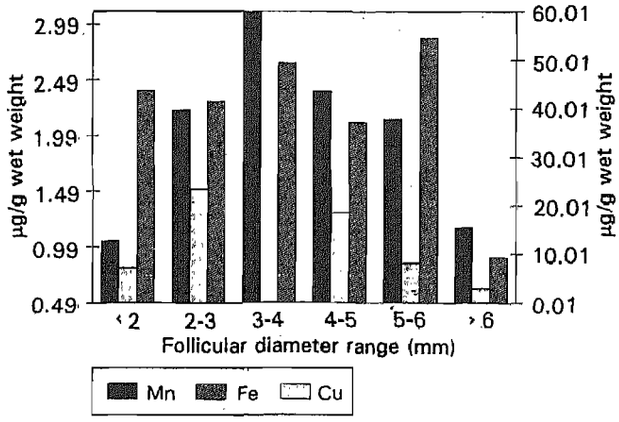


Fig. 2. Changes in trace elements during folliculogenesis.

i.e. preovulatory follicles. These variations are attributed to increased levels of steroid hormones (oestrogen and progesterone) that induce increased haemodynamic pulses in the vascular shunt of the developing follicles (Sangha *et al.* 1993). These biochemical findings, further endorse the earlier findings of Guraya *et al.* (1991). The increase in iron content corresponds with the development of smooth musculature around the preovulatory follicles (Ishwar *et al.* 1987). The variation recorded in iron level corresponds positively to the increased biosynthetic activities of the follicle (Sangha *et al.* 1993). In ovulatory follicles, the iron declined may be due to ischaemia leading to the rupture of follicle wall at stigma (a vascular-bed deficient portion). These findings further suggest that the variations in iron content are in accordance with the haemodynamic changes observed in the follicular wall during follicular development and maturation and opines that these changes are regulated by intrafollicular factors (Guraya *et al.* 1991). The overall average concentration of manganese (Mn) in follicles of different categories differ significantly. The maximum concentration of Mn was observed in follicles of category 3 and minimum in category 1. These variations are indicative of its utilization in enzyme activities like bone and blood phosphatase, arginase required for urea formation and as an activator of carboxylase, choline esterase, muscle adenosine-triphosphatase and other enzymes (Sangha *et al.* 1993). The lower values observed in follicles of categories 1 and 6 are possibly due to the poor uptake of Mn in the presence of high calcium and phosphorus level (Sherman 1965). The maximum copper concentration was 1.5 mg/g in small antral follicles. This increased copper concentration can be correlated with the increased oestrogen levels which presumably relates to the increases in ceruloplasmins in synthetase (Sharma and Sharma 1997). The variations observed in different categories of follicles are possible due to the synergistic effect of progesterone and oestrogen (Singh *et al.* 1991, Sangha *et al.* 1993, Sharma and Sharma 1997) as well as mobilization of copper in erythrocuprein, hepatocuprein, cytochrome-C oxidase, tyrosinase monoamine oxidase, ascorbic acid oxidase and a number of co-enzymes in dehydrogenases required in fatty acid catabolism (Sharma and Sharma 1997). These results fix stage-specific norms of the trace elements for specific stage of normal developing follicle can, therefore, provide additional information that deficiency of a single or combined trace elements can induce reproductive failure.

ACKNOWLEDGEMENTS

We thank Director, Central Soil Salinity Research Institute, Karnal, and Chairman, Department of Zoology, Kurukshetra University, Kurukshetra, for providing facilities. University scholarship rendered to Rajeev Vats by Kurukshetra University, Kurukshetra, is acknowledged.

REFERENCES

Guraya S S, Parminder Kaur and Sharma R K. 1991. Histochemical and biochemical studies on esterase activity in the rat ovary. *European Journal of Morphology* **29** (3): 161-72.

Hafez E S E. 1987. *Reproduction in Farm Animals*. 5th edn. Lea and Febiger, 600 Washington Square Philadelphia, USA.

Ishwar S, Sankarnarayan A and Bawa S R. 1987. Neurogenic involvement in follicular development probability. *International Journal of Fertility* **32** (5): 388-93.

Ludmilla D. 1976. *Chemical Analysis by Atomic Absorption Spectroscopy*. Varian Tecthran Pvt. Ltd., Melbourne, Australia.

Sangha G K and Guraya S S. 1989a. Biochemical changes in lipids during follicular growth and corpora lutea formation and regression in rat ovary. *Journal of Experimental Biology* **27**: 998-1000.

Sangha G K and Guraya S S. 1989b. DNA, RNA and Protein changes in rat ovarian follicles. *Proceedings of Indian National Science Academy* B55 No.3, pp.155-58.

Sangha G K, Sharma R K and Guraya S S. 1993. Distribution of trace elements in blood and ovary during the oestrous cycle and pregnancy in house rat. *Indian Journal of Animal Sciences* **63**(2): 142-45.

Sharma R K and Guraya S S. 1990. Granulosa heterogeneity: A histochemical, lectin staining and scanning electron Microscopic study of *Rattus rattus* ovary. *Acta Embryologica Morphologica Experimentalis* n.s., **11**(2): 107-21.

Sharma R K and Sharma M. 1997. Physiological perspectives of copper: A review. *Indian Journal of Experimental Biology* **35**: 696-713.

Sharma R K, Vats R and Sawhney A K. 1996. Changes in follicular lipids during follicular growth in the goat (*Capra hircus*) ovary. *Small Ruminant Research* **20** (2): 177-80.

Sherman W C. 1965. *Manganese*. National Livestock and Meat Board. Food and Nutrition News, **36**: No.8.

Zar J H. 1984. *Biostatistical Analysis*. Prentice Hall. Inc., Englewood Cliffs, N.J.

Indian Journal of Animal Sciences **68**(4) : 332-336, April 1998

Ultrastructure changes in the oocytes of caprine antral follicles

R K SHARMA[1] and S CHOWDHURY[2]

Kurukshetra University, Kurukshetra, Haryana 136 119

Received : 19 May 1997 ; Accepted : 28 October 1997

ABSTRACT

Ultrastructure analysis of the oocytes from medium antral follicles (MAF-3-5 mm diameter) and large antral follicles (LAF, >5 mm diameter) have been made during the follicular phase of goat *(Capra hircus)*. The variations in the spatial distribution of cell organelles like mitochondria, microvilli, lipid droplets, glycogen bodies and vocalization in the peripheral ooplasm of both the categories of oocytes revealed that as the oocytes advanced in size the spatial distribution of the organelles was altered and in large antral oocytes all the cell organelles were found concentrated near the zona pellucida. These ultrastructural variations within the oocytes of 2 categories shall be discussed in relation to their development, maturational and fertilizational competence.

Key words : Goat , Ovary, Oocyte, Ultrastructure

The oocyte maturaion, fertilizabiltiy and early embryogenesis largely depends upon several cytological and molecular events occurring within the developing oocyte during folliculogenesis (Buccione *et al.* 1990, McGaughey *et al.* 1990, Philips and Dekel 1991). The cytological maturation of the oocyte is a must for the success of *in vitro* fertilization and its subsequent development up to the blastocyst stage (Tan and Lu 1990, Pavlok *et al.* 1992, Lonergan *et al.* 1994a, Carolan *et al.* 1996). Fine structural details of the ooplasmic components from the antral follicles of different sizes have been investigated only in case of cattle and sheep (Cran *et al.* 1980). Such investigations are lacking in the goat. In the present study, an attempt has been made to point out the ultrastructural details of the ooplasmic maturational status in 2 categories of antral follicles of goat.

MATERIALS AND METHODS

Goat ovaries were collected from the abattoir of Delhi (28°38'N, 77°12'E) and were brought to the laboratory on ice. The follicles were devided in 2 categories on the basis of their daughter as medium-antral follicles (3-5 mm diameter) MAF and large antral follicles (>5 mm diameter) LAF. The follicular contents were aspirated out by 24 G syringe from both MAF and LAF. The contents were washed in phosphate buffer saline (PBS) having pH 7.2 and was fixed in 2.5% glutaraldehyde. The samples were processed thereafter for electron microscopy (Sharma *et al.* 1996).

Present address : [1] Lecturer, [2]Research Scholar, Department of Zoology.

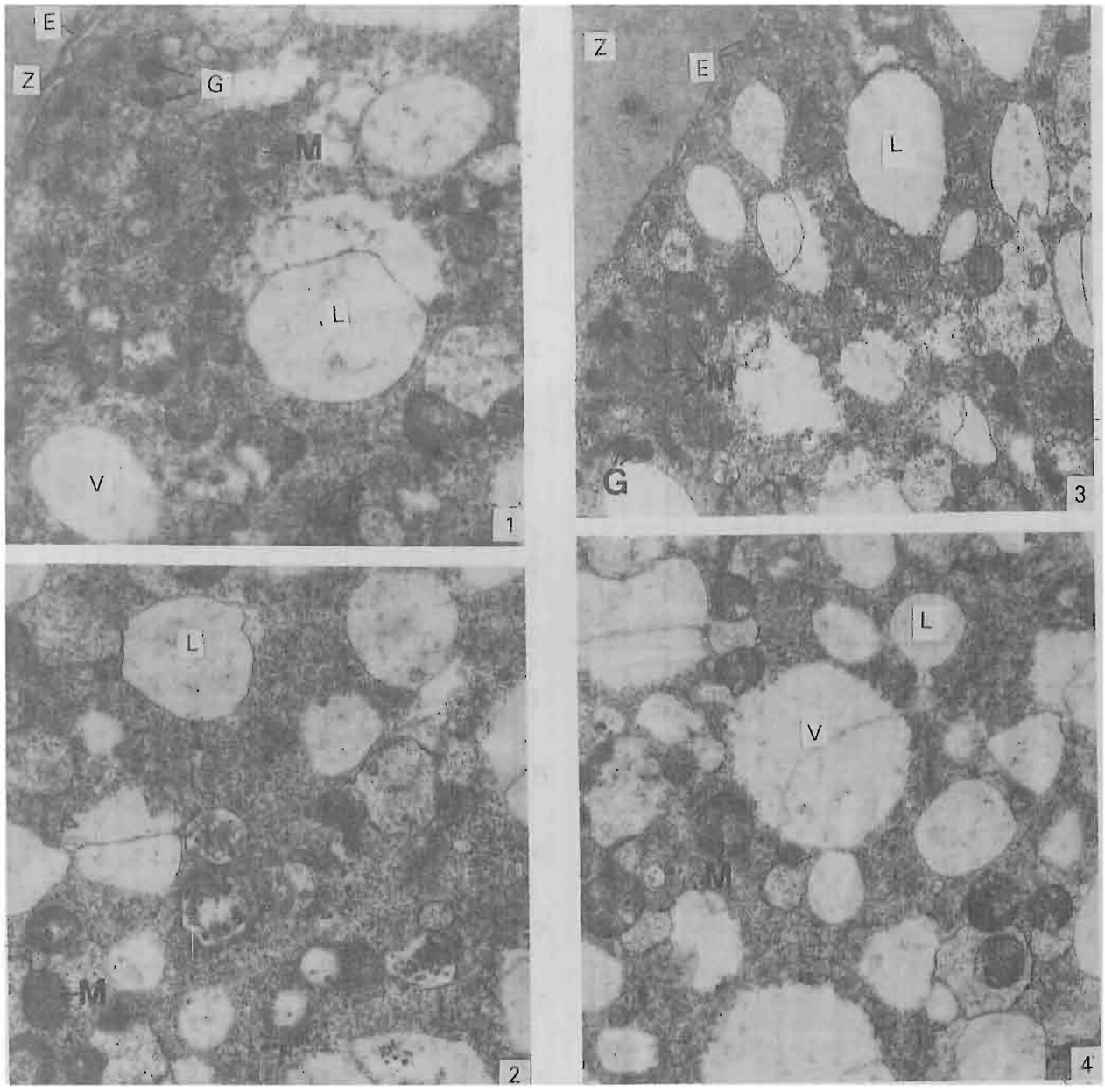
Ultrathin section were viewed under electron microscope installed at All India Institute of Medical Sciences, New Delhi, and selected photographs were taken.

For the analysis of the cell organelles, the method of Cran *et al.* (1980) was followed.

RESULTS

The presence of scattered mitochondria, endoplasmic reticulum, vacuoles, glycogen and lipid droplets in the ooplasm of both the categories of follicles was observed. The oocyte membrane showed numerous slender cytoplasmic processes microvilli and micropapillae. The number and size of microvilli increased as the oocyte advanced in size from medium to large antral follicles (Figs. 1, 3 ; Graph 1). Thick processes from surrounding cumulus cells were seen entering the zona pellucida and some of these even made contact with the oolemma. Most of the Golgi complexes were localized in the vicinity of oolemma and comprised number of stacks of cisterns with numerous vesciles.

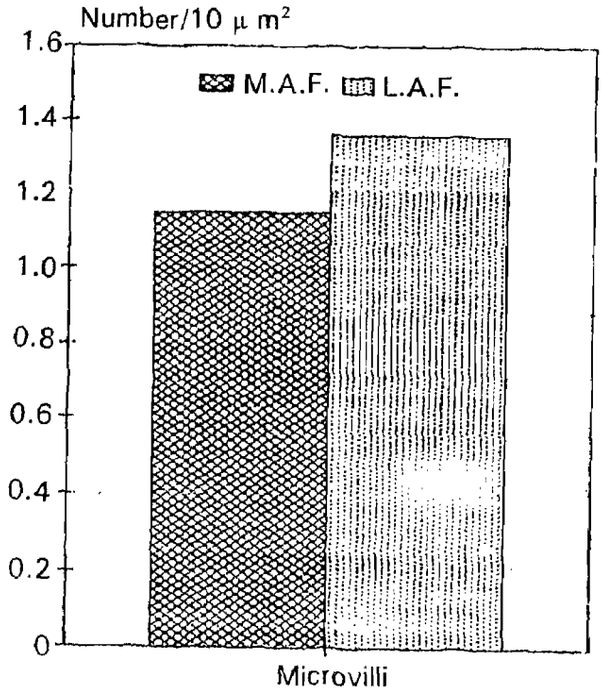
The mitochondria appeared round to oval in morphology but no significant change in size was observed in both the categories of follicles. The distribution of mitochondria was random throughout the ooplasm of medium antral follicles, whereas, in large antral follicles , they were concentrated in the peripheral zone (Figs 1-4; Fig 6) . Proliferation of mitochondria by fission was also seen in the ooplasm of large antral follicles. This may be the reason for rapid increase in their number. The number of vacuoles near the peripheral ooplasmic zone were higher in frequency in large antral follicles as compared to those observed in medium antral follicles (Figs 1, 3; Fig. 7).

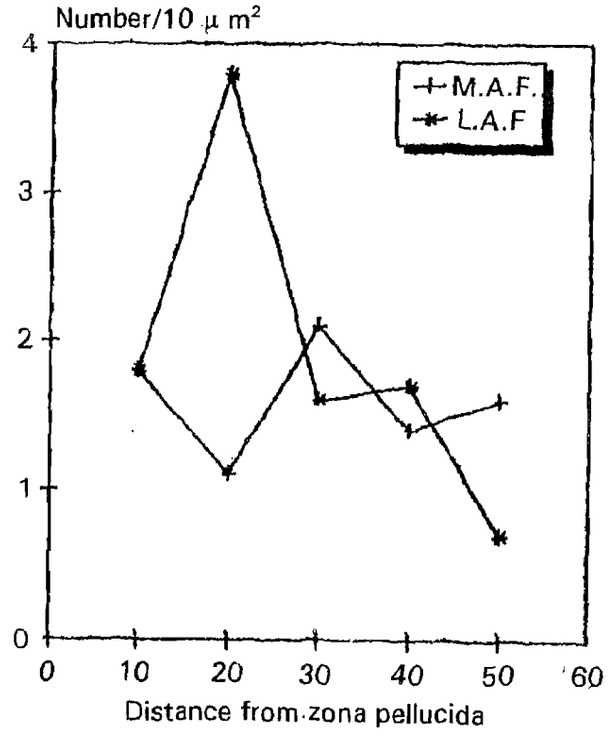


Figs 1-2. **1**. Electron micrograph of the peripheral portion of the oocyte from medium antral follicle showing microvilli (E), mitochondria (M), glycogen (G), lipid droplets (L) and vacuoles (V). Microvilli project from oocyte surface into the zona pellucida (Z).**2**. A high power view of the central portion of the cytoplasm from an oocyte of medium antral follicles with the cell organelles more dispersed.

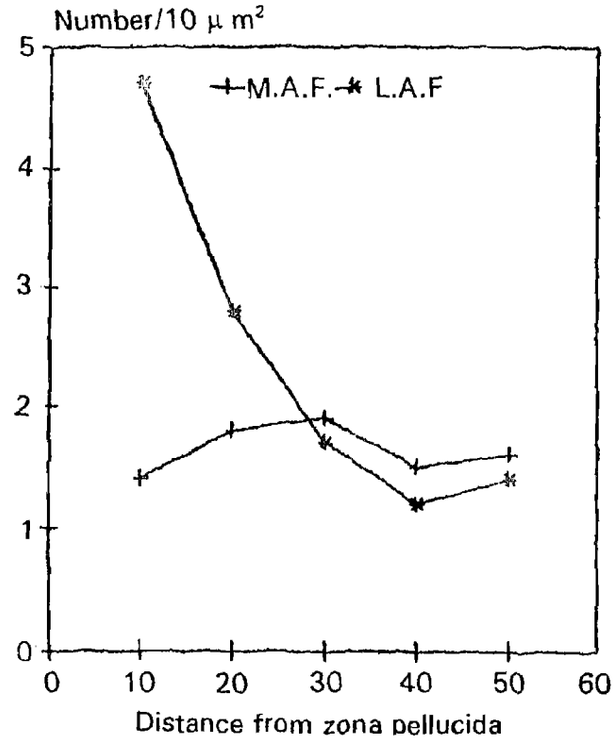
Figs 3-4. **3**. Peripheral portion of the ooplasm from the large antral follicles giving the details like microvilli (E), aggregation of mitochondria (M), glycogen (G) and lipid (L) droplets near the oolemma. Note the dividing mitochondria (arrow). **4**. Central magnified portion of ooplasm from large antral follicles with sparsely distributed cell organelles like mitochondria (M), vacuoles (v) and lipid droplets (L).

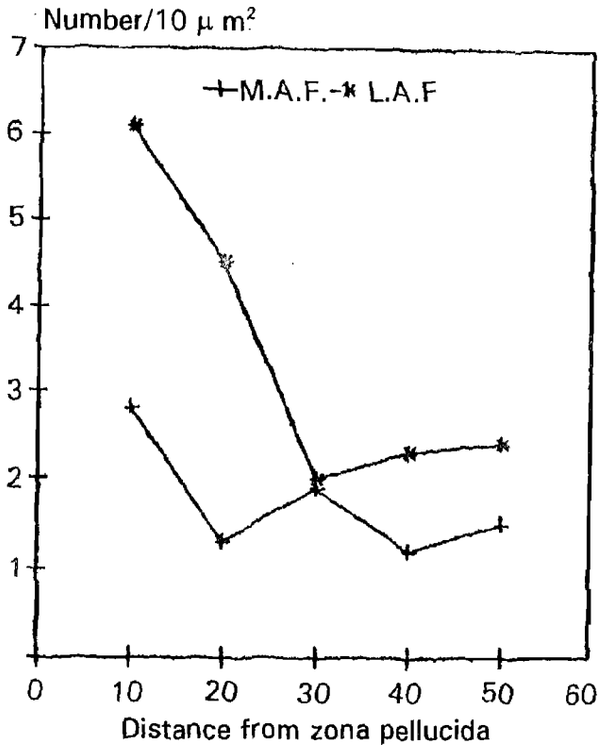
Lipids were seen as round to oval droplets in medium antral follicles but they appeared more elongated in large antral follicles. The distribution of glycogen and lipid droplets was uneven in medium antral oocyte, however, the number and size of these droplets increased and were found aggregated beneath the ooplasmic membrane in large antral follicles (Figs 1-4; Fig. 4). Similar phospholipid bodies and small vesicles of glycogen were seen in the corona radiata and other granulosa cells which suggests that these cells contribute lipid and glycogen to the developing oocyte.





Figs 5-6. 5. A graph depicting the increase in the number of microvilli projecting from oocyte surface into zona pellucida (Z) with the advancement of follicle from medium to large antral size. 6. Graphic representation of mitochondria in the peripheral ooplasm of medium and large antral follicles.





Figs 7-8. 7. Distribution of vacuoles in the ooplasma of medium and large antral follicles showing aggregation near the oolemma in LAF. 8. Patterm of distribution of glycogen and lipid droplets in the cytoplasm of oocyte of medium and large antral follicle.

Most of the cell organelles like mitochondria, Golgi complexes, phospholipid droplets and glycogen bodies were scattered in the cortical ooplasm in large antral follicles thus leaving the central area of oocyte less populated.

DISCUSSION

The increase in number and size of microvilli in oocyte from medium to large antral follicles indicated that uptake of the nutritional components from the oocyte continue to increase throughout the later phases of oogenesis (Guraya 1996). These findings strongly endorse the findings of Moor and Smith (1979), who have demonstrated a constant rate of uptake of amino acids in the oocytes of follicles having diameter more than 3.5 mm. The uptake increases only during second meiotic metaphase. The close association between the mitochondria, endoplasmic reticulum and Golgi complex observed in the present investigation is suggestive of the fact that like most mammalian oocytes in goat also endoplasmic reticulum (ER) temporarily dilate of ER is observed in most of the ooplasm (Cran *et al.* 1980). These variations are transitory phases of storage of products acquired from surrounding granulosa cells as suggested by Norrevang (1968).

Localization of mitochondria beneath the ooplasmic membrane of goat oocytes shows a great similarity to that of rat (Sotelo and Porter 1959), hamster (Hadek 1969), cow (Fleming and Saake 1972) and sheep (Cran *et al.* 1980). The goat oocytes differ from that of cow (Fleming and Saake 1972) oocytes in terms of mitochondrial dispersal as it is not associated with the development which is common feature of cattle oocyte. The presence of hooded mitochondria in oocyte from LAF strongly supports the earlier observations of Senger and Saake (1970) and Fleming and Saake (1972) who have assigned hooding of mitochondria to increase the surface area for providing specific micro-environment to efficate the exchange of metabolites with the ER. It may, therefore, be a reason of increase in mitochondrial activity in later stages of development.

The peripheral placement of ER, mitochondria, Golgi complex and glycogen lipid droplets in the present findings of goat, strongly advocates the findings of Wassarman and Albertinin (1994) for mammals in general.

Cortical granules were also reported beneath the plasma membrane in large antral oocytes. In this regard, goat oocyte largely resembles that of bovine oocyte (Szollosi *et al.* 1978).

Presence of any large glycogen and lipid droplets in the oocyte of goat suggest that this is a characteristic feature of bovine species in general (Fleming and Saake 1972). These vesicles greatly increase in number with the advancement in follicle diameter, indicating the increase of yolk deposition in the oocyte in LAF required to sustain life after fertilization. Similar phenomenon of vesicle deposition have been reported in rabbit (Zamboni and Mastroianni 1996a,b, Kruaskopf 1968a,b, Longo and Anderson 1969, Gulyas 1971) and pig-tailed monkeys (Szollosi *et al.* 1978) oocytes , thereby suggesting the mode of incorporation of lipid and glycogen droplets is the same in mammals in general , but varied rates lead to the species specific differences in oocytes.

ACKNOWLEDGEMENTS

Authors are thankful to the University Grants Commission for sponsoring the Project, and Professor A K Susheela, Officer-in-Charge, EM Facility, All-India Institute of Medical Sciences, New Delhi, for rendering the facilities for Electron Microscopy.

REFERENCES

Buccione R, Schreoeder A C and Eppig J. 1990. Interactions between somatic cells and germ cells throughout mammalian oogenesis. *Biology of Reproduction* **43** : 543-47.

Carolan C, Lonergan P, Monget P, Monniaux D and Mermillod P. 1996. Effect of follicle size and quality on the ability of the follicular fluid to support cytoplasmic maturation of bovine oocytes. *Molecular Reproduction and Development* **43** : 477-83.

Cran D G, Moor R M and Hay M F. 1980. Fine structure of the sheep oocyte during antral follicle development. *Journal of Reproduction and Fertility* **59** : 125-32.

Fleming W N and Saake R G. 1972. Fine structure of the bovine oocyte from the mature Grafian follicle. *Journal of Reproduction and Fertility* **29** : 203-13.

Gulyas B. 1971. The rabbit zygote : formation of annulate lamellae. *Journal of Ultrastructural Research* **35** : 112-26.

Guraya S S. 1997. *Ovarian Biology of Buffaloes and Cattle.* ICAR Publication, New Delhi .

Hadek R. 1969. *Mammalian Fertilization.* Academic Press, New York.

Krauskopf C. 1968a. Elektronenmkroskopische Untersuchungen uber die struktur de Oocyte und des Z-Zellstadium beim Kanichen. I. Oocyte. *Z – Zellforsch mikrosk Anatomy* **92** : 275-95.

Krauskopf C. 1968b. Elektronenmikroskopische Untersuchungen uber die Struktru der Oocyte und des Z-Zellstadiums beim Kaneihen. 2. Blastomeren. *Z Zellforsch mikrosk Antatomy* **92** : 296-312.

Lonergan P, Monaghan P, Rizos D, Boland M P and Gordon I. 1994a. Effect of follicle size on bovine oocyte quality and development competence following maturation, fertilization and culture *in vitro*. *Molecular Reproduction and Development* **374** : 48-53.

Longo F J and Anderson E. 1969. Cytological events leading to the formation of the two cell stage in the rabbit : association of the maternally and paternally derived genomes. *Journal of Ultrastructural Research* **29** : 86-118.

McGaughehy R W, Racowksy C, Rider V, Baldwin K, DeMarais A A and Webster S D. 1990. Ultrastrutural correlates of meiotic maturation in mammalian oocytes. *Journal of Electron Microscopy Techniques* **16** : 257-80.

Moor R M and Smith M W. 1979. Amino acid transport in mammalian oocytes. *Experimental Cell Research* **119** : 333-41.

Norrevang A. 1968. Electron microscopic morphology of oogenesis. *International Review of Cytology* **23** : 114-86.

Pavlok A, Lucas-Hahn A and Niemann H. 1992. Fertilization and development competence of bovine oocytes derived from dif-

ferent categories of antral follicles. *Molecular Reproduction and Development* **31** : 63-67.

Philips D M and Dekel N. 1991. Maturation of the rat cumulus-oocytes complex : structure and function. *Molecular Reproduction and Development* **28** : 297-306.

Senger P L and Saake RG. 1970. Unique mitochondria in the bovine oocyte. *Journal of Cell Biology* **46** : 405-8.

Sharma R K, Sawhney A K and Vats R. 1996. Ultrastructure of thecal components in caprine ovary. *Small Ruminant Research* **22**(3) : 249-53.

Sotelo J R and Porter K R. 1959. An electron microscope study of the rat ovum. *Journal of Biophysics Biochemical Cytology* **5** : 327-41.

Szollosi D, Gerard Y, Menezo Y and Thaibault C. 1978. Permeability of ovarian follicle ; corona cell-oocyte relationship in mammals . *Annals of Biology Animal Biochemistry and Biophysics* **18** : 511-21.

Tan S J and Lu K H. 1990. Effects of different oestrous stage of ovaries and sizes of follicles on generation of bovine embryos *in vitro*. *Theriogenology* **33** : 335.

Wassarman P M and Albertini D F. 1994. The mammalian ovum. *The Physiology of Reproduction* . Vol. 1, pp. 79-122.

Zamboni L and Mastroianni L. 1966a. Electron microscopic studies on rabbit ova. 1. The follicular oocyte. *Journal of Ultrastructural Research* **14** : 95-117.

Zamboni L and Mastroianni L. 1966b. Electron microscopic studies on rabbit ova. 2. The penetrated tubal ovum. *Journal of Ultrastructural Research* **14** : 118-32.

Indian Journal of Animal Sciences **68** (4) : 337-341, April 1998

Production of mouse embryos under *in vivo* system using exogenous gonadotropins

MAHESH DATT[1], ARJAVA SHARMA[2] and ASHWANI SHARMA[3]

National Dairy Research Institute, Karnal, Haryana 132 001

Received: 19 August 1996 ; Accepted : 28 October 1997

ABSTRACT

To study monthly and seasonal variations in the quantity as well as quality of mouse embryos, 949 ± 0.67 animals were used for superovulation and subsequent flushing in 237 trials spread over 12 months. Out of these 498 ± 0.62 mice had confirmed copulation plug and 312 ± 0.30 animals responded to superovulation. A total of 3 631 ± 1.19 embryos were recovered of which 2 892±1.09 and 739 ± 0.32 were of good and poor quality respectively. It was concluded that the month of March was most ideal time for obtaining preimplantation stage embryos.

Key words : Embryo production, Exogenous gonadotropins, Mouse embryo

Mouse embryos at preimplantation stage are excellent models for conducting pilot experiments on *in vitro* fertilization, micromanipulation, cloning, cryopreservation and gene transfer,because these are economical, compact ,transparent and sturdy.Their culture conditions are also well standardized. However,since superovulation is one of the most unpredictable aspects of reproduction, the availability both in terms of quality and quantity is restricted by some factors beyond our control.

This experiment was planned to study retrieval rate and quality of embryos produced under *in vivo* system using exogenous gonadotropins.

MATERIALS AND METHODS

The present investigations were undertaken with Swiss-Albino strain of mouse procured from Disease-free Animal House of CCS Haryana Agricultural University, Hisar, Haryana, and later maintained at Small Animal House of National Dairy Research Institute, Karnal, Haryana. During the study, temperature and photoperiodicity of the Animal House was kept under control. Virgin females, 8-16 week old, were induced to superovulate with intraperitoneal injections of 5-7 IU PMSG followed 48 hr later by similar dose of hCG. Females were kept with healthy males immediately after hCG injection and copulation plugs were confirmed the next morning. Laparotomy was performed and reproductive tract was flushed to collect embryos at preimplantation stage. The embryos at <8-cell and 8-16-cell stage were collected from the oviducts whereas uterine horns were flushed to obtain embryos at 16-32-cell and >32-cell stage. The embryos were examined under inverted microscope which was fitted with camera for photomicrography.

Present address:[1]Assistant Professor, Department of Gynaecology, Rajasthan Agricultural University Bikaner.[2]Principal Scientist. [3]Scientist(SS) (DCB).

RESULTS AND DISCUSSION

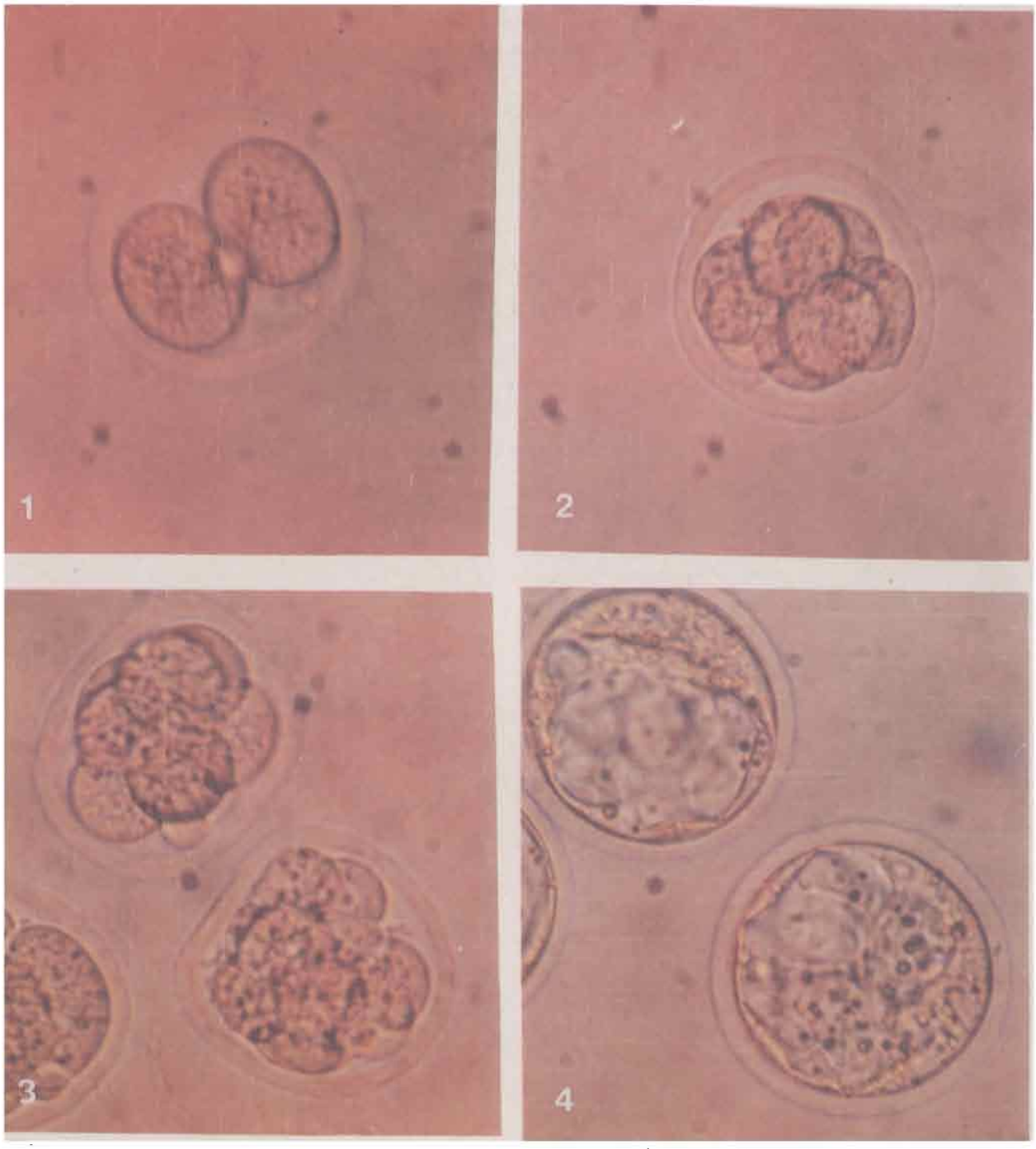
Details of animals used for superovulation has been presented in Table 1. Quality-wise details of embryos produced has been given in Table 2 (Figs 1-4).

Quantity of embryos

In this study 949 ± 0.67 animals were used in 237 trials spread over 12 months, out of which 498 ± 0.62 animals had confirmed copulation plug and 312 ± 0.30 animals responded to superovulation (Table 1). The number of animals used for embryo production during different month varied from 20 to 160 in to 36 trials. Number of animals used for oocyte retrieval ranged between 3-6.In 2 recent studies conducted for the retrieval of mouse embryos, Singh (1990) and Das (1991) also used 3-6 females for inducing superovulation.

The number of animals having confirmed copulation plug over different months varied between 12±0.51 to 96±0.14. When the number of animals having confirmed copulation plug was expressed as percentage of animals treated, it ranged between 34.14% (December) and 63.04% (June) with the overall average being 52.47%.

On an average 32.87% of the animals treated for superovulation responded to embryo production, the maximum being in January (45.00%) and minimum in March (23.12%). After examination of confirmed copulation plug, 62.65% of the animals yielded embryos, the maximum being in January (92.30%) and minimum during March (38.54%).



Figs 1-4. **1.** Mouse embryo at cell state. **2.** Mouse embryo at 4-8 cell stage. **3.** Early morula. **4.** Showing late blastocyst stage.

Quality of embryos

The quality of embryos (3631±1.19) recovered in different months in 158 trials was studied. Of these 2 892±1.09 and 739±0.32 were of good and poor quality respectively (Table 2). The distribution of good and poor quality embryos over different months ranged between 31 (August) to 889 (March) and 3 (August and October) to 169 (March) respectively. Maximum number of good, poor and total embryos were recovered during March, whereas minimum number of good, poor and total embryos were produced during August as well as in October (Table 2). This may be because of role of photoperiodicty on superovulation response and subsequent

Table 1. Details of animals used for superovulation

Month	No. of trials	Animals treated N ± (S.E) (CV)	CP confirmed N ± S.E. (CV)	CP confirmed %	Animals responded N ± S.E. (CV)	Animals responded Animals treated (%)	Animals responded CP confirmed (%)
1	25	80 ± 0.13 (20.17)	39 ± 0.13 (48.02)	48.75	36 ± 0.10 (35.18)	45.00	92.30
2	8	31 ± 0.23 (16.54)	15.± 0.22 (34.18)	48.38	10 ± 0.18 (37.03)	32.25	66.66
3	36	160 ± 0.18 (24.91)	96 ± 0.14 (32.32)	60.00	37 ± 0.28 (16.22)	23.12	38.54
4	26	80 ± 0.53 (8.83)	47 ± 0.16 (46.99)	58.75	28 ± 0.53 (25.23)	35.00	59.57
5	35	118 ± 0.18 (23.95)	63 ± 0.13 (42.18)	53.38	39 ± 0.55 (28.27)	33.05	61.90
6	18	92 ± 0.23 (18.85)	58 ± 0.22 (29.26)	63.04	28 ± 0.12 (32.87)	30.43	48.27
7	26	123 ± 0.12 (12.76)	56 ± 0.14 (33.97)	45.52	44 ± 0.92 (27.81)	35.77	78.57
8	5	20 ± 0.00 (0.00)	12 ± 0.51 (47.50)	60.00	8 ± 0.24 (34.23)	40.00	66.66
9	26	123 ± 0.12 (12.76)	56 ± 0.14 (33.97)	45.52	44 ± 0.92 (27.81)	35.77	78.57
10	5	20 ± 0.00 (0.00)	12 ± 0.51 (47.51)	60.00	8 ± 0.24 (34.23)	40.00	66.66
11	16	61 ± 0.33 (34.82)	30 ± 0.25 (49.72)	49.18	18 ± 0.85 (30.36)	29.50	60.00
12	11	41 ± 0.14 (12.53)	14 ± 0.19 (50.81)	34.14	12 ± 0.91 (27.64)	29.26	85.71
Total	237	949 ± 0.67 (25.86)	498 ± 0.62 (45.12)	52.47	312 ± 0.30 (35.40)	32.87	62.65

CP: Copulation plug.

embryo production (Sharma *et al.* 1994).

A total of 3 631 embryos were recovered from 312 animals which responded to superovulation. Thus, the actual recovery rate of embryos from these animals was 11.64 (Table 2). Vickers (1967, 1969) in 2 different reports obtained a wide range of embryo recovery i.e. 7.14 - 13.94 due to delayed fertilization. Similar recovery rates were reported by Fujimoto *et al.* (1975) in rats and by Das (1991) in mice. However, the recovery rate of Takagi and Sasaki (1976) was much higher (21.5) than that obtained in this study. Differences in strains, superovulation protocols and other experimental design might have contributed to this variation. Production of total and good quality embryos per animal was maximum in March, whereas this was minimum during July and September. The possible reason may be that there was availability of light during summer and ratio of light and dark cycle is known to influence the superovulation and subsequent availability of embryos.

Besides good and poor qualities of embryos recovered, there were many instances when flushing contained some unfertilized oocytes or degenerated embryos. Overall, 396±0.29 and 919+0.80 embryos, recovered were unfertilized and degenerated in 128 and 129 trials respectively.

These results provide some information about planning and conduct of experimental trials for producing embryos at preimplantation stages. It seems that March is most optimum

Table 2. Quality of total embryos produced in vivo

Month	Good embryos N ± S.E (CV)	Good embryos %	Poor embryos N ± S.E. (CV)	Poor embryos %	Total embryos N ± S.E. (CV)
1	306 ± 3.87 (66.32)	81.60	69 ± 0.87 (58.77)	18.40	375 ± 4.55 (63.58)
2	75 ± 4.02 (60.00)	75.00	25 ± 0.75 (44.04)	25.00	100 ± 3.68 (54.16)
3	889 ± 2.61 (61.00)	81.93	169 ± 0.39 (47.25)	15.57	1058 ± 2.85 (58.87)
4	448 ± 2.63 (70.68)	85.33	77 ± 0.38 (57.38)	14.67	525 ± 2.86 (64.00)
5	519 ± 2.22 (73.83)	82.78	108 ± 0.91 (104.75)	17.22	627 ± 2.51 (68.97)
6	93 ± 4.76 (57.23)	80.87	22 ± 0.80 (53.63)	19.13	115 ± 5.02 (64.11)
7	71 ± 6.19 (128.10)	54.20	60 ± 1.72 (52.98)	45.80	131 ± 5.78 (81.72)
8	31 ± 9.50 (86.68)	91.18	3 ± 0.00 (0.00)	8.82	34 ± 11.00 (91.51)
9	71 ± 6.15 (128.10)	54.20	60 ± 1.72 (52.98)	45.80	131 ± 5.78 (81.72)
10	31 ± 9.50 (86.68)	91.18	3 ± 0.00 (0.00)	8.82	34 ± 11.00 (91.51)
11	224 ± 2.64 (62.28)	75.93	71 ± 1.86 (95.65)	24.07	295 ± 3.58 (63.63)
12	134 ± 3.91 (78.79)	65.05	72 ± 1.53 (67.35)	34.95	206 ± 4.66 (71.61)
Total	2892 ± 1.09 (71.93)	79.65	739 ± 0.32 (76.91)	20.35	3631 ± 1.20 (65.62)

for obtaining maximum as well as best quality mouse embryos at preimplantation stages.

REFERENCES

Das S K. 1991. 'Cytogenetic studies on preimplantation embryos.' M.Sc. Thesis submitted to NDRI (Deemed University) Karnal (Haryana).

Fujimoto S, Passantino T J, Koenczoel I and Segal S J. 1975. A simpified method for chromosome preparations of rat preimplantation embryos. *Cytologia* **40** : 469-75.

Sharma A, Datt M, Sharma A and Balakrishnan C R. 1994. *In vitro* maturation, fertilization and cytogenetic evaluation of buffalo embryos-A review. *Journal of Dairy Science* **47**(9) : 545-52.

Singh Y P. 1990. '*In vitro* fertilization, culture and development of mouse oocytes.' M.Sc. Thesis submitted to NDRI, (Deemed University) Karnal (Haryana)

Takagi N and Sasaki M. 1976. Digynic triploidy after superovulation in mice. *Nature,London* **264**: 278-85.

Vikers A D. 1967. A direct measurement of sex ratio in mouse blastocysts. *Journal of Reproduction and Fertility* **13** : 375-76.

Vikers A D. 1969. Delayed fertilization and chromosomal anomalies in mouse embryos. *Journal of Reproduction and Fertility* **20** : 69-76.

Indian Journal of Animal Sciences 68(4) : 341-343, April 1998

Thyroid functions, testosterone profiles and preputial microbes in cow and buffalo bulls

N S BUGALIA[1], INDERJEET[2], B R GULATI[3], S L GARG[4] and S K VERMA[5]

CCS Haryana Agricultural University, Hisar, Haryana 125 004

Received : 21 July 1996 ; Accepted : 28 October 1997

ABSTRACT

Thyroid function, testosterone levels and preputial microbial load were studied in pubertal buffalo bull calves (n=7), mature buffalo bulls (n=8) and mature cow bulls (n=6). Thyroid hormones (T_3 and T_4) in pubertal buffalo bull calves were comparable to breeding buffalo bulls and cow bulls, suggesting stable thyroids activity from puberty to sexual maturity for initiation of pubertal endocrine and exocrine testicular functions. Testosterone profiles, however, were distinctly low in buffalo bulls/bull calves as compared to cow bulls ($P<0.05$) indicating species variations and may be associated with poor libido of buffalo bulls. A variety of microbes, mostly mixed infections, were harbouring the preputial cavity of all categories of males, though slightly greater incidence was recorded in breeding cow bulls, while buffalo bull calves tended to be having relatively cleaner preputial cavity. *Escherichia coli* was the most frequent isolate, and the rare occurrence of *Klebsiella* spp. in preputial washings was also recorded in the present study.

Key words : Cow and buffalo bulls, Preputial microbes, Testosterone, Thyroid hormones

Delayed puberty in buffalo calves, as compared to cow bulls, is a major concern of buffalo breeders and scientists endeavouring improvement in the reproductive efficiency of this species. Low testosterone levels were recorded in pubertal buffalo bulls experiencing delayed puberty (Perara *et al.* 1979). Induced hypothyroidism resulted in impaired pituitary gonadotrophin secretion and delayed puberty in rams and ewes (Chandrasekhar *et al.* 1985) and thyroxine administration restored cyclicity and spermatogenesis in thyroidectomised ewes and rams respectively (Brooks *et al.* 1964).

In addition, preputial micro-organisms often contaminate ejaculates with resultant poor fertility results. Preputial microbial load increases in mature bulls with age (Kher and Dholakia 1984), but no literature is available on preputial microbial load of pubertal buffalo bulls. Present study therefore apart from determining peripheral plasma concentrations of thyroid hormones and testosterone, also looked at the preputial microbial load in pubertal buffalo bull calves, and these parameters were compared with mature buffalo and cow bulls.

Present address : [1]Associate Professor, Department of Veterinary Gynaecology and Obstetrics, [2]Assistant Scientist, Department of Veterinary Gynaecology and Obstetrics, [3]Assistant Professor, Department of Veterinary Microbiology, [4]Research Officer, Department of Veterinary Physiology.

MATERIALS AND METHODS

Experimental animals and management

Present study was conducted in January 1995 on pubertal buffalo bull calves (n=7), mature buffalo bulls (n=8) and mature cow bulls (n=6) maintained under optimal conditions of feeding and management of semen bank, bhiwani and Hisar.

Blood and preputial sampling

Morning (9:00 to 10:00 AM) jugular venous blood samples were collected in heparinized glass tubes, centrifuged and plasma stored at –20°C pending radio-immuno assay of hormones. Taking all aseptic precautions, preputial cavity of each bull/bull calf was flushed with 100 ml sterile phosphate buffered saline (PBS, pH 7.2) for isolation of micro-organisms. Preputial washings could not be obtained from 3 aggressive buffalo bulls.

Radio-immunoassay (RIA) of hormones

Blood plasma samples were analyzed for determining thyroid hormones (T_3 and T_4) and testosterone concentrations using RIA kits supplied by Bhabha Atomic Research Centre, India and Diagnostic Products Corporation , USA respectively . Inter-assay and intra-assay variations for T_3 were 10.6 and 4.65% and for T_4 these were 14.69 and 2.96% respectively. The assays used highly specific antisera and sensitivity of the kits for T_3 , T_4 and testosterone were 0.24 ng/ml , 0.50 ng/ml and 0.04 ng/ml, respectively.

Table 1. Plasma hormonal concentrations and preputial microbial load in buffalo bull calves, buffalo bulls and cow bulls

Animals	Mean ±SE plasma concentrations (ng/ml)			Preputial microbial load		
	Testosterone	T_3	T_4	Sterile isolates	Single infection	Mixed infection
Buffalo bull calves (n=7)	0.24±0.09[a]	0.56±0.08	51.37±5.24	4	2	1
Buffalo bulls (n=8)	0.69±0.30[a]	0.66±0.07	48.24±4.48	3	Nil	2
Cow bulls	6.59±1.60[b]	0.67±0.09	58.40±8.27	2	2	2

a/b Values with different superscripts in a column differ significantly ($P<0.05$).

Microbial examination

Preputial flush samples were centrifuged at 4 000 rpm for 30 min at 4°C and the supernatant was decanted and sediment was responded in 1.0 ml sterile PBS for microbial isolation. Each sample was inoculated on a set of 3 media (10% Sheep Blood Agar, MacConkey Agar and Nutrient Agar) and incubated at 37° C for 24-48 hr. Smears were prepared for each isolated colony, heat fixed and stained by Gram's method for morphological evaluation and Gram's reaction. Further identification was done by the method described by Cowan and Steel (1974).

RESULTS AND DISCUSSION

Endocrine parameters

Plasma testosterone concentrations in pubertal buffalo bull calves and buffalo bulls were similar, though these were significantly ($P<0.05$) lower than cow bulls (Table 1), suggesting possible genetic / species variations. Low testosterone concentrations may also be associated with the reported poor libido and prolonged reaction time in buffalo bulls (Perera *et al.* 1979), in view of a positive correlation of plasma testosterone profiles and libido in cow bulls (Henny *et al.* 1990). The lower testosterone concentrations, though non-significantly, in buffalo bull calves as compared to mature buffalo bulls, may indicate delayed pubertal steroidogenesis, as reported in Holstein bulls (Aman and Walker 1983).

Thyroid hormones regulate gonadal steroidogenesis (Hagino 1976, Brooks *et al.* 1964) and thyroidectomy at 4 months of age abolished libido in Jersey bulls (Peterson *et al.* 1941). In the present study, libido was not directly recorded but poor libido in buffalo males (Perera *et al.* 1979), as also suggested by low plasma testosterone concentrations, could not implicate thyroid -mediated effects since plasma T_3 and T_4 concentrations were similar in buffalo bulls, buffalo bull calves and cow bulls (Table 1). Adult level function of thyroids in pubertal buffalo bull calves elucidates maturation of the hypothalamo-hypophyseal-gonadal axis for pubertal testicular functions as reported in rats (Hagino 1976). In addition , it corroborates the concept of stable thyroidal activity from puberty to sexual maturity for initiation of pubertal endocrine and exocrine testicular functions (Agarwal *et al.* 1983).

Table 2. Bacterial isolates from the preputial cavity of cow and buffalo bulls

Bacterial isolate	Total isolations	Single isolates	Mixed infection
Bacillus spp.	2	1	1
Pseudomonas spp.	2	Nil	2
Escherichia coli	4	1	3
Staphylococcus aureus	2	1	1
Klebsiella spp.	2	1	1
Streptococcus spp.	1	Nil	1
Corynebacterium pyogenes	1	Nil	1

Preputial microbes

A majority of buffalo bull calves (4/7) which were yet to be introduced in the artificial insemination programme, were negative for bacterial infection in the preputial cavity and 2 were infected with a single isolate (Table 1). Similarly, mature buffalo bulls were also less prone to preputial infections as the preputial fleshings from 3 to 5 buffalo bulls were sterile, though the rest 2 were having mixed infection. Most of the cow bulls (4/6), however, harboured bacteria in their preputial cavity, either single or in mixed infection. The most common bacterial isolate was *Escherichia coli*, which was isolated from 4 cases, mostly occurring in mixed infection (Table 2) *Pseudomonas* spp., *Streptococcus* spp. and *Corynebacterium pyogenes* were isolated only in mixed infections from 2, 1 and 1 case respectively. *Pseudomonas* spp. has previously been recorded in mixed infection of bovine prepuce (Reddy *et al.* 1971). Kher and Dholakia (1984) recovered *Corynebacterium pyogenes* from buffalo bull semen. However, *Klebsiella* spp. recovery from prepuce is rare in literature. *Staphylococcus aureus* and *E. coli* were the most common preputial microbes recovered from bovine semen (Gangadhar *et al.* 1986). Recorded highest frequency of preputial infection in older cow and buffalo bulls than in pubertal buffalo bull calves explains frequent services and preputial folds rendering them more suseeptible to preputial infection (Reddy *et al.* 1971).

ACKNOWLEDGEMENT

We thankfully acknowledge the co-operation of In-charge, Semen Bank, Bhiwani, Hisar during these investigations.

REFERENCES

Agarwal V K. Agarwal S P. Narinder Singh and Dwarkanath P K. 1983. Levels of serum thyroid hormone in relation to age and

sexual development of crossbred bulls. *Indian Journal of Animal Sciences* **53** : 1063-65.

Aman R P and Walker O A. 1983. Changes in pituitary-gonadal axis associated with puberty in Holstein bulls. *Journal of Animal Science* **57** : 437-42.

Brooks J R, Ross C V and Turner C W. 1964. Effect of thyroidectomy on reproductive performance of ewe and semen quality in rams. *Journal of Animal Science* **23** : 54-58.

Chandresekhar Y, Holland N K, Diocchio M J and Setchell B P. 1985. Spermatogenesis, seminal characteristics and reproductive hormone induced hypothyroidism and hyperthyroidism. *Journal of Endocrinology* **105** : 39-46.

Cowan S T and Steel K J. 1974. *Mannual for Identification of Medical Bacteria*. 2nd edn. University Press, Cambridge, U K.

Gangdhar K S, Rao A R and Subbiah G . 1986. Effect of antibiotics on bacterial load in frozen semen of buffalo bulls. *Indian Veterinary Journal* **63** : 489-93.

Hagino M. 1976. Effect of thyroid hormone on initiation of pubertal oestrus in the rat. *Acta Biologica Academy of Science* **27** : 93-99.

Henny S R, Killian G J and Deaver D R. 1990. Libido, hormone concentrations in blood plasma and semen characteristics in Holstein bulls. *Journal of Animal Science* **68** : 2784-92.

Kher H N and Dholakia P M. 1984. Bacteriological studies on bovine semen. *Indian Journal of Animal Research* **5** : 81-84.

Perera B M A O, Pathraja N, Motha M X J and Weerasekera D A. 1979. Seasonal differences in plasma testosterone profiles in buffalo bulls. *Theriogenology* **12** : 33-39.

Peterson W E A, Spielmen A , Pomeroy B S and Boyd W L. 1941. Effect of thyroidectomy upon sexual behaviour of male bovine. *Proceedings of Society of Experimental Biological Medicine* **46** : 16-30.

Reddy J C, Krishnamurthy P S and Venkataswamy V. 1971. Bacterial flora of prepuce and the effect of intra-preputial treatment on the bacteriological quality of semen. *Indian Veterinary Journal* **48** : 722-27.

Indian Journal of Animal Sciences **68** (4) : 344-345, April 1998

Transplacental transmission of *Trypanosoma evansi* in mice

P C SARMAH[1]

Punjab Agricultural University, Ludhiana ,Punjab 141 004

Received : 20 April 1997; Accepted :27 October 1997

Key words : Mice, Parasitaemia, Transplacental transmission, *Trypanosoma evansi*

There are few sporadic case reports on the congenital transmission of *Trypanosoma evansi* resulting termination of pregnancy in naturally infected animals (Paikne and Dhake 1972).The present communication reports the results of investigation on such transmission through experimental infection.

White albino mice were used to study the congenital transfer of infection as laboratory animal model. Adult female mice were allowed to mate with their counterpart in individual cages. Five such mice in their near term pregnancy were infected each with 1×10^3 live *T.evansi* (Equine strain) intraperitoneally in 0.5 ml of Alsever's solution. Parasitaemia in these mice were checked daily by examining wet film preparation of tail blood. On day 5 post-infection when the mice developed teeming parasitaemia, they were euthenized by cervical dislocation. Foetuses were carefully dissected away from the mothers' uterus. Maternal blood from the placentome area and the foetal blood from the cord and tail were examined microscopically in wet film preparation and the parasitaemia was graded following the method of Gill (1965) as R (rare - less than 1 parasite) , + (1 - 5 parasites) , ++ (5 - 10 parasites) ,+++ (10 -20 parasites) ,++++ (20 - 40 parasites) and T (teeming -more than 40 parasites / high power microscopic field). Each of the 20 foetuses recovered was washed thoroughly in distilled water to remove contamination with mothers' blood.

Foetuses were ground separately with 1-2 ml of 0.85 % NaCl solution containing 1000 IU penicillin and 100 /µg streptomycin/ ml in pestle and mortar for a minute. The homogenate after filtered through 2 - layered gauze was examined under microscope and subinoculated in 0.5 ml amount intraperitoneally into mice. The recipient mice were observed for parasitaemia through tail blood examination and mortality.

Present address : [1]Associate Professor, Department of Parasitology, College of Veterinary Science, Assam Agricultural University, Guwahati , Assam 781 022.

Microscopic examination of blood from foetuses was negative to the parasite. Similar examination of saline suspension of foetal homogenates indicated rare parasitaemia in only 30% foetuses . However ,subinoculation test was 100% positive as evidenced by appearance of parasites in blood of recipient mice within 5-6 days and death of all due to teeming parasitaemia in the next 5 - 7 days. The subinoculation test was more sensitive compared to microscopic examination of blood for detection of parasites in this study conforming to the report of Pegram and Scott (1976). However, Kraneveld and Mansjoer (1954) in their experimental study failed to demonstrate the parasite in foetuses of infected rat and rabbit. Such failure might attribute to the test adopted for detection of parasite similar to the negative findings of microscopical examination of blood of foetuses in the present study.

Results of the present study confirm the congenital transmission of *T.evansi* (Paikne and Dhake 1972) in a naturally infected she buffalo. Although no satisfactory explanation is available in regards to the mechanism by which the parasite crosses the placenta, it has been reported that the African trypanosomes possess a vascular permeability increasing factor (Goodwin and Guy 1973) and biologically active products (Tizard *et al.* 1978) for alteration and destruction of mammalian cells. In spite of teeming parasitaemia in the experimental mother mice at the placentome with least intervening layers in their endotheliochorial placentation , very few trypanosomes have been able to reach the foetus. This might be because of presence of some additional factors in the placenta which restrict the free entry of trypanosomes into foetus.

Lohr *et al.* (1986) observed abortion as the most frequent clinical sign in natural *T.evansi* infected buffaloes during later stage of pregnancy . Edeghere *et al.* (1992) in their experimental infections with *T. vivax* and *T. brucei brucei* reported abortion still birth , premature delivery with no gross placental lesion in heifer and ewes under the stress of pregnancy ,infection induced anaemia, weight loss and hormonal imbalance. However, no abortion or still birth in any of the infected pregnant mice of present study was noticed by day 5

post-infection when they were sacrificed . This variation might be due to the strain of *T. evansi* and the species of experimental host. It is well established that mice has the highest susceptibility to *T.evansi* compared to cattle and buffaloes which are chronic carriers of infection. The strain of *T.evansi* used in the present study was highly virulent since infection with single parasite caused death of mice within 10 days. Hence, infection with 1×10^3 parasites in the present study otherwise would have been expected to cause death of pregnant mice without abortion normally in another 1 or 2 days (Sarmah *et al.* 1985) . This paper forms the basis of further study on pathophysiology of transplacental transmission of *T.evansi* in large animals.

ACKNOWLEDGEMENT

The author is grateful to the Punjab Agricultural University for facilities provided.

REFERENCES

Edeghere H , Elhassan E , Abenga J , Osue H O , Lawani F A G and Falope O. 1992. Effects of infection with *Trypanosoma brucei brucei* on different trimesters of pregnancy in ewes. *Veterinary Parasitology* **43** : 203-09.

Gill B S. 1965. Studies on protective immunity of *Trypanosoma evansi* . *Journal of Comparative Pathology* **75** : 233-40.

Goodwin L G and Guy M W. 1973. Tissue fluid in rabbits infected with *Trypanosoma (Trypanozoon) brucei* . *Parasitology* **66**: 499-513.

Kraneveld F C and Mansjoer M. 1954 Intrauterine infection in Surra. *Hemera Zoa* **61** : 97 -108.

Lohr K F , Pholpark S, Siriwan P, Leesirikul N , Srikitjakarn L and Staak C .1986. *Trypanosoma evansi* infection in buffaloes in North -East Thailand. 2. Abortions. *Tropical Animal Health and Production* **18** : 103-08.

Paikne D L and Dhake P R. 1972. Abortion due to *Trypanosoma evansi* in a she- buffalo *(Bubalus bubalis)* . *Indian Veterinary Journal* **49**: 1091-92.

Pegram R G and Scott J M .1976. The prevalence and diagnosis of *Trypanosoma evansi* infection in camels in Southern Ethiopia .*Tropical Animal Health and Production* **8**: 20 -27.

Sarmah P C ,Bhttacharyulu Y and Gupta P P. 1985. Experimental *Trypanosoma evansi* (Steel, 1985) Balbiani ,1988 in mice and guineapigs. *Acta Veterinaria* (Beograd) **35** : 333-40.

Tizard I , Nielsen K H , Seed J R and Hall J E. 1978. Biologically active products from African trypanosomes .*Microbiology Review* **42**: 661-81.

Indian Journal of Animal Sciences **68** (4) : 346-347, April 1998

Mycoplasmal pneumonia in goats

N S KANWAR[1], O P PALIWAL[2], N C SRIVASTAVA[3] and RAM KUMAR[4]

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122

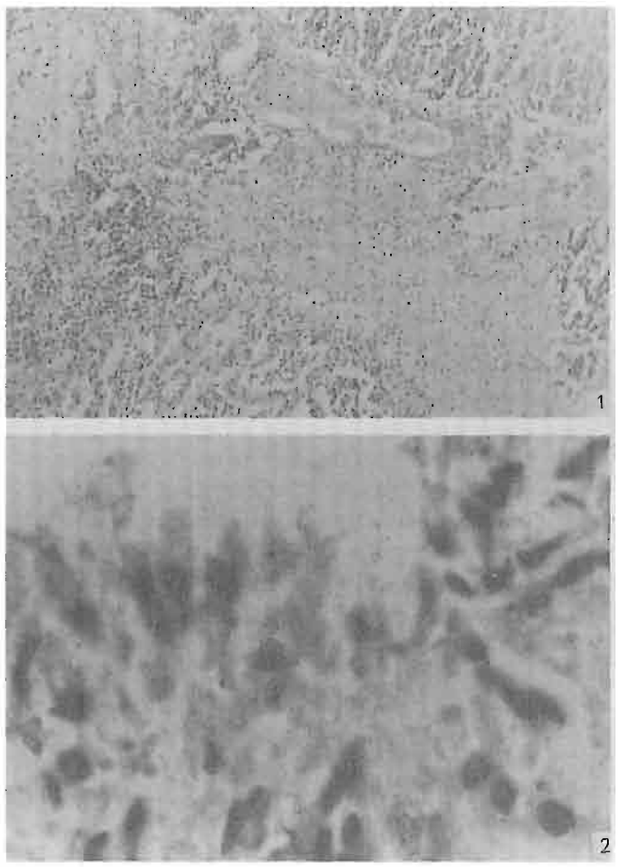
Received : 18 August 1997 ; Accepted : 28 October 1997

Key words: Goats, Mycoplasma, Pneumonia

Mycoplasma induces pleuropneumonia in goats. Various species of mycoplasma, viz . *Mycoplasma mycoides* sub sp. *capri, M. mycoides* sub sp. *(LC), M. capricolum, M. capricolum* sub sp. *capripneumoniae, M. agalactiae, M. ovipneumoniae* etc. have been associated with pneumonia in goats causing high morbidity and mortality in different parts of the country (Uppal *et al.* 1987, Mukherjee *et al.* 1990, Srivastava *et al.* 1996). In the present investigation attempt has been made to isolate mycoplasma species from pneumonic lesions and pathomorphological alterations have been described.

An etiopathological investigation on mycoplasma pneumonia was conducted by examining 9432 apparently healthy goats slaughtered in various abattoirs of the defence establishment of North and Eastern parts of the country during the period from March 1995 to May 1996. Affected lungs (374) were fixed in 10% neutral buffered formalin and Vandegriffts solution, processed through conventional procedure for tissue sectioning and stained with haematoxylin and eosin. Duplicate sections were stained with modified Brown and Brenn and Giemsa's method of staining for demonstration of organism (Luna 1972). Suspected specimens (21) were subjected for isolation of mycoplasma on liquid as well as solid mycoplasma medium and the isolates were identified on the basis of biological, biochemical and serological tests using standard techniques (Alluotto *et al.* 1970).

Out of 9432 lungs examined 374 (3.97%) specimens revealed pneumonia, of which 7 specimens (1.87%) had lesions of mycoplasma pneumonia based on histopathological examination and microbial investigation. The incidence observed in this study (1.87%) was much lower than 9.94% recorded by Mukherjee *et al.* (1990). The lowered incidence



Figs 1-2. 1. Mycoplasma pneumonia: Thickened and widened interlobular septa, engorgement of blood vessels and infiltration of polymorphs and mononuclear cells. H&E ×.100. 2. Mycoplasma organism on peribronchiolar epithelium Giemsa × 1000.

can be explained by the fact that apparently healthy animals declared by antemortem examination were only examined.

The pathological changes of lungs were variable amount of congestion and consolidation in different lobes alongwith thickened, prominent pleura and interlobular septa. Histologically, lung parenchyma had variable degree of

Present address: [1] Second-in-command (Pathologist), Central Military Veterinary Laboratory, Meerut Cantt , Uttar Pradesh 250 001.

[2] Principal Scientist,[4] Senior Scientist, Division of Pathology;[3]Principal Scientist, Division of Bacteriology and Mycology.

hepatization, serofibrinous exudation with infiltration of polymorphs, mononuclear cells in pleura and interlobular septa (Fig.1). Accumulation of inflammatory cellular exudate mainly neutrophils, mononuclear cells and alveolar macrophages in alveoli, bronchi and bronchiolar lumen, peribronchial / peribronchiolar lymphoid hyperplasia and perivascular lymphocytic cuffing were the main features. In all the specimens presence of minute coccoid organism morphologically indistinguishable from mycoplasma were seen in special stained sections (Fig. 2). Almost similar lesions were also observed by earlier workers (Alka-Sharan 1986, Srivastava *et al.* 1989). In 2 cases mixed infection of pasteurella and hydatid cyst were encountered and in 1case isolation of *Corynebacterium pyogenes* alongwith mycoplasma indicated concurrent infection mainly due to secondary bacterial complication. Nayak and Bhowmik (1988) suggested endotoxin librated by mycoplasma organism produces toxic injury to vascular endothelium which may help in proliferation of secondary bacteria thus complicate the picture by masking mycoplasmal lesions. The possibility of lowered incidence as observed presently also can not be ruled out.

Out of 21 specimens processed for isolation of mycoplasma organisms, only in 2 cases showed changes in pH towards acidic side on liquid medium and fried egg colonies on solid media. On the basis of biological, biochemical and serological tests both the cultures were *Mycoplasma agalactiae*. Although this species of organism is known to produce mastitis in adult goats and septicaemia, arthritis in kids but its involvement in pneumonia is rarely reported (Srivastava *et al.* 1996). This could be explained due to sharing affinity of this organism for connective tissue and lymphatics (Jubb *et al.* 1993) which are affected in the pneumonic lesions of mycoplasma.

ACKNOWLEDGEMENTS

We thank Director of the institute for the facilities provided and Professor J Necolet, Director, Institute of Veterinary Bacteriology, Berne, Switzerland for serotyping of mycoplasma isolates.

REFERENCES

Alka-Sharan. 1986. Studies on the pathology of pneumonia in goats with special reference to mycoplasma infection. *Indian Journal of Veterinary Pathology* **10** : 122-23.

Alluotto B B, Wittler R G, Willium C O and Faber J E. 1970. Standardized bacteriological techniques for the characterization of mycoplasma species. *International Journal of Systemic Bacteriology* **20** : 35-38.

Jubb K V F, Kennedy P C and Palmer N. 1993. *Pathology of Domestic Animals*. 4th edn, Vol. 3, pp. 467. Academic Press, Inc, California.

Luna L G. 1972. *Histological Staining Methods of Armed Forces Institute of Pathology*. 3rd edn. McGraw-Hill Book Co., New York.

Mukherjee S, Som T L and Bhowmik M K. 1990. Caprine mycoplasmal pneumonia in West Bengal (India). Isolation, identification *in vitro* drug sensitivity and pathological studies. *Indian Journal of Animal Health* **29** : 3-4.

Nayak N C and Bhowmik M K. 1988. Pathology of spontaneous mycoplasmal polyarthritis in goats. *Indian Journal of Veterinary Pathology* **12** : 1-6.

Srivastava N C, Chattopadhyaya S K, Singh V P and Tripathi B N. 1996. Isolation of *Mycoplasma agalactiae* from pneumonic sheep. *Indian Journal of Animal Sciences* **61** : 1000-02.

Srivastava N C, Sikdar A and Uppal P K. 1989. Pathogenicity of *Mycoplasma mycoides* sub sp. *capri* in goats by intratracheal route. *Indian Journal of Animal Sciences* **59** : 491-93.

Uppal P K, Srivastava N C and Kumar A A. 1987. Isolation of pathogenic strains of *Mycoplasma mycoides* sub sp. *capri* from an outbreak of contagious caprine pleuropneumonia in India. *Israel Journal of Medical Sciences* **23** : 231.

Indian Journal of Animal Sciences **68** (4) : 348-349, April 1998

Pasteurellosis in a hippopotamus in captivity

T G PRABHAKAR[1], B PREMKUMAR DAVID[2], ANDREW CHANDRAMOHAN[3] and R A VENKATESAN[4]

Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu 600 007

Received: 25 June 1997; Accepted : 28 October 1997

Keywords : Hippopotamus, *Pasteurella* spp., Pasteurellosis

Pasteurellosis is widely distributed in nature and is associated with a wide range of septicaemic infections in different species of animals including wild animals. Occurrence of pasteurellosis was reported in an elephant (De Alwis and Thambithurai 1965), zebra and tapir (Wallach and Boever 1983), big horn sheep, bison, black tailed deer and wild swine (Rosen 1970), snow leopard (Chaudhuri *et al.* 1992) and wild ruminants (Rhoades and Rimler 1992). In the present investigation, pasteurellosis was identified from a hippopotamus in captivity.

The carcass of an adult male hippo, about 4 years old was brought to the Department of Pathology, Madras Veterinary College, for postmortem examination from the Zoological Park, Vandalur (Chennai), Tamil Nadu. The animal was febrile and anorectic for a day. On the next morning it developed respiratory distress and died. Thorough postmortem examination revealed septicaemic changes throughout the gastrointestinal tract. Inflammatory changes with severe congestion were prominent in the stomach and intestines. Heart blood smears, heart blood swabs and a piece of spleen were collected for microscopic and cultural examination. Leishman stain revealed bipolar organisms. Biological testing (heart blood swab and spleen) of the cultured organisms inoculated subcutaneously confirmed the presence of bipolar organisms in the heart blood in 2 rabbits that succumbed 24 hr post inoculation (PI). Haemorrhagic tracheitis, the typical pathognomonic lesion of pasteurellosis and mild splenomegaly was noticed.

The rabbit heart blood was inoculated into broth and incubated at 37°C. The 8-hr-old broth culture as well as the rabbit heart blood containing *Pasteurella* species produced minute pin-head -sized dew drop-like colonies on blood agar within 6 hr of incubation at 37°C. The colonies were gram negative coccobacillary in morphology. There was no growth

Present address:[1]Assistant Professor, Department of Microbiology, Veterinary College and Research Institute, Namakkal, Tamil Nadu 637 007.

[2]Assistant Professor, Radioisotope Laboratory, [3]Assistant Professor, Department of Animal Biotechnology, [4]Former Professor and Head, Department of Microbiology, Madras Veterinary College.

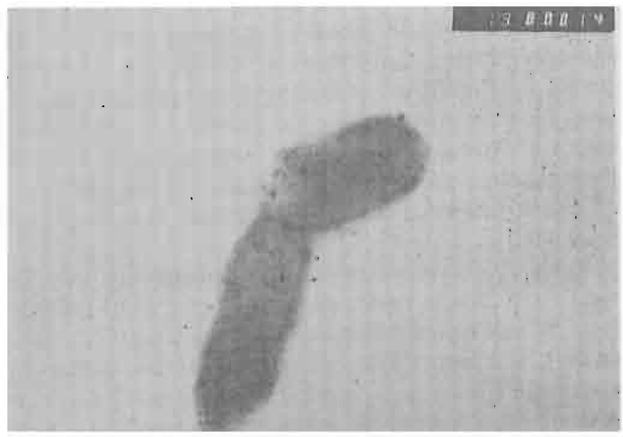


Fig. 1. *Pasteurella* spp. - Electron micrograph - Nonflagellated coccobacilli. (PTA- 13,000 ×).

on MacConkey agar and no haemolysis was observed in blood agar. The organism was further characterized biochemically (Buchanan and Gibbons 1974) in the genus *Pasteurella*. Other gram negative species like *Salmonella* strains from 287 hippo autopsy were isolated and 8 serum samples out of 144 gave positive agglutination test for brucellosis (Guilbride *et al.* 1962).

The 8 hr-old broth culture was charged on to carbon coated grids for transmission electron microscopy for the observation of morphological features using phosphotungstic acid negative staining and ammonium molybdate capsular staining. This revealed pure coccobacillary forms of bacteria without flagella (Fig. 1). A thin microcapsule was observed using ammonium molybdate staining.

Thus, on the basis of morphology, biochemical study by standard procedures and biological tests in rabbits, including electron microscopy, following isolation of the organism from the heart blood and spleen in pure culture, the organism was recognized as the genus *Pasteurella*.

REFERENCES

Buchanan R E and Gibbons. 1974. *Bergey's Manual of Determinative Bacteriology*. 8th edn. The Williams and Wilkins

Co., Baltimore.

Chaudhuri S, Mukherjee S K, Chatterjee A and Ganguli J L. 1992. Isolation of *P.multocida* F-3.4 from a still born snow leopard. *Veterinary Record* **130**: 36.

De Alwis M and Thambithurai U. 1965. *Ceylon Veterinary Journal* **13**: 17.*(fide.* Wallach J D and Boever W J. 1983.)

Guilbride P D L, Coyle T J, Mc Anulty E G, Barber L and Lomax G D. 1962. Some pathogenic agents found in hippopotamus in Uganda. *Journal of Comparative Pathology* **72**: 137.

Rhoades K D and Rimler R B. 1992. Serological characterization of *Pasteurella multocida* strains isolated from wild ruminants as capsular serogroup B. *Veterinary Record* **130**: 331.

Rosen M N. 1970. *Pasteurellosis. Infectious Diseases of Wild Mammals*. pp. 215 (Eds) Davis J W, Kanstad L H and Trainer D O. The Iowa State University Press. Ames, USA.

Wallach J D and Boever W J. 1983. *Diseases of Exotic Animals*. Chapter 18, 7th edn, p.789. W B Saunders Co., Philadelphia/ London/Mexico City/Riode Jarin/Sydney/Tokyo.

Indian Journal of Animal Sciences **68** (4) : 350-353, April 1998

Pathohistomorphology of swine male genitalia in India

R SINGH[1] and N S PARIHAR[2]

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122

Received : 16 June 1997;Accepted : 29 October 1997

Key words : Histomorphology, Pathology, Pigs, Swine male genitalia

Pathomorphological studies on affection of male pig genitalia were undertaken on abattoir materials in view of the meagre information available (Rao 1991).

The genitalia, from testicles to penises including prepuce and preputial diverticulum, from 82 entire (18 *desi*, 14 Landrace and 50 crossbreds) and 336 castrated (210 *desi*, 2 Landrace and 124 crossbreds) pigs, 3 to 18 months old at Bareilly and Aligarh abattoirs were examined and their representative specimens from different portions with or without lesions were processed conventionally for histopathological examination.

A total of 127 (30.38%) male pigs out of 418 (82 entire and 336 castrated) revealed occurrence of testicular degeneration 15.85% (13 cases), chronic epididymitis 1.22% (1 case), seminal vesiculitis 1.19% (5 cases), cystic prostate 1.91% (8 cases), bulbourethral adenitis 1.19% (5 cases), serrated penis 0.95% (4 cases), scarred penis 0.24% (1 case), nodulated penis 0.24% (1 case), calculus in preputial diverticulum 1.91% (8 cases), preputial diverticular ulcers 16.02% (67 cases) and interstitial cell tumor 1.21% (1 case) either singly or in combination (Tables 1, 2). The preputial diverticulum was affected in the highest number of the cases (75/162).

Characteristics of testicular degeneration consisting of smaller testicles, wrinkled tunica albuginea and mild to moderate degenerative changes in seminiferous tubules were similar to those observed by Shyu *et al.* (1985), McEntee (1990) and Ladds (1993).

Chronic granulomatous reaction, in the markedly enlarged left caudal epididymis with small grey foci of homogenous semi-solid tissue in an adult crossbred boar, occurred around extravasated spermatozoa with necrosis and calcification but without giant cells. A similar lesion was also noticed by Thomas and Raja (1974) in the tail of the right epididymis of 1 out of 137 boars examined. Escape of spermatozoa into the adjacent connective tissue has been attributed to infection, and the acid-fast material from the extravasated sperms to bring about the granulomatous response (McEntee 1990).

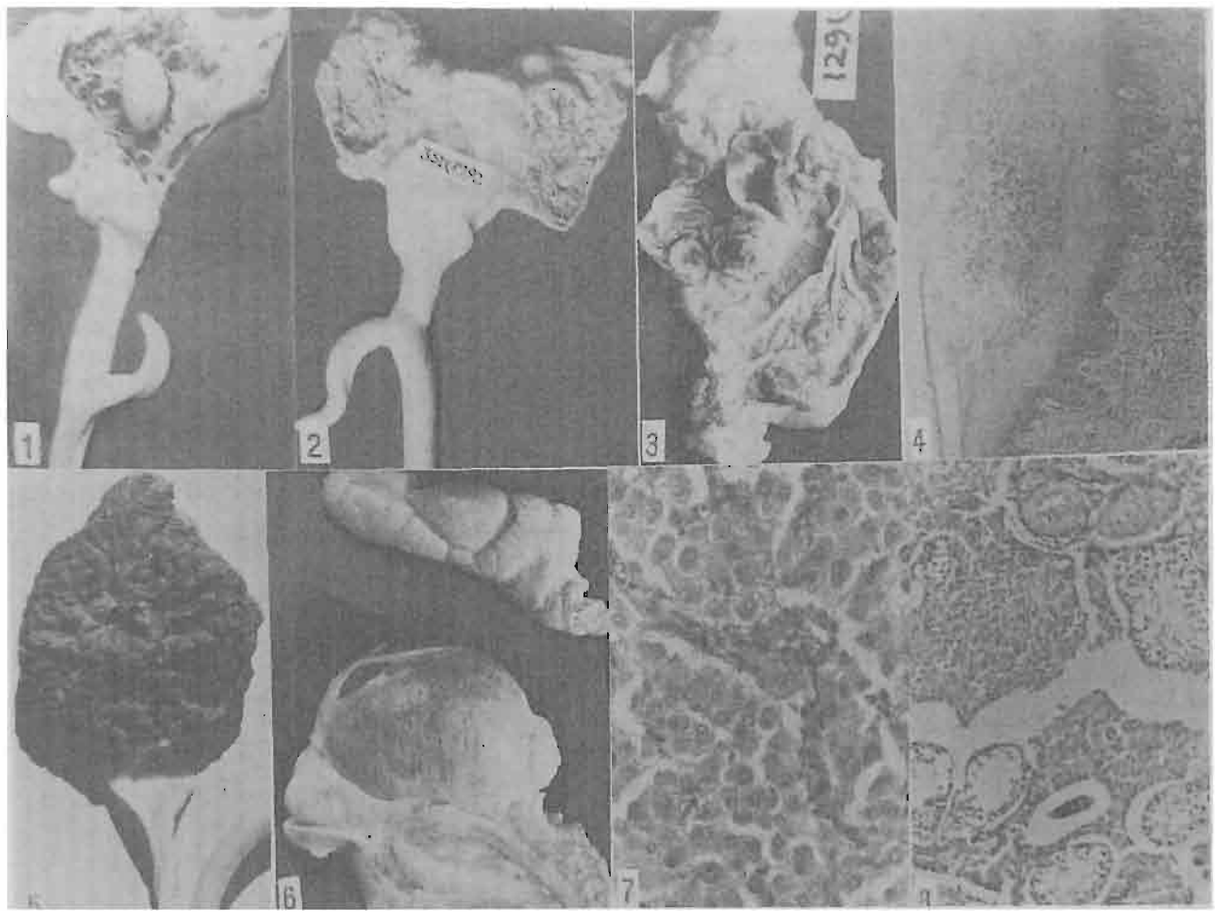
The accessory sex glands are known for showing lesions rarely (Ladds 1993). In the present study seminal vesiculitis (1.19%, 5/418), prostatitis (3.11%, 13/418), cystic body of the prostate glands (1.19%, 8/418) and bulbourethral adenitis (1.19%, 5/418) were encountered in castrated pigs conforming in histological details with Ball *et al.* (1964) in bulls. The changes could have been due to infection following inadequate asepsis under field conditions.

Penises have been reported to suffer from injuries in all species of domesticated animals (McEntee 1990) and the 2 (0.84%) cases encountered did not appear to be any exception. Tip of the glans penis in 1 case was missing with flattened whitish grey end. In another case a pin-head-sized nodule, made up of fibrous connective tissue, was present at the tip of glans penis. Serrations on the sides of glans penises in 4 (3 castrated, 1 entire) cases revealed irregular hyperkeratinization and could have been due to lack of normal sexual activity and consequent lack of wear and tear.

Calculi - one each in preputial diverticulum in 8 (1.91%) castrated pigs occurred as brown, smooth or rough, ovoid or spherical, hard structures about 10-20 mm × 5 mm (Fig. 1). The lining of diverticula was usually wrinkled and occasionally eroded leading to ulcer or plaque formation. McEntee (1990) had reported that calculi occasionally developed in preputial diverticula in boars. The occurrence of calculi in the diverticula of castrated pigs appeared to be rather distinct possibility in view of the continued accumulation of debris in the sexually non-active males.

Ulcers occurred in preputial diverticulum in 16.02% (67/418) cases. Their occurrence compared well with the incidence reported by Bollwahn and Schoon (1980, 15.7%, 6/38) but was distinctly lower than those reported by Ladds (1993, 25-40%) and Wieringa and Mouwen (1983, 43.33%). The ulcers were seen distributed irregularly on the lining, longitudinal septum or at the neck of the diverticulum. The lining of the diverticulum having ulcers was generally highly wrinkled with dirty brown debris. The ulcers were small pits up to 2 mm in diameter, single or multiple in 30 cases,

Present address: [1] Scientist (Senior Scale). [2]Acting Joint Director (CADRAD).



Figs1-8. **1.**A large calculus in the preputial diverticulum showing craterous ulcers in a castrated pig. **2.** Scattered yellowish brown ulcers with pitted centres in the lining of the preputial diverticulum of a castrated pig. **3.** Enlarged ulcers (plaques) in the preputial diverticulum. **4.** Preputial diverticulum. Dermis congested and infiltrated densely with neutrophilic leucocytes. Vacuolar degeneration and neutrophilic infiltration in stratum spinosum. H. & E. x 65. **5.** Preputial diverticulum with irregular rough folds in the thickened mucosa. **6.** Left testis (tumorous) highly reduced in size than the right normal testis in a *Desi* boar. **7.** Interstitial cell tumor. Highly proliferated interstitial cells arranged in sheets. H. & E. x 250. **8.** Interstitial cell mass containing degenerating and disappearing seminiferious tubules. H. & E. x 100.

craterous, 2 to 5 mm, in diameter, in 14 cases and plaque like, 10 to 15 mm wide, in 3 cases. In 20 cases both pits as well as plaques were encountered (Figs 2,3). The microscopic changes consisted of necrosis in the epidermis accompanied with dense infiltration with polymorphs in varying stages of degeneration, Occasionally the stratum germinativum had formed downward projections into the dermis which was also engorged and infiltrated with neutrophils, lymphocytes, plasma cells and occasionally eosinophils (Figs 4,5). The ulcerative with lesions of hyperkeratosis, parakeratosis and acanthosis in the adjoining lining were comparable with those reported by Bollwahn and Schoon (1980), and Ladds (1993). The precise etiology and the mechanism of formation of preputial diverticular ulcers have not yet been elucidated and need thorough investigation.

A case of interstitial (Leydig) cell tumor was encountered in a *desi* adult boar in the left testis which was severely reduced in size and weight (3.3 cm × 2.0 cm × 1.1 cm; 11.75g) compared to right normal testis (7.5 cm × 4.8 cm × 4.1 cm; 75.0 g; Fig. 6). On incision the left testis revealed large areas of yellow discoloration. Microscopically these yellowish foci consisted of varying sized masses of interstitial endocrine cells arranged in sheets exhibiting remnants of seminiferous tubules (Fig. 7). The cells were placed close to each other in the scanty but vascular stroma. The seminiferous tubules were few, compressed and atrophied (Fig. 8). McEntee (1990) reported comparable histological details in such tumors in dogs and bulls. Any report of the interstitial cell tumor in porcine testis, however, was not traceable in the available literature.

ACKNOWLEDGEMENTS

We are grateful to the Head, Division of Pathology, and

Table 1. : Spontaneously occurring pathological conditions in male pig genitalia

Lesion	Entire pigs (82)									Castrated pigs (336)			Freuency occurance (%)
	Desi (18)			Landrace (14)			Crossbred(50)			(210)	(02)	(124)	
	R	L	B	R	L	B	R	L	B	D	L	DXL	
Testicular degeneration	-	-	1	-	-	1	-	-	11	-	-	-	*13(15.85)
Chronic epidymitits	-	-	-	-	-	-	-	1	-	-	-	-	*1(1.22)
Seminal vesiculitis	-	-	-	-	-	-	-	-	-	5	-	-	5(1.19)
Postatitis	-	-	-	-	-	-	-	-	-	10	-	3	13(3.16)
Cystic prostate gland	-	-	-	-	-	-	-	-	-	5	-	3	8(1.91)
Bulbourethral adernitis	-	-	-	-	-	-	-	-	-	4	-	1	5(1.91)
Serrated penis	-	-	1	-	-	-	-	-	-	3	-	-	4(0.95)
Scarred penis	-	-	-	-	-	-	-	-	-	-	-	1	1(0.24)
Nodulated penis	-	-	-	-	-	-	-	-	-	-	-	1	1(0.24)
Calculi in preputial diverticulum	-	-	-	-	-	-	-	-	-	8	-	-	8(1.91)
Preputial diverticular ulcers	-	-	1	-	-	2	-	-	3	34	-	27	67(16.02)
Interstial cell turnor	-	1	-	-	-	-	-	-	-	-	-	-	*1(1.21)

Figures in parentheses indicate the number of animals *out of 82 entire pig genitalia.
D = *Desi*, L = Landrace, DXL = crossbred, R=right, L= left, B=bilateral.

Table 2 : Distribution of pathological lesions in male pig genitalia

Organ		Lesion	*Desi*	Landrace	Crossbred	Total
Entire male pigs						
Testis	(i)	Testicular degeneration	1	1	11	13
	(ii)	Interstital cell tumor	1	-	-	1
Testis + preputial diverticulum	(i)	Testicular degeneration + preputial diverticular ulcer	-	1	2	3
Epididymis	(i)	Chronic epididymitis	-	-	1	1
	(ii)	Total in entire males	2	2	14	18
Castrated male pigs						
Vesicular gland	(i)	Seminal vesiculitis	3	-	-	3
Vesicular gland + bulbourethral gland	(i)	Seminal vesiculitis + bulbourethral adenitis	1	-	-	1
Vesicular gland + penis	(i)	Seminal vesiculitis + scarred penis	1	-	-	1
Prostate gland	(i)	Prostatitis	8	-	3	11
	(ii)	Cystic prostate gland	5	-	3	8
Prostate + preputial diverticulum	(i)	Prostatis + preputial diverticular ulcer	2	-	-	2
Bulbourethral gland	(i)	Bulbourethral adenitis	3	-	-	3
Bulbourethral gland + preputial diverticulum	(i)	Bulbourethral adentis + preputial diverticular ulcer	-	-	1	1
Penis	(i)	Serrated penis	3	-	-	3
	(ii)	Nodulated penis	-	-	1	1
Preputial diverticulum	(i)	Calculi in preputial diverticulum	8	-	-	8
	(ii)	Preputial diverticular ulcers	36	2	26	64
	(iii)	Total in castrated males	70	2	34	106
	(iv)	Total number of cases in entire and castrated male pigs	72	4	48	114

Director of the Institute for providing the necessary facilities.

REFERENCES

Ball L, Young S and Carrol E J. 1968. Seminal vesiculitis syndrome: Lesions in genital organs of young bulls. *American Journal of Veterinary Research* **29** : 1173-84.

Bollwahn W and Schoon H A. 1980. Clinical and histological findings in preputial ulcers of boars. *Deutsche Tierartliche Wochenschrift* **87**: 50-53. *(fide Veterinary Bulletin* **50** : 7657.)

Ladds P W. 1993. The Male Genital System. *Pathology of Domestic Animals*. Vol. 3, 4th edn., pp. 471-528.(Eds) Jubb K V F, Kennedy P C and Palmer N., Academic Press, Inc., London.

McEntee K. 1990. *Reproductive Pathology of Domestic Mammals.* Academic Press, San Diego, California.

Rao Ramamohana A. 1991. *Reproductive Disorders in Indian Livestock.* Part 2. Reproductive disorders in the swine. Indian Council of Agricultural Research, New Delhi.

Shyu J J, Wu F M and Lin H K. 1985. Pathological studies of the genital system of young boars culled from a boar testing station. *Journal of the Chinese Society of Veterinary Science* **11** : 75-84.

Thomas V P and Raja C K S V. 1974. Spermatic granuloma in boars. *Kerala Journal of Veterinary Science* **5** : 113-20.

Wieringa W and Mouwen J M V M. 1983. Ulceration of the preputial diverticulum in Swine. *Tijdschrift voor Diergeneeskunde* **108** : 751-60. *(fide Veterinary Bulletin* **54** : 901.)

Indian Journal of Animal Sciences 67 (4) : 354-356, April 1998

Pathological investigations on tubal abnormalities in infertile buffaloes

C T KHASATIYA[1], S K SINGH[2], A J DHAMI[3] and F S KAVANI[4]

Gujarat Agricultural University, Anand Campus, Anand 388 001

Received : 18 August 1997 ; Accepted : 27 October 1997

Key words : Buffalo genitalia, Genitalia, Histopathological investigation, Tubal abnormalities

The information available on aetiopathological aspects of tubal affections as a cause of infertility or sterility in bovine is very meagre. Autopsy findings of the uterine tubes and ovarian bursae reveal approximately twice as many lesions as are detected clinically on rectal palpation (Dawson 1977). The present study was therefore undertaken to know the incidence and nature of tubal blockage by gross and histopathological investigations of tubal lesions in abattorial genitalia of buffaloes.

Genital organs of 250 nongravid freshly slaughtered buffaloes (46 heifers ; 204 buffaloes) were collected from the local abattoirs of Anand and Ahmedabad. Tubal potency was tested by hydro-airo-tubation as per Kavani *et al.* (1982). The gross structural abnormalities whether congenital or acquired, and of unilateral or bilateral involvement of oviducts and bursae were recorded following macroscopic examination of the dissected organs. The fallopian tubes of 21 apparently pathological genitalia found were collected in 10% formalin for studying microscopic lesions. Histopathological investigations of tubal lesions like salpingitis, hydro-salpinx and ovarobursal adhesions / blockage were undertaken as per the standard procedures with haematoxylin and eosin staining.

Grossly, the oviduct abnormalities were present in 63 (25.2%) of 250 genitalia investigated. Salpingitis was found bilaterally in 3 (1.2%) and unilaterally in 5 (2.0%) genitalia. Hydrosalpinx and pyosalpinx both were detected only bilaterally in 4 (1.6%) and 2 (0.8%) genitalia respectively. Whereas ovarobursal adhesions were present bilaterially in 4 (1.6%) and unilaterally in 7 (2.8%) genitalia. These plus some other unidentified lesions resulted in overall bilateral and unilateral tubal blockage in 18 (7.2%) and 45 (18.0%) of the genitalia. Among the later, the incidence of right and left tube involvement was 57.8 and 42.2% (26 vs 19 tubes). All these findings agreed well with the reports of Elsawaf and Found (1956), Sharma *et al.* (1968), Kavani *et al.* (1982, 1986), Nagarajan *et al.* (1987) and Khana *et al.* (1995). The later authors, however, reported a very high incidence of ovarobursal adhesions (19.4%) in buffalo genitalia. The higher incidence of tubal affections observed in pluriparous buffalo genitals as compared to heifers (80 vs 20%) could be due to age associated changes and repeated infection at each breeding/calving. The overall high occurrence of unilateral blockage than the bilateral one and more involvement of right than the left tube noticed in the present study was in agreement with the reports of Koike and Kawate (1959) and Kavani *et al.* (1982). The reason for this could be relatively more active nature of the right ovary and also the right tube being handled more often than the left during gynaecological examination (Roberts 1971).

Histopathological aspects of lesions studies in 35 fallopian tubes of 21 excised buffalo genitalia (Figs 1 to 4) revealed major structural alterations as hydrosalpinx (7), pyosalpinx (3), chronic salpingitis without (7) or with adhesion (8), and normal structures (10). These microscopic lesions were similar to the number of earlier reports (Dwivedi and Singh 1971, Kavani *et al.* 1986).

Hydrosalpinx

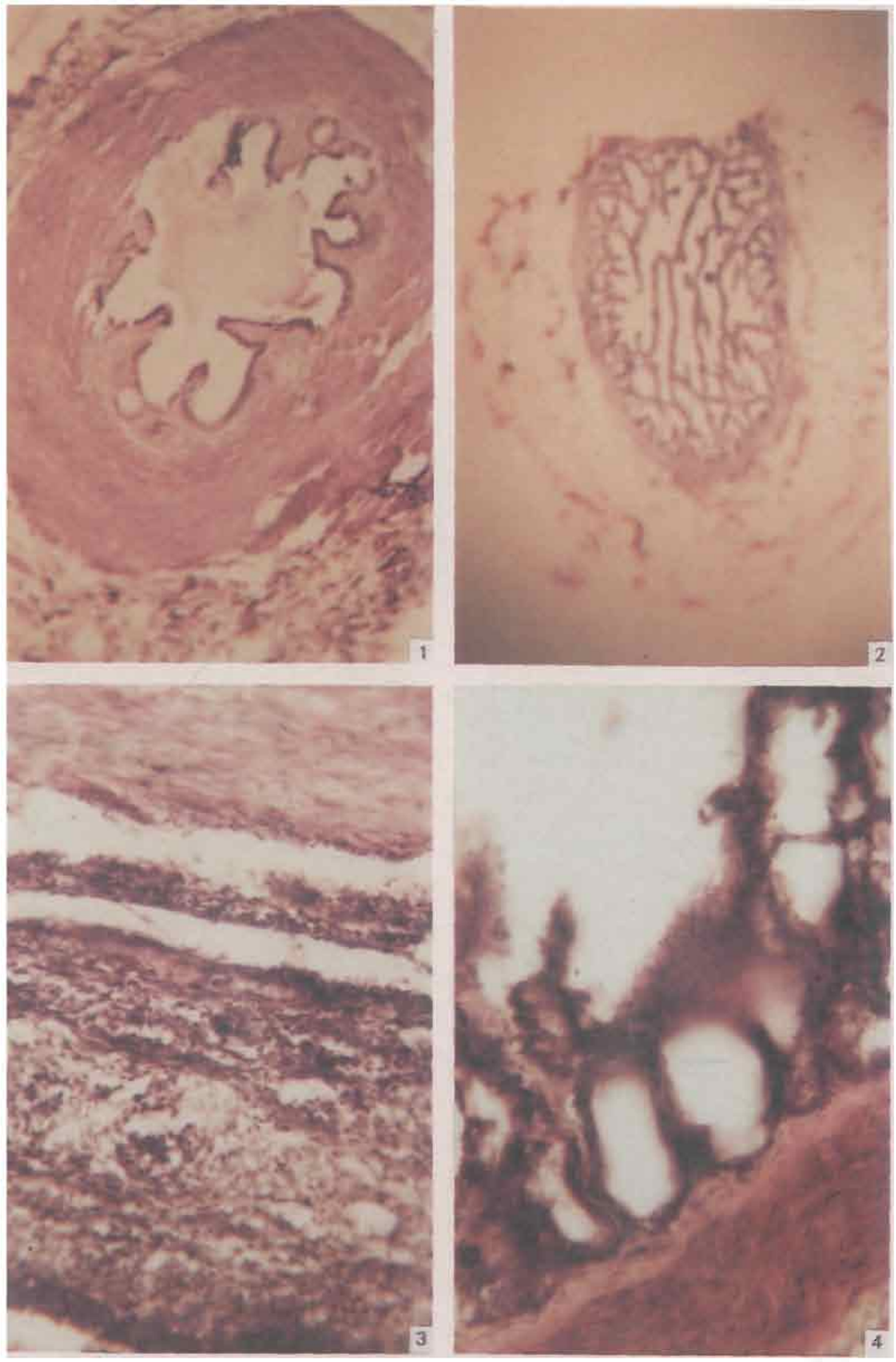
The most common histological changes noticed in hydrosalpinx (Fig. 1) were degeneration, desquamation atrophy of epithelia linings of mucosal and submucosal folds. The lumen contained eosinophilic homogenous material. The mucosal folds were either reduced or had last secondary folds. The muscularis layer revealed hyalinization. The lumen of the isthmic region of some hydrosalpinx revealed pink coloured serous material in addition to atrophy and loss of secondary folds and hyalinization of tubal wall. Sections through ampullae revealed ampullae revealed multiocular lumen laying across with many trabaeculae linked by cuboidal cells (follicular hydrosalpinx Fig, 2).

Pyosalpinx

Majority of pyosalpingitis cases revealed moderate to

*A part of M.V. Sc thesis of first author approved by Gujarat Agricultural University, Anand.

Present address : [1]Post-graduate student, Department of Gynaecology; [2]Assistant Professor, Department of Pathology; [3]Associate Professor, Department of Animal Sciences ; [4] Professor and Head, Department of Gynaecology and Obstetrics.



Figs 1-4. **1.** Section through isthmus. The lumen contained pink coloured homogenous material (hydrosalpinx). Mucosal folds are reduced in size with loss of secondary folds and hyalinization of muscularis. H & E × 60. **2.** Section through ampulla. Multiocular lumen laying across with many trabeculae lined by cuboidal epithelial cells (Follicular hydrosalpinx). H & E × 24. **3.** Section of fallopian tube. Marked thickening of the mucosal layer due to heavy infiltration of neutrophils, mononuclear cells, histiocytes and fibrocytes (pyosalpinx) H & E × 240. **4.** Section of fallopian tube showing desquamation of mucosal epithelium and submucosal cyst formation (chronic salpingitis). H & E × 60.

marked infiltration of neutrophils, mononuclear cells and hoisting mucosal and muscularis layers. Some cases revealed marked thickening of mucosal layer due to infiltration of neutrophils, macrophages, histocytes and fibrous tissue formation (Fig. 3).

Salingitis

Sections from infundibulum of some tubes revealed degeneration and desquamation of secondary folds and detachment of some mucosal folds from muscularis characteristic of salpingitis. Majority of oviducts (left 8 and right 4) revealed chronic salpingitis and histopathological lesions consisted of complete loss/reduction of mucosal folds, infiltration of mononuclear cells, histiocytes, fibrocytes and hyalinization of muscularis and moderate to massive fibrosis in some sections of usthmic region. However, sections from ampullae revealed denudation complete loss mucosal folds with hyalinization and fibrosis of tubal wall (Fig. 3). These lesions very well confirmed the reports of Dwivedi and Singh (1971), Kavani *et al.* (1986) and Khanna *et al.* (1995). Further, the association of salpingitis with infectious pyometra/metritis seen in 11 cases under study suggested the possibility of ascending infection as a basic cause of repeat breeding in buffaloes.

Submucosal cysts : Some sections (6) from cases of salpingitis and pyosalpingitis also revealed atrophy/ denudation of mucosal folds and moderate to massive fibrosis of tubular wall with multiple submucosal cyst formation in ampullary region (Fig. 4). Multiocular intramuscular cyst formation by the fusion of adjacent folds and denuded epithelial linings due to salpingitis that could account for tubal blockage in cows and buffaloes have also been reported by Lombard *et al.* (951), Roberts (1971), Dwivedi and Singh (1971) and Kavani *et al.* (1986).

Thus it is concluded that the autopsy findings of uterine tubes and ovarian bursa revealed approximately twice as many lesions as were detected clinically on rectal palpation, the tubal blocks were more frequent on right than the left side and chronic genital infection could lead to inflammatory and adhesive lesions in the oviduct leading to infertility or sterility in female bovines.

ACKNOWLEDGEMENTS

Authors express their thanks to the Principal, College of Veterinary Science and Animal Husbandry, Anand, for the facilities provided; and to the ICAR, New Delhi for the financial support extended in the form of Junior Research Fellowship to the first author.

REFERENCES

Dawson F L M. 1977. Reproductive potential in female cattle discarded as infertile. *Journal of Reproduction and Fertility* **51** : 53-56.

Dwuvedi J N and Singh C M. 1971. Studies on the pathology of the female reproductive organs of the Indian buffalo. 4. Observations on bursitis and hydrosalpinx cases. *Indian Journal of Animal Sciences* **41** : 27-32.

Elsawat S A and Found K A. 1965. Morphology of normal and diseased buffalo genital tracts as an aid to clinical diagnosis. *Veterinary Medical Journal (Giza)* **10** : 155-82.

Kavani F S, Bhavsar B K and and Kodagali S B. 1982. Studies on tubal patency in Surti buffaloes. *Indian Journal of Animal Reproduction* **2** : 19-22.

Kavani F S, Heranjal D D, Prajapati K S and Kodagali S B. 1986. Histopathological investigations of genitalia in repeat breeder cows. *Indian Journal of Animal Reproduction* **7** : 48-52.

Khanna A K, Sharma N C and Kali Charan. 1995. Fallopian tube normalities in buffaloes. *Indian Journal of Animal Sciences* **65** : 282-84.

Koike T and Kawate K. 1959. Studies on the tubal patency of the cow. I. Experiments made with slaughterhouse material. *Japanese Journal of Veterinary Research* **7** : 61-69.

Lombard L, Morgan B B and Menutt S H. 1951. Some pathological alterations of the bovine oviducts. *American Journal of Veterinary Research* **12** : 69-74.

Nagarajan Y, Neduncharalthan B, Kathiresan D, Quayam S A and thangaraj T M. 1987. The incidence of tubal abnormalities in shebuffaloes. *Cheiron* **16** : 137-40.

Roberts S J. 1971. *Veterinary Obstetrics and Genital Diseases.* 2nd edn., Edward Brothers Inc, Michitan.

Sharma O P, Bhalla R C and Soni B K. 1968. Studies on the abnormalities of ovarina bursa and oviduct in buffalo cows. *Indian Veterinary Journal* **45** : 293-96.

Indian Journal of Animal Sciences **68** (4) : 357-363, April 1998

Pathohistomorphology of swine female genitalia

R SINGH[1] and N S PARIHAR[2]

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122

Received : 8 May 1997; Accepted :27 October 1997

Key words : Female genitalia, Pathological lesions, Swine

Efficient reproduction is prerequisite for deriving maximum benefit from domestic animals particularly fast breeding pigs. Decline in reproductive efficiency has led to 18-70% culling of breeding stock in pigs (Viswanath *et al.* 1979, Kudlac 1980, Nath *et al.* 1982, Sing *et al.* 1983, Das *et al.* 1986, Kunneken *et al.* 1989, Obwolo and Lawson 1992). In spite of important role of genital disorders in pigs, information on their nature and occurrence has been meagre. The present communication attempts to supplement information on this fact and details the pathological abnormalities encountered in female pigs.

The genital tracts from ovaries to vaginal vestibule from 218 females (90 gilts and 128 non-gravid sows) were collected, observed and then representative tissue pieces from different portions/areas were processed conventionally for histopathological studies.

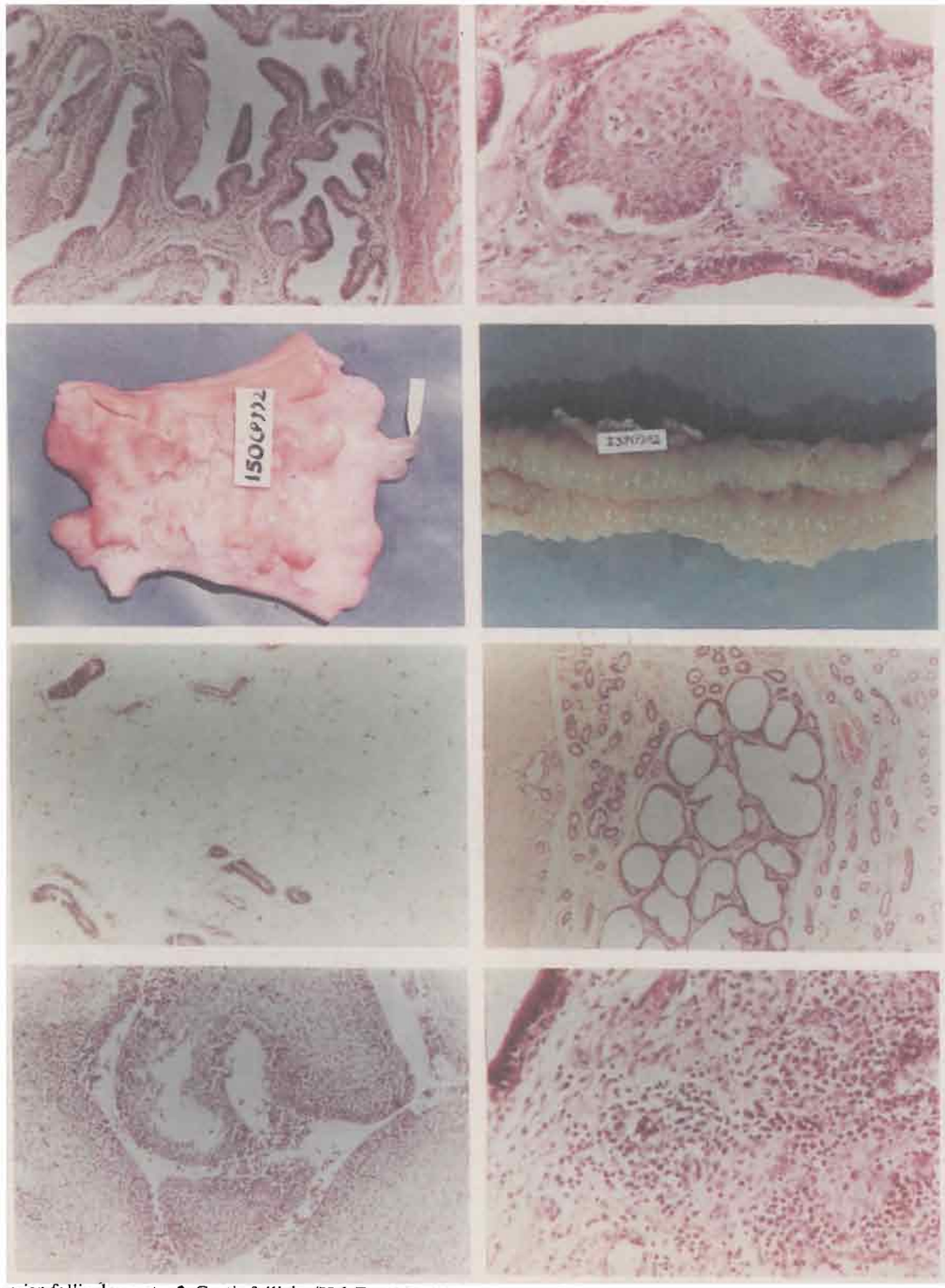
Examination of 218 female genitalia (90 gilts : 33 *desi*, 7 Landrace and 50 crossbreds and 128 non-gravid sows : 62 *desi*, 4 Landrace and 62 crossbreds) revealed occurrence of lesions in 103 (47.24%) cases in 27 (12.38%) gilts and 76 (34.86%) sows (Tables 1,2). The occurrence of the pathological conditions, other than congenital lesions (ovarian hypoplasia, uterine tube aplasia and hemiuterus, uterine tube diverticula, segmental aplasia of uterus, parovarian cysts and Gartner's ducts), was found almost at the same level as those reported by Viswanath *et al.* (1979) and Sing *et al.* (1983). Relatively lower occurrence (19.9 to 28.8%) was reported by Das *et al.* (1986), Nath *et al.* (1982), and Obwolo and Lawson (1992), which could have been due to the fact that they had not looked for microscopic lesions also. Silobad (1971), in his analysis of postmortem findings however, reported higher (59%) incidence of genital tract abnormalities. Amongst gilts 30% (27/90) and amongst sows 61.71% (76/128) genitalia revealed the lesions. Thus, the sows were found more commonly affected than gilts obviously due to their being subjected increasingly to stress factors connected with reproduction. The abnormalities recorded presently were higher in *desi* (60/95) than in crossbreds (39/112) and Landrace (4/11) females which is not in consonance with observations of Das *et al.* (1986).

The lesions occurred in ovaries (71/103; 68.93%), uterine tubes (38/103; 36.89%), and uteri (21/103; 20.38%) in decreasing order. Silobad (1971) and Gamcik and Sevcik (1961) reported comparable incidences of ovarian affections. But the occurrence of uterine tube affections was relatively low and the uterine affections quite high (Gamcik and Sevcik 1961).

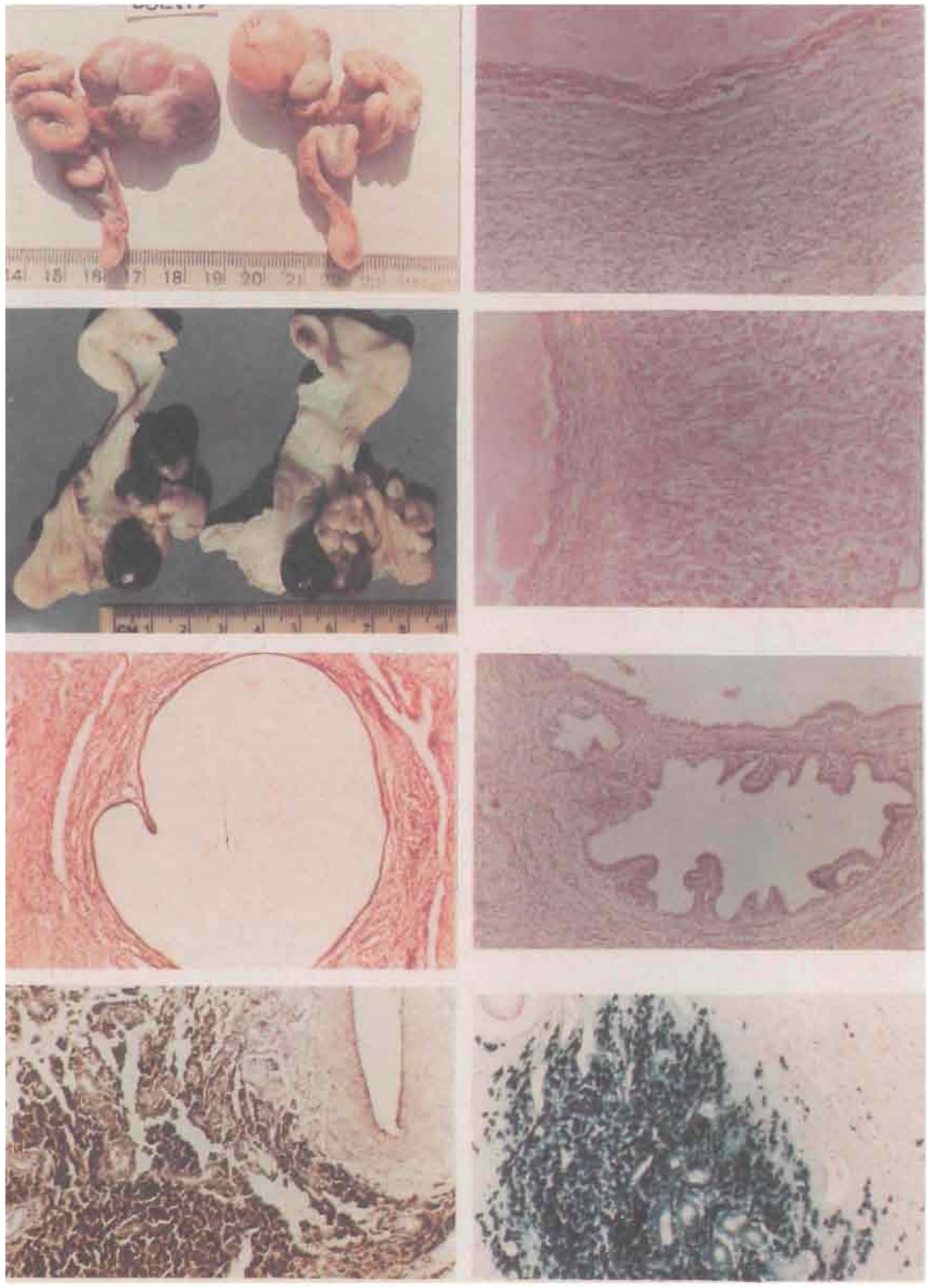
According to McEntee (1990) cystic ovaries accounted for about 10% of the reproductive problems in sows. Presently in 28 (12.82%) cases, 8 gilts and 20 sows, the ovaries revealed cystic follicles (11 cases), haemorrhagic follicular cysts (8 cases) and cystic corpora lutea (9 cases) (Figs1-4). The mechanisms of occurrence of cysts in individual cases is an area for further investigation. In 3 cases (1 gilt and 2 sows) variably distended rete ovarian cystic spaces lined with flattened to low columnar, usually non-ciliated epithelium rarely forming projections (Figs 5, 6) were detected microscopically in the medullary region in ovaries as observed in other domestic animals including swine (Gelberg *et al.* 1984). Though the role of cystic rete ovarii in reproductive process is not known, the condition is being reported in pigs most probably for the first time in the country. Oophoritis in 9 (4.12%) cases with infiltration with lymphocytes, plasma cells and eosinophils in connective tissue stroma and theca layers of follicles and corpora lutea, was akin to that described by McEntee (1990) and Sing *et al.* (1983). Haemosiderosis in ovaries recorded in 11 (5.04%) cases was also similar (Sing *et al.* 1983). The golden brown pigment within the macrophages in diffuse or aggregated fashion in the ovarian substance around receding corpora haemorrhagica, corpora lutea, corpora albicans and blood vessels formed blackish brown minute spots (Figs 7,8).

Microscopically oviducts in 28 (12.84%) cases (1 gilt, 27 sows) revealed foci of flattened stratified squamous epithelium on longitudinal folds (Figs 9, 10). Sing *et al.* (1983) observed lower occurrence (6.6%; 8/120) of cases of squamous metaplasia of uterine tubes with similar histological details.

Present address:[1] Scientist (Senior Scale), [2]Principal Scientist, CADRAD.



Figs 1-8. **1** Ovarian follicular cysts. **2.** Cystic follicle. (H & E ×100). **3.** Haemorrhagic follicular cysts. **4.** Cystic corpus luteum. (H & E ×100).**5.** Cystic rete ovarii. (H & E × 100). **6.** Cystic rete ovarii with papillary infoldings. (H & E × 65). **7.** Haemosiderin deposits in the ovarian cortex. (H & E × 100). **8.** Same as in Fig. 7. (Perl's stain × 100).



Figs 9-16. **9.** Uterine tube. Focal squamous metaplasia. (H & E × 65). **10.** Same as in Fig. 9. Proliferation and stratification of epithelial cells evident. (H & E × 250) **11.** Cystic elevations on the uterine mucosa. **12.** Uterine mucosa velvety and folded. **13.** Same as in Fig. 12. Myxomatous lamina propria-submucosa. (H & E × 100). **14.** Cystic endometrial glands. (H & E ×65). **15.** Acute endometritis. Neutrophilic infiltration prominent. (H & E × 100). **16.** Subacute endometritis. Lamina propria-submucosa infiltrated with mononuclear cells. (H & E × 250).

Table 1. Spontaneously occurring lesions in female pig genitalia

Lesion	Gilt (90)									Sow (128)									Frequency
	Desi (33)			Landrace (07)			Crossbred (50)			*Desi* (62)			Landrace (04)			Crossbred (62)			B occurrence (%)
	R	L	B	R	L	B	R	L	B	R	L	B	R	L	B	R	L	B	
Cystic follicle	-	-	3	-	-	-	-	-	-	2	-	4	-	-	1	-	-	1	11 (5.04)
Haemorrhagic follicular cyst	-	-	3	-	-	-	-	-	-	-	3	-	1	-	-	-	1	-	8 (3.66)
Cystic corpus luteum	4	-	-	-	-	-	-	-	-	-	1	5	-	-	-	1	-	-	11 (5.04)
Cystic rete ovarii	-	-	1	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	3 (1.37)
Oophoritis	-	-	-	-	-	1	-	-	3	-	-	4	-	-	-	-	-	1	9 (4.12)
Haemosiderosis in ovary	-	-	3	-	-	1	-	-	1	-	-	3	-	-	1	-	-	2	11 (5.04)
Ovarian hypoplasia	-	-	1	-	-	-	-	-	-	-	-	3	-	-	-	-	-	1	5 (2.29)
Parovarian cyst	3	-	-	-	-	-	2	-	-	5	2	6	-	-	-	6	3	3	30 (13.76)
Uterine tube squamous metaplasia	-	-	-	-	-	-	-	-	1	-	-	18	-	-	1	-	-	8	28 (12.84)
Salpingitis	-	-	1	-	-	-	-	-	1	-	-	2	-	-	-	-	-	1	5 (2.29)
Uterine tube aplasia and hemi-uterus	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1	-	2 (0.91)
Uterine tube diverticulum	1	-	-	-	-	-	-	-	-	2	-	1	-	-	-	2	-	-	6 (2.75)
Endometrial oedema	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-	2 (0.91)
Cystic endometrial glands	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	4	6 (2.75)
Endometritis	-	-	-	-	-	-	-	-	-	-	-	5	-	-	1	-	-	5	11 (5.40)
Segmental aplasia of uterus	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1	-	-	2 (0.91)
Cervicitis	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	2	3 (1.37)
Vaginitis	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	2 (0.91)
Gartner's duct	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 (0.45)

Figures in parentheses indicate the number of animals; R, right; L, left; B, bilateral.

The porcine fallopian tube has been considered comparatively more prone to develop squamous metaplasia than the uterine tube in other domestic animals (Kuttel 1935). Palludan (1996) opined that the changes were due to vitamin-A deficient diets which could also not be ruled out in the present studies. Salpingitis in 5 (2.29%) cases (1 gilt, 4 sows) was akin to those described by Kennedy and Miller (1993). The condition has been reported in 1.3 to 10% swine (Kuttel 1935, Silobad 1971, Viswanath *et al.* 1979, Sing *et al.* 1983, Das *et al.* 1986). Even the mildest form of salpingitis has been reported to create unfavourable environment for the survival of the ova and sperms (McEntee 1990) and the same could not be ruled out in these cases also.

Due to endometrial oedema uterine mucosa was swollen, glistening and covered with mucus in a *desi* sow (Fig. 11) and was turgid with bulging conglomeration of mucosal folds in a crossbred gilt (Fig. 12). Microscopically the connective tissue in deeper layers was scanty and rarefied between the endometrial glands (Fig. 13). The condition in bitches, cats and cows has been stated to occur mostly due to excessive and prolonged oestrogenic stimulation (Kennedy and Miller 1993). The uteri in 6 cases (2.75%) revealed dilatation of deeply situated endometrial glands with sparse mononuclear cell reaction in the adjacent connective tissue stroma (Fig.

Table 2 . Distribution of pathological lesions in female pig genitalia

Sl. No.	Organ	Lesion	Gilt			Sow			Total
			Desi	Landrace	Crossbred	*Desi*	Landrace	Crossbred	
1.	Ovary	Cystic follicle	3	-	-	3	-	1	
		Haemorrhagic follicular cyst	1	-	-	1	-	-	
		Cystic corpus luteum	1	-	-	3	-	-	
		Oophoritis	-	-	1	1	-	-	
		Haemosidrosis in ovary	1	-	1	2	1	1	
		Ovarian hypoplasia	-	-	-	2	-	-	
		Ovarian hypoplasia + cystic rete ovarii	1	-	-	-	-	-	
		Parovarian cysts	2	-	1	6	-	6	
		Parovarian cyst + cystic corpus luteum	-	-	1	-	-	1	
		Haemorrhagic follicular cyst + haemosidcrosis in ovary+salpingitis	1	-	-	-	-	-	
		Oophoritis + haemosiderosis in ovary	-	1	-	-	-	-	
		Cystic rete ovarii + oophoritis	-	-	-	1	-	-	
			10	1	4	19	1	9	44
2.	Ovary + uterine tube	Cystic follicle + uterine tube sq. metaplasia	-	-	-	3	1	-	
		Haemorrhagic follicular cyst + haemosiderosis in ovary	1	-	-	-	-	-	
		Cystic corpus luteum + uterine tube sq. metaplasia	1	-	-	1	-	-	
		Cystic corpus luteum+parovarian cyst+oophoritis+uterine tube sq. metaplasia	-	-	-	1	-	-	
		Oophoritis + uterine tube sq. metaplasia + salpingitis	-	-	1	-	-	-	
		Ovarian hypoplasia + uterine tube sq. metaplasia	-	-	-	1	-	-	
		Ovarian hypoplasia + uterine tube sq. metaplasia + salpingitis	-	-	-	-	-	1	
		Parovarian cyst + uterine tube diverticula	-	-	-	1	-	1	
		Parovarian cyst + uterine tube diverticula	-	-	-	1	-	1	
		Parovarian cyst + uterine tube sq. metaplasia	-	-	-	1	-	2	
		Parovarian cyst + uterine tube diverticula + cystic corpus luteum	-	-	-	1	-	-	
		Parovarian cyst + haemorrhagic follicular cyst + uterine tube squamous metaplasia + haemosiderosis in ovary	-	-	-	1	-	-	
		Parovarian cyst + cystic corpus luteum + oophoritis + uterine tube sq. metaplasia	1	-	-	-	-	-	
		Parovarian cyst + oopharitis + uterine tube sq. metaplasia	-	-	-	1	-	-	
			3	-	1	11	1	4	20
3.	Ovary + uterus	Oophoritis + cystic endometrial gland	-	-	1	-	-	-	
		haemorrhagic follicular cyst + endometritis	-	-	-	1	-	-	
		Parovarian cyst + endometritis	-	-	-	-	-	1	
			-	-	1	1	-	1	3
4.	Ovary + cervix	Parovarian cyst + oophoritis +	-	-	-	-	-	1	

(Table 2 continued)

(Table 2. Concluded)

Sl. No.	Organ	Lesion	Gilt			Sow			Total
			Desi	Landrace	Crossbred	*Desi*	Landrace	Crossbred	
		Haemosiderosis in ovary + cervicitis	-	-	-	-	-	1	1
5.	Ovary + uterine tube + uterus	Parovarian cyst + haemorrhagic follicular cyst + uterine tube sq. metaplasia + endometritis	-	-	-	1	-	1	
		Haemorhagic follicular cyst + segmental aplasia of uterus + uterine tube sq. metaplasia	-	-	-	-	-	1	
		Cystic rete ovarii + uterine tube sq. metaplasia + endometritis	-	-	-	-	1	-	
			-	-	-	1	1	1	3
6.	Uterine tube	Salpingitis	-	-	-	1	-	-	
		Uterine tube squamous metaplasia	-	-	-	5	-	1	
		Uterine tube aplasia and hemiuterus + uterine tube squamous metaplasia	-	-	-	-	-	1	
		Uterine tube aplasia and hemiuterus	-	-	1	-	-	-	
		Uterine tube diverticulum	1	-	-	1	-	1	
			1	-	1	7	-	3	12
7.	Uterine tube + uterus	Uterine tube sq. metaplasia + endometrial oedema	-	-	-	1	-	-	
		uterine tube sq. metaplasia + cystic endometrial gland	-	-	-	-	-	1	
		Salpingitis + Endometritis	-	-	-	1	-	-	
			-	-	-	2	-	1	3
8.	Uterus	Endometrial oedema	-	-	1	-	-	-	
		cystic endometrial gland	-	-	-	1	-	3	
		Endometritis	-	-	-	2	-	4	
		Segmental aplasia of uterus	-	-	1	-	-	-	
			-	-	2	3	-	7	12
9.	Cervix	Cervicitis	-	-	-	1	-	1	
			-	-	-	1	-	1	2
10.	Vagina	Vaginitis	-	-	2	-	-	-	
		Gartner's duct	1	-	-	-	-	-	
			1	-	2	-	-	-	3
		Total number of cases	15	1	11	45	3	28	103

14). The condition has been opined to occur following resolution of mild endometritis (Kennedy and Miller 1993). Endometritis with distinct leukocytic cell infiltration in the lamina propria and focal degeneration and denudation of mucosa were encountered in 11 (5.04%) sows (Figs 15, 16). Endometritis even of mildest magnitude has been considered of great significance in animal infertility (Kennedy and Miller 1993).

The pathological changes in 3 cases of cervicitis also resembled those described by Kennedy and Miller (1993). The low occurrence of vaginits in 2 (1.37%) cases simulated the findings of Viswanath *et al.* (1979) and Sing *et al.* (1983) and was in concordance with the fact that the vagina remained resistant to myriads of bacterial infections by virtue of oestrogenic effect, lactic acid production and stratified lining (Kennedy and Miller 1993).

ACKNOWLEDGEMENTS

The authors thank the Head, Division of Pathology, and Director of the Institute for providing necessary facilities.

REFERENCES

Das P K, Singh B K, Singh M P and Sinha A K. 1986. Incidence of reproductive abnormalities in female swine of different breeds - abattoir study. *Indian Veterinary Journal* 63 : 762-66.

Gamcik P and Sevcik A. 1961. Lesions in genital organs of slaughtered sows in relation to sterility. *Folia Veterinaria, Kosica* **5** : 187-200. *(fide Veterinary Bulletin* **32** : Abst. 3870.)

Gelberg H B, McEntee K and Heath E H. 1984. Feline cystic rete ovarii. *Veterinary Pathology* **21** : 304-07.

Kudlac E. 1980. Causes of impaired fertility in pigs. *Monatschefte fur Veterinaermedizin* **35** : 432-36. *(fide Veterinary Bulletin* **51** : Abst. 1303).

Kennedy P C and Miller R B. 1993. The female genital system. *Pathology of Domestic Animals.* 4th edn, Vol. 3,pp. 349-452.(Eds) Jubb K V F, Kennedy P C and Palmer N. Academic Press Inc., London,

Kunneken J, Peterson B and Ellinghaus . 1989. Importance of different fertility and health disorders as criteria for culling of sows. *Tierartliche Umschau* **44** : 145-50. *(fide Veterinary Bulletin* **60** : Abst. 1030.)

Kuttel J. 1935. Data concerning the pathologic anatomy of the bovine and porcine oviducts. Dissertation. Gyor, Hungary *(fide* McEntee 1990.)

McEntee K. 1990. *Reproductive Pathology of Domestic Mammals.* Academic Press, San Diego, California.

Nath K C, Purbey L N and Luktuke S N. 1982. Genital disorders of porcine female. *Indian Veterinary Journal* **59** : 878-81.

Obwolo M J and Lawson G. 1992. Abattoir survey of abnormal female porcine genital tracts in Zimbabwe. *Veterinary Record* **130** : 122-23.

Palludan B. 1966. Direct effect of vitamin A on boar testis. *Nature, London* **211** : 639-40.

Silobad B. 1971. Sterility in the sow with reference to pathological changes in genital organs. *Veterinaria Sarajevo,* **20** : 375-96. *(fide Veterinary Bulletin* **42** : Abst. 4168).

Sing S A, Nath K C, Rajkonwar C K and Chakraborty A K. 1983. Genital abnormalities in indigenous and exotic female pigs : Prevalence, gross and microscopic appearance. *Indian Journal of Animal Reproduction* **4** : 13-20.

Viswanath K S, Rama Rao P and Sastry G A. 1979. A note on pathoanatomy of female genital tract of pigs. *Indian Journal of Animal Sciences* **49** : 868-71.

Indian Journal of Animal Sciences 68 (4) : 364-365, April 1998

Preservability of Large White Yorkshire boar semen with BTS, Kiev and BL 1 dilutor from 18° to 20°C

R P PANDEY[1] and B K SINGH[2]

Ranchi Veterinary College, Ranchi, Bihar 834 007

Received: 28 April 1997;Accepted : 28 October 1997

Key words : BTS, Kiev, BL1 dilutor, White Yorkshire boars

Several extenders are currently in use by commercial artificial insemination centres and Kiev is the most widely used dilutor among them (Plisko 1965). In some experiments Beltsville thaw solution (BTS) has shown better fertility than Kiev (Aalbers *et al.* 1983). In India very limited work on preservation of boar semen (Murthy and Rao 1975, Tamuli *et al.* 1986, Rao *et al.* 1991, and Rao *et al.* 1992) has been conducted. The present study was carried out to compare the preservability of diluted boar semen with BTS, Kiev and BL1 dilutor and stored for 72 hr from 18° to 20°C.

Sperm rich portion of the ejaculates were collected from 5 Large White Yorkshire boars following the method of Zavos and Liptrap (1987) and were apportioned into 3 parts and diluted with BTS, Kiev and BL 1 dilutor at semen to extender ratio of 1:2. The diluted semen samples were kept separately in 100 ml flexible plastic bottle and stored at 180°C. Seminal characteristics like sperm motility, live sperm percentage, normal acrosome percentage, head and tail abnormalities were assessed at 24 hr interval following the standard procedure and results were analyzed statistically according to Snedecor and Cochran (1967).

Mean value of seminal characteristics is presented in Table 1. Highly significant effect of dilutors ($P < 0.01$) on sperm motility and percentage of live spermatozoa was recorded between 48 and 72 hr of preservation which is similar to the finding of Bhuyan *et al.* (1992). Vijaykumar and Iyar (1980) however, recorded lower motility with Kiev dilutor at 0-12, 12-36 and 36-48 hr of preservation. Tamuli *et al.* (1986) recorded lower percentage of live sperms in diluted semen during different stages of preservation to GPSE, a dilutor containing EDTA and thus substantiate the present finding. The average percentage of normal acrosome with BTS, Kiev and BL1 dilutor during different hours of preservation showed highly significant effect of dilutors at 24, 48 and 72 hr of preservation with lower value in BL 1 dilutor than other dilutors which is in accordance to the finding of Bhuyan *et al.* (1992). Nonsignificant effect of dilutors and preservation time on incidence of head abnormality was recorded which is agreeable with finding of Ruiz Gonzales (1986). The incidence of tail abnormalities varied from 1.837 to 12.183% with different dilutors at different hours of preservation. Highly significant effect of both hours of preservation and dilutors on the incidence of tail abnormalities was recorded. Increase in the incidence of tail abnormality after freezing and thawing has been reported by Baviskar (1985) and Sinha (1986) which supports the present findings.

Table1. Mean percentage (± SE) of seminal characteristics during preservation

Dilutors		Motility (hr)	Live sperm	Normal arsome	Head abnormality	Tail abnormality
BTS	0	81.465	83.641	98.166	2.625	2.025
		±0.196	±0.264	±0.133	±0.114	±0.195
	24	71.225	80.183	95.250	2.490	3.125
		±0.221	±0.460	±0.238	±0.140	±0.064
	48	67.308	45.058	85.333	2.540	5.100
		±0.312	±0.288	±0.329	±0.103	±0.073
	72	63.785	40.075	78.250	2.560	11.413
		±0.289	±0.492	±0.601	±0.099	±0.228
Kiev	0	80.291	84.258	98.083	2.650	1.837
		±0.266	±0.238	±0.126	±0.133	±0.229
	24	70.583	80.900	95.750	2.560	3.425
		±0.248	±0.194	±0.169	±0.101	±0.192
	48	66.250	74.983	85.416	2.430	5.858
		±0.129	±0.294	±0.187	±0.130	±0.341
	72	61.525	70.433	79.916	2.660	11.533
		±0.520	±0.503	±0.384	±0.005	±0.479
BL1	0	81.358	83.408	98.333	2.545	2.133
Dilutor		±0.297	±0.205	±0.127	±0.085	±0.187
	24	70.925	81.525	85.933	2.515	4.583
		±0.480	±0.298	±0.335	±0.085	±0.288
	48	62.725	70.925	80.833	2.535	7.216
		±0.411	±0.480	±0.335	±0.098	±0.255
	72	59.816	63.141	74.583	2.520	12.183
		±0.533	±0.422	±0.252	±0.095	±0.383

Present address:[1] Scientist (Animal Reproduction), Animal Production Research Institute, Pusa, Samastipur 848 125.

[2] Formerly Professor and Head.

The superiority of BTS and Kiev dilutor over BL 1 dilutor might be due to presence of sodium salt of EDTA in the 1 and 2 dilutors. The EDTA is not toxic to boar spermatozoa and also inhibits enzyme activity during storage (Plisko 1965). The inhibitory action of antirelaxin serum on porcine sperm motility is neutralized by EDTA (Juang *et al.* 1990) and prevent the decrease of aldolase enzyme action (Kurilo 1968) and thus may help in preserving sperm motility for longer period.

ACKNOWLEDGMENT

Authors thank the Dean (Veterinary Science), Birsa Agricultural University, Ranchi, for providing necessary facilities.

REFERENCES

Aalbers J G, Rademaker J H M, Groten H J G and Johnson L A. 1983. Fecundity of boar semen stored in BTS and Kiev. Zorlesco and Modeena extenders under field conditions. *Journal of Animal Science* **57**: 314-15.

Baviskar S J. 1985. 'Studies on effect of certain extenders on survival of goat spermatozoa.' M.V.Sc. Thesis, Punjabrao Krishi Vidyapeeth, Akola.

Bhuyan O, Bergohain B N, Ahmad K and Deka B C. 1992. Effect of Kiev, GPSE 1 and BL 1 extenders on the semen quality of crossbred boars. *Indian Journal of Animal Reproduction* **13** : 41-43.

Juang H H, Musah A I and Anderson L L. 1990. Ethylene diamine tetra acetic acid (EDTA) and caffeine are antagonistic to antirelaxin serum inhibition of porcine sperm motility. *Animal Reproduction Science* **22** : 253.

Konig I and Tschinkel I. 1970. Artificial insemination of pigs in German Democratic Republic after the Introduction of diluents containing EDTA. *Animal Breeding Abstract* **111** : 4076.

Kurlio J V G. 1968. Activity of aldolase in fresh and stored boar semen. *Svinarstro Kygiv* **7** : 41-52.

Murthy P R and Rao A R. 1975. Preservation of boar semen. *Indian Veterinary Journal* **52**: 415-20.

Plisko N T. 1965. A method for prolonging the viability and fertilizing ability of boar spermatozoa. *Svinovodstvo* **19** : 37-41.

Rao B V, Venkatamuni Chetty A, Ramachandraiah S V and Sreeraman P K. 1991. Preservation of native boar semen. *Indian Journal of Animal Reproduction* **12**: 148-50.

Rao M M, Ramachandraiah S V, Venkatamuni Cheety A and Sreeraman P M. 1992. Studies on crossbred boar semen characteristics and preservation. *Indian Journal of Animal Reproduction* **13**: 141-42.

Ruiz Gonzales M A. 1986. Dilution, storage and *in-vitro* evaluation of the potential fertility of boar semen. *Animal Breeding Abstract* **54**: 4582.

Sinha S N. 1986. 'Studies on freezability, certain characteristics and fertility of buck spermatozoa.' Ph.D. Thesis. Birsa Agricultural University, Ranchi.

Snedecor G W and Cochran W G. 1967. *Statistical Methods.* 6th edn. Oxford and I.B.H. Publishing Co., Calcutta.

Tamuli M K, Rajkonwar C K and Goswami B K. 1986. Preservation of boar semen. *Indian Journal of Animal Reproduction* **7**: 70-72.

Vijay Kumar V and Iyer C P N. 1980. Extenders for preservation of boar semen. *Kerala Journal of Veterinary Science* **11**: 215.

Zavos P M and Liptrap D O. 1987. Procedure for collection, evaluation, dilution and artificial insemination of boar spermatozoa. *Agriculture Practice - Swine Reproduction* **8** : 268.

Indian Journal of Animal Sciences 68 (4) : 366-367, April 1998

Comparative performance of Black Australorp, Malawian local chicken and their F_1 crossbred roasters

H J S KADIGI [1], R K D PHOYA[2] and A C L SAFALAOH[3]

Bunda College of Agriculture, University of Malawi, P.O. Box 219 Lilongwe, Malawi

Received: 20 Setpember 1996 ; Accepted : 28 October 1997

ABSTRACT

A study was conducted to assess and compare the growth performance and carcass yield traits of Black Australorp (BA), Malawian local chicken (LC) and their F_1 crosses at 8 weeks of age. For each genotype BA × BA, LC × LC, BA × LC and LC × BA, 45 birds were used. Commercial growers' mash was fed to experimental roasters *ad lib*. BA × BA roasters were significantly ($P< 0.05$) superior for live body weight at 20 weeks and average daily weight gain to 20 weeks of age. BA × LC and LC × BA were intermediate and significantly heavier than LC × LC. LC × LC consumed the least amount of feed, whereas BA × BA, BA × LC and LC × BA consumed similar amount of feed. LC × LC was significantly inferior ($P< 0.05$) for warm carcass, drumstick, thigh, breast meat and remaining carcass yields. Drumstick and thigh yields did not differ significantly ($P< 0.05$) between BA × BA and BA × LC. LC × BA had similar breast meat yield to BA × BA.BA × BA was significantly superior ($P< 0.05$) for remaining carcass yields while BA × LC and LC × BA were intermediate. There were no significant differences ($P> 0.05$) due to genotypes for proportion (%) of carcass parts yields except wing yields. Wings constituted a significantly higher proportion of LC × LC warm carcass, crossbreds were intermediate and BA × BA was significantly inferior.

Key words: Black Australorp, F_1 crosses, Malawian local chicken, Roasters

In Malawi, the Department of Veterinary Services, in its policies and plans for development of the livestock industry, recommended Plymouth Rock, Black Australorp and Rhode Island Red as suitable breeds of chickens to crossbreed with the Malawian local chicken (Upindi 1989). However, information on the exotic breeds tested with local chicken so as to come out with suitable recommendations is limited. This study was aimed at assessing and comparing the growth performance and carcass yield traits in Black Australorp, Malawian local chicken and their F_1 crossbred roasters under deep litter system of management.

MATERIALS AND METHODS

Black Australorp (8 cocks and 77 hens) at 32 weeks of age and mature Malawian local chicken (83) were purchased. Parent chickens were assigned to mating groups (Table 1), 903 eggs were collected and sent to Central Hatchery - Lilongwe for incubation. Day-old chicks hatched (706) were brooded. Wood shavings was used as litter material and charcoal burner as source of heat. Chicks were fed on commercial chick mash (20% CP) *ad lib.* and clean drinking water provided daily. Oxyvit chick formula was given in drinking water daily for the first 2 weeks and chicks were vaccinated against gumboro disease at 3 weeks of age. Brooding period lasted at 8 weeks of age and sexing of chicks was carried at this stage.

Table 1. Mating groups, number of eggs collected for incubation and number of chicks hatched from parent chickens.

Mating parents	Number of cocks	Number hens	Number eggs collected	Number chick hatched
BA × BA	4	38	232	152
LX × 4	37	253	192	
BA × LC	4	38	248	208
LC × BA	4	39	220	154
Total	16	152	953	706

BA, Black Australorp; LC, Malawian local chicken. Fertility, 81.90%; hatchability 91.80%.First pair of letters represents the sire and the second pair stands for dam.

From each of the 4 genotypes, 45 male chicks were reared as roasters under deep litter system to 20 weeks of age. Fifteen birds were assigned in pen measuring 2.00 m × 1.95 m covered with wood shavings. Chickens were vaccinated against Newcastle disease 3 days after being assigned into experimental units. Commercial growers' mash (16% CP) was fed to roasters *ad lib.* As group feeding was practised,

Present address: [1] Ministry of Agriculture, Livestock Division, P.O. Box 852, Shinyanga, Tanzania.

[2,3] University of Malawi, Bunda College of Agriculture, P.O. Box 219, Lilongwe, Malawi.

Table 2. Means and standard errors of growth performance in roasters

Parameter	BA × BA	LC × LC	BA × LC	LC × BA
Live weight (kg)				
8 weeks	0.34±0.12[a]	0.26±0.11[b]	0.32±0.15[ab]	0.3±0.19[a]
20 weeks	2.28±0.03[a]	1.783±0.03[c]	2.14 ± 0.01[b]	2.09±0.03[b]
Total feed intake (kg/roaster)	9.05±1.03[a]	8.16±1.01[b]	8.82±0.95[a]	8.79±0.89[a]
Daily weight gain (g/roaster)	22.92±0.93[a]	18.75±0.83[c]	21.69±0.78[ab]	20.66±0.99[b]
Feed : gain ratio	4.90±0.48	5.34±0.49	4.88±0.36	5.29±0.47

[a-c] Means of the same parameter in a row with different superscript are significantly different (P<0.05).

group weighing record for body weight, feed consumption and daily weight gains per roaster were kept per unit. At 20 weeks of age, 6 roasters from each genotype were slaughtered and dressed as per standard procedure and data were recorded for each roaster.

A Complete Randomized Design (CRD) was used in the study. Data were subjected to analysis of variance using Statistical Analysis System (SAS 1985) and General Linear Model procedure, Duncan's Multiple Range test (Montgomery 1984) method was adopted to test significant difference between means.

RESULTS AND DISCUSSION

LC × LC had significantly (P<0.05) the lowest body weight at 8 weeks of age (Table 2). Main cross roasters weighed lighter compared to its reciprocals probably due to BA heavier dam egg size (Al-Murrani 1978). However, at 20 weeks BA × BA roasters were significantly (P< 0.05) superior among genotypes (2.28 kg). Crossbreds were intermediate and significantly (P< 0.05) heavier than local roasters. Probably the fairly high heritability of body weight (0.54-0.62) might have influenced this trait in crossbred roasters, similar to what was reported by Goodman (1973). Daily weight gain was significantly higher in BA × BA (22.92 g/day) compared to that in crossbreds. However, local roasters (18.75g/day) were significantly inferior. This was reflected in live body weight at 20 weeks of age. Feed:gain ratio did not differ significantly among genotypes (4.90 for BA × BA, 5.34 for LC × LC, 4.88 for BA × LC and 5.29 for LC × BA).

Significant differences (P< 0.05) were observed for carcass traits among genotypes (Table 3) due to growth body weight differences among genotypes. Dressing percentage, neck and wing yields did not differ significantly among genotypes. Dressing percentages ranged from 70.34 to 71.62. No significant differences (P< 0.05) were observed for neck yield, drumstick and thigh yields between Black Australorp and main cross roasters when expressed as percentage of warm carcass yield. Lowest breast, drumstick and thigh yields were observed in local roasters. There was no significant difference

Table 3. Means (kg) of carcass part yields expressed as portions (%) of warm carcass weight and their standard errors of roasters at 20 weeks of age

Parameter	BA × BA	LC × LC	BA × LC	LC × BA
Wcwt. (kg)	1.71±0.04[a]	1.41±0.05[c]	1.57±0.04[b]	1.53±0.04[b]
Ds %	70.49±0.62	71.62±0.97	70.60±0.78	70.34±0.77
Dwt (kg)	0.27±0.02[a]	0.23±0.01[b]	0.27±0.01[a]	0.25±0.01[ab]
%	15.99±0.73	16.10±0.39	17.15±0.41	16.38±0.42
Thwt (kg)	0.34±0.01[a]	0.28±0.01[b]	0.32±0.01[a]	0.29±0.01[b]
%	19.66±0.40	9.78±0.63	20.45±0.39	18.83±0.49
Bwt (kg)	0.26±0.01[a]	0.22±0.01[b]	0.24±0.01[ab]	0.24±0.01[a]
%	15.37±0.31	15.46±0.65	15.17±0.35	16.29±0.66
Nwt (kg)	0.11±0.01	0.10±0.00	0.10±0.00	0.09±0.01
%	6.54±0.35	6.75±0.18	6.55±0.08	6.07±0.32
Wwt (kg)	0.19±0.01	0.18±0.01	0.19±0.01	0.18±0.00
%	11.20±0.42[b]	12.56±0.31[a]	12.19±0.27[ab]	11.79±0.23[ab]
Rcwt (kg)	0.53±0.03[a]	0.42±0.02[a]	0.47±0.01[b]	0.45±0.01[b]
%	31.24±1.28	29.36±0.08	28.50±0.57	30.63±0.24

[a-c] Means and proportions of the same trait in a row followed by different superscripts are significantly (P<0.05).

Wcwt, Warm carcass yield; Ds%, dressing percentage : Thwt, thigh yields ; Bwt, breast meat yield ; Nwt, neck yield; Wwt, wing yield; Rcwt, remaining carcass.

for breast meat yield between BA × BA and LC × BA genotypes. Local roasters had the least breast meat yield (0.22 kg). BA × BA roasters were significantly superior for remaining carcass yield while crossbreds were intermediate but superior to local roasters. Remaining carcass yields were 0.53 kg, 0.42 kg, 0.47 kg and 0.45 kg for BA × BA, LC × LC, BA × LC and LC × BA roasters respectively.

When carcass cut-up yields were expressed as proportion of warm carcass yield (Table 3), it was interesting to note that despite lower wing yield in LC × LC roasters, wings constituted significantly the higher proportion of warm carcass yield among genotypes (12.56%) while BA × BA was the least (11.20%). BA × LC and LC × BA (12.19% vs 11.79%) were intermediate.

ACKNOWLEDGMENTS

We thank GTZ and the British Council (ODA - Malawi) for supporting this study financially and Department of Veterinary Services for providing the Black Australorp parent chickens.

REFERENCES

AL-Murrani W K. 1978. Maternal effects on embryonic growth in poultry. *British Poultry Science* **19:** 277.

Goodman B L. 1973. Heritabilities and correlations of body weight and dressing percentage in broilers. *Poultry Science* **52:** 379.

Montgomery D C. 1984. *Design and Analysis of Experiments.* 2nd edn. John Wiley and Sons, New York.

SAS, 1985. *Statistical Analytical System. User's Guide,* SAS Institute Inc, Cary, NC, USA.

Upindi B G. 1989. Smallholder rural poultry production in Malawi (Unpublished paper).

Indian Journal of Animal Sciences **68** (4) : 368-372, April 1998

Estimation of combining ability in egg type chickens from a diallel cross

P K MISHRA[1], S C MOHAPATRA[2] and B N PATRO[3]

Orissa University of Agriculture and Technology, Bhubaneshwar, Orissa 751 003

Received : 25 February 1996; Accepted: 28 October 1997

ABSTRACT

Six single crosses and 6 reciprocal crosses were produced in the fashion of a modified diallel using 4 White Leghorn strains. Sires (20) from each strain were mated with 20 dams from each of the other 3 strains simultaneously. General combining ability was more important compared to other measures of genetic variability in the inheritance of all the traits considered except for egg weight. Specific combining ability appeared to be important only for body weight at 500 days of age and yolk index. Significant maternal effects were evident for egg production to 280 and 500 days of age, egg weight, yolk index and shell thickness. Sex-linked effects were important for body weight at 280 days of age, egg production to 260 and 280 days of age and average egg weight.

Key words : Combining ability, Diallel cross, Maternal effects, Sex-linked effects

The diallel analysis has become a common method to evaluate the kind and magnitude of genetic variability influencing different economic traits. It helps to identify superior cross combinations, provides information regarding the relative importance of certain kinds of specific combining abilities and indicates whether extensive crossing is useful to exploit non-additive genetic variation. A diallel analysis that allows the estimation of maternal and sex-linked effects is needed in choosing sire or dam line in a cross. With the above objectives a diallel mating experiment was conducted with 4 strains of White Leghorn chickens.

MATERIALS AND METHODS

Six single crosses and 6 reciprocal crosses (734 pullets) were produced as per a modified diallel (Griffing 1956) using 4 White Leghorn strains such as A, B, C and D. These strains had been maintained with IDS method of index selection (Osborne 1957 a,b) for increasing egg production to 280 days of age. Twenty sires from each strain were mated with 20 dams from each of the other 3 strains simultaneously. The progeny were obtained in 2 hatches. A standard method of feeding and management was followed throughout the experiment.

Diallel analysis on hatch corrected data was performed utilizing 2 methods. These are Henderson's (1948) method as described by Harvey (1966) with the mathematical model 7 and Griffing's (1956) method 3 in which F1s and the reciprocal crosses constitute the experimental material. Fixed model was used for analysis of data in both the methods since the lines used constituted the entire set of lines on which inferences are to be drawn.

Present address : [1] Associate Professor,[3]Professor and Head, Department of Animal Breeding and Genetics, Orissa Veterinary College.

[2]Formerly Director, Central Avian Research Institute, Izatnagar, Uttar Pradesh 243 122.

RESULTS AND DISCUSSION

The results from Henderson's analysis were compared with Griffing's analysis.

Henderson's method

Body weight (BW)

General combining ability (GCA) for body weight at all the 3 ages of measurement resulted in significant variation (Table 1). Variation due to specific combining ability (SCA) and residual reciprocal effects (sex-linkage) was significant only for BW at 500 days of age and 280 days of age respectively. The GCA estimate of strain A was positive and highest for BW at all the ages of measurement (Table 2). The estimates of SCA for BW at 500 days of age was positive and highest for AB and CD. The sex-linked effect at 280 days of age was positive in AC, CB, BA, DA, CD and BD in descending order, differing significantly from all other crosses except DB. GCA was found more important compared to other sources of variation at all the 3 ages of measurement. This was consistent with the findings of Hill and Nordskag (1958) and Verma (1980). Contrary to this Goto (1968) observed greater contribution from SCA than GCA for body weight at different ages.

Age at sexual maturity

Variation due to GCA only was significant for age at sexual

Table 1. Mean squares for combining ability from Henderson's method (H) and Griffing's method (G)

Traits	Sources								
	General combining abilities		Maternal abilities	Specific combining abilities		Residual reciprocal effects (sex- linkage)	Reciprocal effects	Error	
	H	G	H	H	G	H	G	H	G
BW at									
140 days of age	251867.77** (3)	6588.21** (3)	320.70 (3)	32341.76 (2)	550.54 (2)	3751.94 (3)	54.92 (6)	15913.85 (722)	261.20 (722)
280 days of age	4142343.26** (3)	7952.48** (3)	68499.71 (3)	10940.45 (2)	189.76 (2)	92835.07* (3)	1338.43 (6)	31229.89 (708)	522.52 (708)
500 days of age	359284.53* (3)	9693.90** (3)	201369.54 (3)	326622.51* (2)	8547.70* (2)	126924.12 (3)	1338.43 (6)	31229.89 (520)	552.52 (520)
Age at sexual maturity	1142.58** (3)	29.21** (3)	32.40 (3)	15.30 (2)	0.25 (2)	241.23 (3)	2.32 (6)	164.71 (707)	2.760 (707)
Surviror's egg production to									
260 days of age	256.92 (3)	14.36** (3)	333.56 (3)	190.84 (2)	3.21 (2)	907.14** (3)	10.59** (6)	175.25 (705)	2.94 (705)
280 days of age	226.51 (3)	13.36** (3)	564.66* (3)	308.51 (2)	5.49 (2)	722.47* (3)	11.06** (6)	198.07 (705)	3.33 (705)
500 days of age	3918.93** (3)	114.96** (3)	19797.54** (3)	428.31 (2)	24.65 (2)	1822.80 (3)	40.91 (6)	855.14 (520)	21.75 (520)
Average egg weight at 280 days of age	22.35 (3)	0.33 (3)	124.82** (3)	14.66 (2)	0.26 (2)	103.22** (3)	2.03** (6)	14.38 (666)	0.25 (666)
Albumin height	3.04 (3)	0.18* (3)	2.65 (3)	2.02 (2)	0.07 (2)	3.28 (3)	0.07 (6)	2.14 (468)	0.05 (468)
Albumin index	24.65* (3)	1.17** (3)	5.80 (3)	11.07 (2)	0.41 (2)	9.59 (3)	0.19 (6)	6.84 (468)	0.17 (468)
Yolk index	25.49** (3)	0.27** (3)	17.00** (3)	7.53* (2)	0.28** (2)	5.13 (3)	0.28** (6)	2.34 (468)	0.06 (468)
Shell thickness	411.95** (3)	7.85** (3)	121.97** (3)	10.87 (2)	0.40 (2)	19.67 (3)	1.77** (6)	13.88 (468)	0.35 (468)

Figures in the parentheses are the degrees of freedom; significant at * P<0005 ; ** P < 0.01.

maturity (Table 1). The GCA estimate of strain 'D' was positive and highest which differs significantly from that of strains B and C (Table 2). Similar to the results obtained in this study Sinha (1977) reported higher contribution of GCA than SCA for age at sexual maturity while Wearden *et al.* (1965) reported lesser contribution of GCA than SCA for this trait.

Egg production

For egg production up to 260, 280 and 500 days of age GCA was significant for 500 days of age, maternal ability was significant for 280 and 500 days of age, sex-linked effect was significant for 260 and 280 days of age and SCA was not significant for egg production irrespective of ages of measurement. GCA estimate for egg production up to 500 days of age was positive and highest in strain B and differed significantly from A and C strain (Table 2). Maternal abilities were positive and highest in strain A than that in others. BD cross had positive and highest sex-linked effect for egg production up to 260 and 280 days of age which differ significantly from other crosses (Table 2). Similarly Kim *et al.* (1975) observed higher contribution of GCA than SCA for egg production but Wearden *et al.* (1965) found that SCA was more important than GCA. Importance of maternal effects in inheritance of egg production as observed in this study is well documented (King and Henderson 1954, Tiwana and Dev 1972). Sex-linked effects in the inheritance of egg production was in agreement with the findings of Rengnathan (1976).

Egg weight

Maternal abilities and sex-linked effects were significant whereas GCA and SCA were nonsignificant for average egg weight at 280 days of age (Table 1). Least-square estimates of maternal ability was highest in A strain which differs significantly from other strains. The estimates of sex-linkage of AC, BA, BD and CD crosses have comparatively higher positive values which do not differ significantly. That the

Table 2. Least-squares estimates (Henderson's method) for overall mean, GCA, maternal abilities, SCA and residual reciprocal effects (sex-linkage) of various traits

Least squares	Body weight (g)			Age at sexual maturity	Survivors egg production to			Average egg weight at 280 days of age (g)	Alubmen height (mm)	Albumen index (angles)	Yolk index (angles)	Shell thickness (mm) × 100
	140d of age	280 d of age	500 d of age		260d of age	280 d of age	500 d of age					
Over-all mean	1095.41	1727.10	1898.68	175.868	58.836	72.881	167.885	55.568	7.449	18.915 (10.5%)	41.045 (43.1%)	29.636
General combining abilities												
A	48.18a	73.28a	77.54a	0.815ab	0.811	0.169	- 4.810b	- 0.274	- 0.065	- 0.341b	0.689a	1.856a
B	17.17c	- 17.69b	- 39.71b	- 3.617c	1.275	1.575	8.228a	0.553	- 0.201	- 0.484b	- 0.465b	1.519a
C	- 30.01b	- 44.85b	- 39.39b	- 0.407b	- 0.409	- 0.247	- 4.067b	- 0.308	- 0.081	0.445a	- 0.088b	- 1.423b
D	- 35.34b	- 10.74b	- 1.156ab	3.209a	- 1.677	- 1.497	0.649ab	0.029	0.185	- 0.380b	- 0.136b	- 1.952b
Maternal abilities												
A	1.21	- 26.78	- 33.77	- 0.722	2.528	3.334a	21.664a	1.239a	- 0.109	- 0.135	- 0.670b	- 1.578c
B	1.55	30.78	62.28	0.489	- 0.480	- 0.790b	- 8.133b	- 1.318c	- 0.067	- 0.008	0.383a	- 0.353b
C	- 1.62	- 5.66	- 39.09	0.243	- 1.239	- 1.333b	- 2.066b	- 0.047b	0.271	0.379	0.151a	0.750ab
D	- 1.14	1.66	10.58	- 0.010	- 0.809	- 1.211b	-11.465b	0.126b	- 0.095	- 0.236	0.136a	1.181a
Specific combining abilities												
AB	12.31	7.29	51.76a	0.114	0.012	-0.303	-1.765	-0.124	6.150	0.030	-0.162b	0.136
AC	-10.22	-6.40	-37.05b	-0.288	-0.902	-0.541	1.522	0.292	-0.094	-0.228	-0.027ab	0.109
AD	-2.59	-0.890	-14.71ab	0.174	0.890	1.344	0.243	-0.168	-6.056	0.198	0.189a	-0.245
BC	-2.59	-0.890	-14.71ab	0.174	0.890	1.344	0.243	-0.168	-6.056	0.198	0.189a	-0.245
BD	-10.22	-6.40	-37.05b	-0.288	-0.902	-0.541	1.522	0.292	-0.094	-0.226	-0.027ab	0.109
CD	12.81	7.22	51.76a	0.114	0.012	-0.803	-1.765	-0.124	6.150	0.030	-0.162b	0.136
Residual reciprocal effects (sex-linkage)												
AB	-1.01	-20.19bfg	-4.38	-0.241	1.082bcde	0.961abe	4.701	-0.907jm	-0.230	-0.352	-0.289	0.500
AC	7.80	33.50ac	43.00	0.037	1.490ae	1.237abcd	-0.805	0.920acd	0.037	-0.019	0.046	-0.358
AD	-6.79	-13.31bcef	-38.62	0.204	-2.572egh	-2.198degl	-3.896	-0.013defje	0.193	0.371	0.243	0.142
BA	1.01	20.19ad	4.38	0.241	-1.082deg	-0.961efg	-4.701	0.907bce	0.230	0.352	0.289	-0.500
BC	-0.86	-21.53beg	-18.23	1.324	-1.927cgh	-1.775cfg	-0.124	0.175ghk	-0.111	-0.051	-0.123	0.548
BD	-0.15	1.34aeg	13.85	-1.565	3.009a	2.736a	4.825	0.732abg	-0.119	-0.301	-0.166	-0.048
CA	-7.08	-33.50ef	-43.00	-0.037	-1.490efgh	-1.237bgh	0.805	-0.920im	-0.037	0.019	-0.046	0.353
CB	0.86	21.53ab	19.23	-1.324	1.927ac	1.775abc	0.124	0.175fh	0.111	0.051	0.123	-0.548
CD	6.94	11.97af	24.77	1.361	-0.437cdeh	-0.538ag	-0.929	0.745abf	-0.074	-0.070	-0.077	0.190
DA	6.79	13.31ae	38.62	-0.204	2.572ab	2.198ab	3.896	0.013ehil	-0.193	-0.371	-0.243	0.144
DB	0.15	-1.34aeg	-13.85	1.565	-3.009h	-2.736fht	-4.825	-0.732hm	0.119	0.301	0.166	0.048
DC	-6.94	-11.97cdef	-24.77	-1.361	0.437bede	0.538hfg	0.929	-0.745ikm	0.074	0.070	0.077	-0.190

Values with atleast one common superscript for an effect within a trait do not differ significantly (P<0.05).Overall mean in angles (arcsin) were reconverted to percentage and are shown in parentheses.

sex-linked effects are important in the inheritance of egg weight was reported by Poggenpoel and Erasmus (1978). Evidence in support of maternal effects influencing egg weight was provided by King and Henderson (1954) and Clayton and Robertson (1966). However, lesser contribution of GCA and SCA to egg weight as observed in this study was contrary to the reports of Goto and Nordskog (1959).

Egg quality traits

From Henderson's analysis it was revealed that GCA was significant for albumen index, yolk index and shell thickness; whereas SCA was significant only for yolk index (Table 1). Maternal effect was significant for yolk index and shell thickness. GCA estimates were positive and highest in A strain for yolk index and shell thickness whereas for albumen index it was C strain (Table 2). For yolk index AD and BC crosses had positive SCA estimates whereas negative for other crosses. As regards to maternal effect strain B, C and D had positive estimates for yolk index, whereas for shell thickness C and D strain had positive estimates. Since egg quality traits are moderate to highly heritable in chickens, significant GCA effects as observed in this study for these traits are not unexpected. Verma (1980) obtained significant GCA, SCA and reciprocal effects

Table 3. Estimation of general combining ability (gi) effects from Griffing's method for various traits

Traits	Parents				SE (gi)	SE (gi-gj) i=j
	A	B	C	D		
Body weight at						
140-day-old	48.78a**	17.94b*	-30.82	-35.91	6.99	11.43
280-day-old	59.98**	-2.30	-47.68	-9.91	9.90	16.16
500-day-old	60.66**	-3.58	-58.91	6.82	21.38	34.92
Age at sexual maturity (d)	0.45	-3.37	-0.28	3.20**	0.72	1.17
Survivor's egg production to(n)						
260-day-old	2.07*	1.03	-1.03	-2.08	0.74	1.21
280-day-old	1.84*	1.18	-0.91	-2.10	0.79	1.29
500-day-old	5.59**	3.55	-4.04	-5.10	2.02	3.30\
Average egg weight at						
280-day-old	0.34	-0.10	-0.33	0.09	0.22	0.36
Albumin height	-0.12	-0.23	0.22*	0.14	0.10	0.16
Albumin index	-0.41	-0.49	0.63**	0.26	0.18	0.29
Yolk index	0.35**	-0.27	-0.01	-0.07	0.10	0.17
Shell thickness	1.07a**	1.34a**	-1.05	-1.36	0.25	0.42

Significant at * P<0.05;** P<0.01.

Values with the same superscript do not differ significantly (P<0.05) in comparison of parents for a particular trait.

for albumen height and albumen index.

Griffing's method and its comparison with Henderson's method

The GCA from Griffing's analysis (Table 1) were significant for all the traits except egg weight. These results were similar with those reported earlier from Henderson's method except for egg production up to 260 and 280 days of age and albumen height. While GCA effect was significant for these traits with Griffing's method it was nonsignificant from Henderson's method. Because GCA estimates from Griffing's analysis is not totally free from the maternal effects. SCA from Griffing's method was in complete agreement with that of Henderson's analysis. Both the methods suggested that SCA effects were relatively less important compared to GCA.

Deviation in 2 methods of analysis was observed only for egg production up to 500 days of age which was significantly influenced by maternal effects. In all other instances there was complete agreement with respect to reciprocal effects (Griffing's method) which is either due to maternal and or sex-linked effects (Henderson's method).

High GCA compared to SCA for most of the characters considered in this study indicated that the performance of single crosses can satisfactorily be predicted from the performance of the pure strains. Difference if any, in performance of single crosses than expected on the basis of GCA would be due to presence of maternal and/or sex-linked effects.

REFERENCES

Clayton G A and Robertson A. 1966. Genetics of changes in economic traits during the laying year. *British Poultry Science* **7** : 143-51.

Goto E. 1968. Genetic parameter estimates and combining ability analysis in meat type chicken populations. *Animal Breeding Abstract* **36** : 4079.

Goto E and Novdskoy A W. 1959. Heterosis in poultry.4. Estimation of combining ability variance from diallel crosses of inbred lines in the fowl. *Poultry Science* **38** : 1381- 88.

Griffing, B. 1956. Concept of general and specific combining ability in relation to diallel crossing systems. *Australian Journal of Biological Science* **9** : 463-93.

Harvey W R.1966. *Least-squares Analysis of Data with Unequal Subclass Numbers.* United States Department of Agriculture, USA.

Table 4. Estimate of specific combining ability (sij) effects (Griffing's method) for various traits

Traits	Parental combination						SE (Sij) i=j
	AB	AC	AD	BC	BD	CD	
BW at 140-day-old	12.80	-10.22	-2.58	-2.58	-10.22	12.80	6.59
280-day-old	7.28	-6.40	-0.88	-0.88	-6.40	7.28	9.33
500-day-old	51.78*	-37.08	-14.69	-14.69	-37.08	51.38*	20.16
Age of sexual maturity (d)	0.11	-0.28	0.17	0.17	-0.28	0.11	0.67
Survivor's egg production to (n)							
260-day-old	0.01	-0.90	0.88	0.88	-0.90	0.01	0.70
280-day-old	-0.80	-0.54	1.34	1.34	-0.34	-0.80	0.74
500-day-old	-2.52	2.44	0.08	0.08	2.44	-2.52	1.90
Average egg weight at							
280-day-old	-0.12	0.29	-0.16	-0.16	0.29	-0.12	0.20
Albumen height (mm)	0.01	-0.14	0.13	0.13	-0.14	0.01	0.09
Albumen index (angles)	0.04	-0.34	0.29	0.29	-0.34	0.04	0.16
Yolk index (angles)	-0.24	-0.04	0.28**	0.28**	-0.04	-0.24	0.09
Shell thickness (mm) × 100	0.20	0.16	-0.36	-0.36	0.16	0.20	0.24

Significant at * P<0.05; ** P<0.01.

Table 5. Estimate of reciprocal (rij) effects (Griffing's method) for various traits

Traits	Parental combination						S.E. (rij) i=j
	AB	AC	AD	BC	BD	CD	
BW at 140-day-old	0.84	6.39	-7.96	-2.44	-1.49	7.18	11.42
280-day-old	8.59	44.06**	0.90	-39.74*	13.22	15.62	16.16
500-day-old	43.49	40.34	-16.26	-68.26	-12.33	49.88	34.92
Age at sexual maturity (d)	0.38	0.47	0.58	1.22	-1.81	1.20	1.17
Survivor's egg production to (n)							
260-day-old	-0.42	-0.39	-4.24**	-2.30	2.84*	-0.22	1.21
280-day-old	-1.10	-1.09	-4.47**	-2.04	2.52	-0.47	1.29
500-day-old	0.21	-2.31	-6.19	2.90	3.15	-3.62	3.29
Average egg weight at							
280-day-old	-2.18**	0.27	-0.57	0.46	-0.01	0.83*	0.35
Albumen height (mm)	-0.20	0.22	0.20	0.05	-0.13	-0.25	0.16
Albumen index (angles)	-0.28	0.23	0.32	0.14	-0.41	-0.37	0.29\
Yolk index (angles)	0.28	0.36*	0.69**	-0.19	-0.28	-0.13	0.17
Shell thickness (mm) × 100	1.11**	0.80	1.23**	1.10**	0.71	0.40	0.41

Significant at * P<0.05 ** P<0.01.

Hendorson C R. 1948. 'Estimation of general, specific and maternal combining abilities in crosses among inbred lines of swine.' Ph.D. thesis, Cornell University, USA. (*fide* Harvey 1966.)

Hill J F and Novdskoy A W. 1958. Heterosis in poultry. 3. Predicting combining ability of performance in the crossbred fowl. *Poultry Science* **37** : 1159-69.

Kim S C, Chung S B, Thak T Y and Sul D S. 1975. A study on estimates of genetic combining ability of economic traits in single crosses of single comb White Leghorns. *Research Reports of the Office of Rural Development, Suwon, Korla, Livestock* **17** (8) : 45-62. (*fide Animal Breeding Abstract* **45** : 6278.)

King S C and Henderson C R. 1954. Variance component analysis in heritability studies. *Poultry Science* **33** : 147-54.

Osborne R. 1957a. The use of sire and dam family averages in increasing the efficiency of selective breeding under a hierachical mating system. *Heredity* **11** : 93-116.

Osborne R. 1957b. Family selection in poultry : The use of sire and dam family averages in choosing male parents. *Proceeding of Royal Society, Edinburgh* B **66** : 374-93.

Poggenpoel G D and Erasmus J E. 1978. Long-term selection for increased egg production. *British Poultry Science* **19** : 111-23.

Renganathan P. 1976. 'Studies on the genetic architecture of 2 While Leghorn strains for economic traits.' M.V.Sc. thesis submitted to Agra University, Agra.

Sinha R.1977. 'Genetic and phenotypic analysis of a closed flock of White Leghorn strain of chicken.' Ph.D. thesis submitted to B.C.K.V., Nadia.

Tiwana M S and Dev D S. 1972. Relative efficiency of different basis of selection for egg production. *Indian Journal of Poultry Science* **7** (3) : 28-34.

Verma S K. 1980. 'Studies on additive and nonadditive genetic effects for some economic traits and construction of selection index in poultry.' Ph.D. Thesis submitted to Rohilkhanda University, Bareilly.

Wearden S, Tindell D and Craig J V.1965. Use of full diallel cross to estimate general and specific combining ability in chickens. *Poultry Science* **44** : 1043-53.

Indian Journal of Animal Sciences 68 (4) : 373-375, April 1998

Nutritional studies on Mongolian sheep for meat production in the Kingdom of Saudi Arabia

A E TAG-EL-DIN[1]

King Saud University, P O Box 2460, Riyadh, Saudi Arabia

Received: 21 April 1997; Accepted: 28 October 1997

ABSTRACT

Mongolian lambs (26), 30 kg live body weight and 4-6-month-old, were distributed into 2 groups, at random, to study the effect of group versus individual feeding on feedlot performance at 2 periods (0-28 day and 29-63 day). Animals were fed *ad lib.* on complete diet for 63 days and water was offered freely. The nutritive values of the diet were 75.6% TDN, 13.8% DCP and 2.7 ME (Mcal/kg DM). Average daily gain (ADG) was higher ($P<0.05$) by 35.9, 10.1 and 24.8% for animals fed in group than those fed individual for periods 1, 2 and the pooled respectively. ADG of period 2 was lower than that of period 1 ($P<0.05$) by 50 and 60.5% for lambs fed in group and individual, respectively. Feed conversion ratio (FCR) was lower ($P<0.05$) for lambs fed in group than those fed individual by 21.7% for period 1, 3.4% for period 2 and 14.9% for pooled period. FCR of period 1 was lower ($P<0.05$) than that of period 2 by 51.4% and 40.1% for animal fed in group or individual respectively.

Key words : Feeding system, Mongolian sheep, Meat production, Nutritional requirement of sheep

The Kingdom of Saudi Arabia depends on foreign sources to meet the current local demand for meat. Large numbers of Mongolian sheep were imported from China for immediate slaughtering and/or fattening. These animals are adapted to temperate environment, strategies for fattening these lambs in the Kingdom of Saudi Arabia (semiarid climate) require knowledge of the extent of the lambs adaptation to the hot environment and the economical weight for slaughtering. Walks *et al.* (1990) and Purwanto *et al.* (1993) reported that hot environments increased the energy requirement for maintenance, which implies that availability of metabolizable energy for production would decrease. This experiment was conducted to study the effect of feeding growing Mongolian lambs in group or individual on the feedlot performance and feed utilization efficiency.

MATERIALS AND METHODS

Imported Mongolian lambs (26) weighing 30 kg and 4-6 months of age were used to study the effect of *ad lib.* feeding in group vs individual system on their performance traits. Lambs were vaccinated, dewormed and vitamin A-D-E injections were given. Initially they were maintained as in one group and gradually conditioned to *ad lib.* experimental diet for 15 days. The experimental complete diet (mash) composed of barley 68.6%, extracted soybean-meal 6.9%, wheat straw 9.9%, alfalfa hay 9.9%, urea 1%, salt and mineral premix 1%, limestone 1.2% and sodium bicarbonate 1.5%; which was considered the least cost diet under the prevailing prices in Saudi Arabia. Thereafter, lambs were distributed into 2 equal groups: the first group was housed in a 25 m^2 pen and fed at *ad lib.* in group. The second group was housed in individual pens, each of 1.5 m^2 and fed individual under *ad lib.* regimen. Water was offered freely for 24 hr daily. Residue was collected weekly, weighed and recorded to estimate the feed consumed. Lambs were weighed in weekly intervals after 12 - 14 hr fasting for 63 days.

Digestibility coefficients and the nutritive value of the experimental diet were estimated using 4 adult Mongolian rams. Animals were kept in 4 metabolic cages to collect faeces and urine in separate. The digestibility trials were conducted according to the official method.

Chemical composition of feed, faeces and urinary nitrogen were analyzed (AOAC 1984). The digestible energy (DE) value was calculated by multiplying the TDN times 4400 Kcal. The metabolizable energy (ME) value was then calculated from DE by the factor 0.82 (NRC 1985). Data for all dependent variables were subjected to analysis by GLM procedures (SAS 1986).

RESULTS AND DISCUSSION

The experimental diet contained 17.4% CP, 13.6% CF, 2.1% EE, 58.9% NFE and 7.9% ash and the digestibility coefficients were 77.9, 79.2, 62.4, 84.5 and 83.6 % for DM,

Present address: [1]Animal Production Department, College of Agriculture, King Saud University.

Table 1 . The effects of feeding system on live body weight, average daily gain , feed intake, crude protein intake and energy intake of Mongolian lambs

Trait	System of Feeding		SEM
	Group	Individual	
Animal weight (kg):			
Initial	29.21c	30.92c	3.04
period 1	36.86b	36.54b	3.18
period 2	41.54a	40.79a	3.55
Average daily gain (gd^{-1})			
period 1	273.00aA	200.89bB	69.08
period 2	133.67c	121.43c	40.22
pooled	195.58bA	156.74bB	38.32
Dry matter intake (kgd^{-1})			
period 1	1.31	1.23	0.12
period 2	1.32A	1.24B	0. 09
pooled	1.32A	1.24B	0.10
Dry matter intake ($g/kg^{0.75}$)			
period 1	95.77aA	87.79aB	7.57
period 2	84.50b	80.21b	6.45
pooled	91.17aA	84.33aB	6.70
Crude protein intake ($g/kg^{0.75}$)			
period 1	16.66aA	15.28aB	1.31
period 2	14.70b	13.96b	1.12
pooled	15.86aA	14.67aB	1.17
ME intake ($Kcal/kg^{0.75}$)			
period 1	261.44aA	239.67aB	3.06
period 2	230.67b	218.97b	17.61
pooled	248.90aA	230.23aB	18.28

SEM, Stander error of mean.

a,b,c Means in the same column within each trait bearing different superscripts are differ ($P<0.05$).

A, B Means in the same row within each trait bearing different superscripts are differ ($P<0.05$)

CP, CF, EE and NFE respectively. The TDN, DCP and ME were 75.6%, 13.8% and 2.7 (Mcal/kg DM) respectively. Nitrogen balance of rams fed the experimental diet was 5.2 g N/head/day. It was 21.1% of nitrogen intake and 26.7% of digested nitrogen.

Live body weight of Mongolian lambs fed in groups were slightly higher than those fed in individual, however, the differences were not significant (Table 1). Animals fed in groups had higher average daily gain (ADG) with 35.9, 10.1 and 24.8% than those fed in individual for 0-28 day (period 1), 29-63 day (period 2) or overall period (pooled) respectively. The differences were significant for period 1 or pooled and nonsignificant at period 2. Also, the results indicated that the ADG was significantly lower by 50.0 and 60.5% in period 2 than in period 1 for lambs fed in groups or those fed individual, respectively, Kraidees *et al.* (1966) reported that increasing the level of feeds from 24 to 30 or 36 g DM/kg body weight significantly ($P<0.01$) increased ADG by 128.8 and 345.8% respectively. In low (24 g DM/kg) and medium

Table 2. The effects of feeding system on feed conversion ratio of dry matter, crude protein and metabolisable energy of Mangolian Lambs

Trait	System of Feeding		SEM
	Group	Individual	
FCR (kg DM/kg gain):			
period 1	4.79aA	6.12aB	1.73
period 2	9.86c	10.21c	5.13
pooled	6.74bA	7.92bB	1.69
CPR (kg CP/kg gain):			
period 1	0.84aA	1.06aB	0.30
period 2	1.72c	1.74c	0.89
pooled	1.17bA	1.38bB	0.29
MER (Mcal/kg gain):			
period 1	13.06aA	16.71aB	4.73
period 2	26.93c	27.86c	13.99
pooled	18.40bA	21.63bB	4.61

SEM, Standard error of mean: FCR, feed conversion ratio; CPR, crude protein ratio; MER, metabolizable energy ratio

a,b,c Means in the same column within each trait bearing different superscripts are different ($P<0.05$).

A,B Means in the same row within each trait bearing different superscripts are different ($P<0.05$).

(30 g DM/kg) feeding levels, ADG were nonsignificantly slower by 10.6 and 9.4% in period 1(0-4 week) than that in period 2 (5-8 week), respectively, however, lambs on high feeding level (36 g/kg) had 67.5% higher ($P<0.01$) ADG in period 1 compared to period 2.

The results of dry-matter intake (kg/day) indicated that animals fed in group consumed significantly higher ($P<0.05$) than those fed individual by about 6.5%. There were no significant differences between the 2 periods . However, dry-matter intake calculated as $g/kg^{0.75}$ was significantly ($P<0.05$) higher for sheep fed in group than those fed individual by 9.0-5.4 and 8.1% for periods 1,2 and pooled respectively. Animals consumed lower dry-matter ($g/kg^{0.75}$) in period 2 by 6.6 and 8.6% than in period 1 for animals fed in group or individual, respectively. Also, the same trend was observed when CP and ME were calculated. They were higher for the first group and in the first period than the second group and in the second period, respectively. The differences were significant ($P<0.05$) between the kind of feeding in the periods 1 or pooled and between both periods 1 and 2.

These results can be explained with the observations of Mongolian lambs (Chinese breed) penned, individual, they were hyper-irritability and, always, moved and jumped in the pen. These reasons may have decreased the feed utilization and the growth rate compared to animals housed in groups. These sheep are natural grazer animals and adapted to temperature climate, when imported to Saudi Arabia, felt various stresses assembly, transportation and hot climate (30-32°C, Kraidees *et al.* 1996). At the beginning of the experiment (February1995) temperature was cold and it

increased by the end of March to become hot during April in Saudi Arabia. Hot climate increased the rate of digesta-outflow from the rumen and decreased the feed utilization. These may be the reasons of decreased in feed conversion ratio during the second period of the experiment.

Feed conversion ratio in terms of (kg DM/kg gain) or (kg CP/kg gain) or ME (M cal/kg gain) were lower (Table 2) for lambs fed in group than those fed individual by 21.7-21.8% for period 1, 1.2 - 3.3% for period 2 and 14.9 - 15.2% for pooled. The differences were significant ($P<0.05$) for period 1 and pooled among the kind of feeding system. These results indicated that Mongolian lambs fed in group utilized feed more efficient compared to those fed individual. The corresponding results of the efficiency of feed utilization during different periods for both feeding systems indicated that period 1 was lower than those of period 2 by 51.2 - 51.5% and 39.1 - 40.1% for Mongolian lambs fed in group and individual respectively. The differences were significant ($P<0.05$). These results explained that imported Mongolian lambs can be fattened in Saudi Arabia until they reached about 36-37 kg live body weight. Wabster (1985) reported that MEm/$kg^{0.75}$/ day in fatter sheep was 16% lower than in thinner ones and that attributed to differing proportions of relatively active protein tissue and relatively inactive fat tissue. He concluded that energy required for fat tissue deposition was greater than that of lean deposition. Purwanto *et al.* (1993) suggested that under heat stress and abnormal activities a proportion of the energy required for maintenance may be increased, which implies that availability of ME for production growth would decrease. Granham (1982) reported that MEm for sheep was 72-190 Kcal/$kg^{0.75}$, while, Thomson *et al.* (1979) and Kraidees *et al.* (1995) reported that MEm for Suffolk crossbred and local Najdi sheep were 95-96 Kcal/$kg^{0.75}$. However, Kraidees *et al.* (1996) reported that the average value of 145 Kcal MEm/$kg^{0.75}$ for Chinese Mongolian sheep which is 51% higher than those reported value for native Najdi sheep.

The present study concluded that imported Chinese Mongolian lambs can be economically fed until they reached 36-37 kg live body weight in group feeding, and used for meat production in the Kingdom of Saudi Arabia.

REFERENCES

AOAC. 1994. *Official Methods of Analysis.* 14th edn. Association of Official Analytical Chemists, Washington, D C. USA.

Granham N M .1982. Maintenance and growth. *World Animal Science, Sheep and Goat Production. Coop* 1 E. pp. 81-101. Elsevier Scientific Publishing Co., Amesterdam.

Kraidees M S, Al-Saiady M Y, Tag El-din A E and Abouheif M A. 1995. Effect of tail docking at birth on energy utilization as well as protein and lipid deposition by fat-tailed Najdi lambs. *Journal of Applied Animal Research* 7:81.

Kraidees M S, Al-Saiady M Y, Tag El-din A E and Abouheif M A. 1996. Feed requirements for maintenance and gain in imported Mongolian ram lambs. *Annals of Agriculture Science, Ain Shams University*, 41 :849.

NRC. 1985. *Nutrient Requirements of Sheep.* 6th edn. National Academy Press, Washington D C. USA.

Purwanto B P, Nakamasu F and Yamamoto S. 1993. Effect of environmental temperature on heat roduction in dairy heifers differing in feed intake level. *Asian-Australian Journal of Animal Science* 6:275.

SAS User's Guide. 1986. *Statistical Analysis System.* Cary, NC. USA.

Thomson D J, Fenlon J S and Cammell S B. 1979. Estimates of maintenance requirement of growing lambs. *British Journal of Nutrition* 41:223.

Wabster A J F. 1985. Differences in the energetic efficiency of animal growth. *Journal of Animal Science* **61**: 92.

Walks D L, Coppock C E, Lanham J K, Brooks K N, Baker C C, Bryson W L, Elmore R G and Stremer R A. 1990. Responses of lactating Holstein cows to chilled drinking water in high ambient temperatures. *Journal of Dairy Science* **73** : 1091-99.

Indian Journal of Animal Sciences 68 (4) : 376-378, April 1998

Quality of refrigerated oil-coated quail eggs during subsequent ambient storage

R P SINGH[1] and S S VERMA[2]

Central Avian Research Institute, Izatnagar, Uttar Pradesh 243 122

Received : 8 May 1997 ; Accepted : 28 October 1997

ABSTRACT

Changes in the internal and sensory quality of quail eggs coated by spraying or dipping in groundnut oil mixed with 200 ppm butylated hydroxytoulene were evaluated under ambient (24±2°C ; 57-58% RH) storage, following 30 days of refrigeration (5±1°C; 80-85% RH). The cumulative loss in the weight of oiled eggs was 2.5 to 2.6% only as against 8.3% in unoiled eggs after 15 days of ambient storage. Both the oiling treatments appeared almost equally effective in retarding the decline in internal and sensory quality of eggs. However, no significant increase in thiobarbituric acid value was found during storage regardless of treatments. Unoiled and oiled eggs held under refrigeration for a month should be marketed within 5 and 15 days, respectively, under ambient condition.

Key words : Ambient storage, Eggs of quail, Oil-coated quail eggs, Storage quality

Oiling shell eggs and / or cold storage are of commercial importance for maintaining their interior quality during storage. Considerable work has been done in this direction on hen eggs (Panda *et al.* 1970, Moorjani and Panda 1977, Reddy and Reddy 1992) but such study in quail eggs is rather very scanty (Tanabe *et al.* 1972). Moreover, no information is available about the qualitative changes occurring in quail eggs after shifting them from cold room to ambient temperature to simulate marketing conditions. Hence, the present study was undertaken to evaluate the effect of coating quail eggs with vegetable (groundnut) oil on weight loss, lipid, oxidation, internal and sensory quality of eggs during refrigeration and subsequent ambient storage.

MATERIALS AND METHODS

Two-hundred fifty fresh Japanese quail (*Coturnix coturnix japonica*) eggs, free from visible dirt and cracks, were obtained from the experimental farm of this institute. Ten eggs were drawn at random for estimating initial egg quality and the remaining eggs were divided equally into 3 groups. One group of eggs was sprayed with groundnut oil mixed with 200 ppm butylated hydroxytoulene as an antioxidant, another group was dipped in this egg coating medium and the third group of unoiled eggs served as control. The eggs were then stored in a refrigerator (5±1°C ; 80-85% RH) for 30 days after which they were taken out and held at ambient temperature (24±2°C ; 57-68% RH) to study subsequent changes in their quality at 5 days intervals.

Present address : [1]Senior Scientist, [2]Head , Division of Post-harvest Technology.

Loss in weight was calculated by the difference between initial and stored egg weights. The air-cell diameter, albumen and yolk indices, per cent thick white and albumen pH were measured as per standard procedures. The formula derived by Kondaiah *et al.* (1983) was used for the determination of interior quality unit (IQU), while the distilation method of Tarladgis *et al.* (1960) was followed for the estimation of thiobarbituric acid (TBA) value in egg yolk. Ten eggs from each treatment at each storage intervals were withdrawn for weight loss and internal quality determination, whereas albumen pH and TBA values were estimated in triplicates. Sensory attributes, viz. appearance, texture, flavour and overall acceptability of hard-boiled eggs were evaluated by 5 taste panelists on a 7- point Hedonic scale. The data were analyzed statistically (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

Table 1 shows per cent weight loss and changes in the internal quality of oil-coated eggs during refrigeration and subsequent storage at room temperature.

As expected, the weight loss and air-cell diameter increased significantly ($P<0.01$) with storage time regardless of treatments. However, the increase was significantly less in oiled than in unoiled eggs. The magnitude of cumulative loss in the weight of untreated eggs reached to 8.3% as against 2.5 to 2.6% losses in oil-coated eggs during one month of refrigeration followed by 15 days of ambient storage. Thus, there was a net reduction of loss by over 5.5% in egg weight by oiling treatments. Although oil dipping offered relatively better protection against moisture loss due to complete coating of egg shell than oil spraying , the differences in weight loss

Table 1. Weight loss and internal quality of oil-coated quail eggs during refrigeration and subsequent ambient storage

Treatments	Days of storage			
	Refrigeration (5±1°C)	Ambient (24±2°C)		
	30	5	10	15
Weight loss (%)				
Untreated	4.26±0.22^{aA}	5.12±0.25^{aA}	6.29±0.32^{bA}	8.32±0.41^{cA}
Sprayed	1.34±0.19^{aB}	1.62±0.26^{abB}	1.98±0.33^{abB}	2.63±0.45^{bB}
Dipped	1.03±0.09^{aB}	1.32±0.13^{aB}	1.64±0.18^{abB}	2.50±0.43^{bB}
Air-cell diameter (cm)				
Untreated	1.44±0.03^{aA}	1.58±0.07^{abA}	1.63±0.05^{bA}	1.74±0.03^{bA}
Sprayed	1.13±0.04^{aB}	1.21±0.03^{abB}	1.25±0.07^{abB}	1.32±0.06^{bB}
Dipped	1.06±0.04^{aB}	1.18±0.07^{abB}	1.20±0.03^{abB}	1.25±0.02^{bB}
Albumen index				
Untreated	0.088±0.008^{aA}	0.083±0.004^{abA}	0.067±0.008^{bcA}	0.055±0.003^{cA}
Sprayed	0.096±0.005^{aA}	0.092±0.005^{abA}	0.081±0.006^{abA}	0.078±0.006^{bB}
Dipped	0.102±0.005^{aA}	0.098±0.004^{abA}	0.084±0.004^{bA}	0.082±0.003^{bB}
Interior quality unit (IQU)				
Untreated	44.85±2.11^{aA}	37.80±2.64^{aA}	29.95±2.14^{bA}	24.49±1.69^{bA}
Sprayed	47.38±1.44^{aA}	43.65±1.70^{aA}	40.57±2.03^{abB}	35.07±1.93^{bB}
Dipped	49.26±1.61^{aA}	43.76±2.16^{abA}	39.26±1.68^{bcB}	35.46±1.73^{cB}
Thick white (%)				
Untreated	33.04±1.42^{aA}	28.05±1.82^{aA}	20.81±1.71^{bA}	14.35±1.17^{cA}
Sprayed	39.91±1.75^{aB}	36.20±1.91^{abB}	31.62±1.92^{bB}	24.98±2.01^{cB}
Dipped	41.06±1.94^{aB}	35.02±1.35^{abB}	33.24±2.03^{bB}	26.39±1.32^{cB}
Yolk index				
Untreated	0.402±0.008^{aA}	0.363±0.006^{bA}	0.270±0.011^{cA}	0.223±0.015^{dA}
Sprayed	0.438±0.015^{aAB}	0.417±0.008^{aB}	0.374±0.012^{aB}	0.356±0.008^{bB}
Dipped	0.452±0.009^{aB}	0.404±0.014^{bB}	0.386±0.011^{BB}	0.346±0.007^{cB}

Initial air-cell diameter, albumen index, IQU, thick white and yolk index of eggs were 0.84 cm, 0.115, 60.52, 48.76% and 0.459 respectively.

and air-cell diameter of eggs between these 2 treatments were nonsignificant.

Oiling treatments also appeared highly effective in retarding the deteriorative changes in albumen quality as reflected by consistently higher albumen index, interior quality unit (IQU) and per cent thick white in oiled then in unoiled eggs throughout the storage. The fall in all these measures of albumen quality of untreated eggs on fifth day was close to the values recorded on 15th of ambient storage in oil-coated eggs. These results substantiate the findings reported for chicken (Schwall *et al.* 1961, Panda *et al.* 1970) and quail (Tanabe *et al.* 1972) eggs and underline the beneficial effect of oiling eggs to minimize the escape of carbon dioxide for maintaining the gel structure of albumen. The pattern of changes observed in yolk index between treatments was more or less similar to that of albumen index.

Changes in albumen pH, yolk lipid oxidation and sensory quality of oil-coated eggs during storage are presented in Table 2.

The pH of albumen of uncoated eggs increased rapidly from an initial value of 8.7 to 9.4 within 5 days of subsequent storage at room temperature and remained almost unchanged thereafter. On the contrary, both egg coating treatments appeared equally effective in restricting the escape of carbon dioxide and thereby slowing down the rate of increase in albumen pH. This slow shift in pH towards alkalinity was a reflection of better albumen quality of oiled eggs (Table 1).

Yolk lipids oxidation, as measured by thiobarbituric acid (TBA) assay, did not differ significantly between treatments. No appreciable increase in TBA value was also found during refrigeration or subsequent ambient storage. This indicated that despite high level (66%) of unsaturated fatty acids (Bitman and Wood 1980), yolk lipids are inherently resistant to oxidative deterioration. The albumen surrounding the yolk might act as a partial barrier against the diffusion of atmospheric oxygen. Moreover, phospholipids and protein in egg yolk are interwoven in the exterior surface layer of low density lipoprotein which can partly exclude oxygen from the inner lipid core of the particle (Burley and Vadehra 1989). Besides, the presence of tocopherol as free-radical terminator and phosvitin as iron-chelator in egg yolk might be responsible for its less susceptibility to oxidative degradation

Table 2. Changes in pH, lipid oxidation and sensory quality of oil-coated quail eggs during refrigeration and subsequent ambient storage

Treatments	Days of storage			
	Refrigeration (5±1°C)		Ambient (24±2°C)	
	30	5	10	15
Albumen pH				
Untreated	9.05±0.04^{aA}	9.38±0.03^{bA}	9.32±0.05^{bA}	9.36±0.03^{bA}
Sprayed	8.79±0.05^{aB}	8.88±0.04^{aB}	9.02±0.02^{bB}	9.13±0.03^{cB}
Dipped	8.82±0.03^{aB}	8.92±0.06^{bB}	8.98±0.07^{bB}	9.08±0.05^{cB}
TBA value (mg malonaldehyde /kg)				
Untreated	0.36±0.03	0.41±0.05	0.39±0.03	0.43±0.03
Sprayed	0.31±0.04	0.28±0.04	0.35±0.06	0.34±0.04
Dipped	0.29±0.03	0.32±0.02	0.28±0.02	0.39±0.01
*Overall acceptability score**				
Untreated	6.2±0.37^{aA}	5.0±0.31^{bA}	3.6±0.24^{cA}	1.8±0.37^{dA}
Sprayed	6.0±0.31^{aA}	5.6±0.40^{abAB}	5.0±0.31^{bcB}	4.6±0.24^{cB}
Dipped	6.2±0.20^{aA}	6.0±0.24^{abB}	5.2±0.37^{bcB}	4.8±0.51^{cB}

*7 = like extremly ; 1 = dislike extremely. Initial albumin pH, TBA value and overall acceptability scores of eggs were 8.68, 0.27 mg/kg and 6.8, respectively. Means bearing similar small letter row-wise and capital letter column-wise or no superscripts do not differ significantly (P<0.01)

(Cotterill *et al.* 1977, Pike and Peng 1985).

For the sake of brevity, only data pertaining to overall acceptability scores (OAS) of sensory traits are presented in Table 2. The mean OAS evinced a progressive decline with storage time. However, oil-coated eggs received consistently higher scores than unoiled eggs at all storage intervals. The latter developed slightly rubbery texture, possibly due to high moisture loss (Table 1), marked alteration in flavour and became organoleptically unacceptable beyond 5 days of holding at room temperature. On the contrary, eggs treated by either the oil spray or dip method remained fairly acceptable up to 15 days of storage.

These results suggest that quail eggs held under refrigeration (5±1°C) for a month should be marketed within 5 days of removal from it. The marketing period of eggs could be extended up to 15 days at room temperature (24±1°C) by spray coating them with easily available groundnut oil mixed with 200 ppm BHT prior to refrigeration or cold storage.

REFERENCES

Bitman J and Wood D L. 1980. Cholesterol and cholesterol esters of eggs from various avian species. *Poultry Science* **59** : 2014-23.

Burley R W and Vadehra D V. 1989. Egg yolk : Structure and Properties. *The Avian Egg*. pp. 171-233. John Wiley and Sons. New York.

Cotterill O J, Marion W W and Naber E C. 1977. A nutrient re-evaluation of shell eggs. *Poultry Science* **56** : 1927-34.

Kondaiah N, Panda B and Singhal R A. 1983. Internal egg quality measure for quail eggs. *Indian Journal of Animal Sciences* **53** : 1261-64.

Moorjani M N and Panda P C. 1977. Changes in the internal quality of eggs brought to atmospheric condition after cold storage. *Indian Journal of Poultry Science* **12** (3) : 24-27.

Panda B, Reddy M S and Rao J J. 1970. Effect of oil-coating and lime sealing on weight loss and internal quality of shell eggs during refrigeration and subsequent marketing periods. *Indian Journal of Animal Sciences* **40** : 368-74.

Pike O A and Peng I C. 1985. Stability of shell eggs and liquid yolk to lipid oxidation. *Poultry Science* **64** : 1470-75.

Reddy L S and Reddy M S. 1992. Effect of oil coating and storage condition on the quality of duck and chicken eggs. *Indian Journal of Animal Sciences* **27** : 208-13.

Schwall DV, Gardner F A and Parnell E D. 1961. Effect of oil treating on shell egg quality during short term refrigerated storage. *Poultry Science* **40** : 583-88.

Snedecor G W and Cochran W G. 1967. *Statistical Methods*. 6th edn. Oxford and IBH Publishing Co., Calcutta.

Tanabe Y, Kamiya M, Nishikawa K, Nakamura T and Takahashi T. 1972. Survey of the methods for the long-term storage of poultry eggs. 6 Effect of paraffin oil coating on quail egg quality. *Research Bulletin, Faculty of Agriculture, Gifu University, Gifu, Japan* **33** : 315-22.

Tarladgis B G, Watts B M, Younathan M T and Dugan Jr. L. 1960. A distillation method for quantitative determination of malonaldehyde in rancid foods. *Journal of American Oil Chemists Society* **37** : 44-48.

Indian Journal of Animal Sciences 68 (4) : 379-381, April 1998

Factors affecting composition of mastitic milk of Friesian cattle in Sudan

IBTISAM E MOHAMED[1], OSMAN A O ELOWNI[2] and GALALEDEIN E MOHAMED[3]

Sudan U of K, Faculty of Animal Production , Sudan P. 032

Received : 10 January 1997; Accepted : 28 October 1997

ABSTRACT

The composition of mastitic cows milk had a highly significant decrease in total solids, caseins, lactose, casein number and fat%, while ash chloride , total proteins and Kositer's value increased. The difference between maximum , minimum mean and standard deviation of milk constituents between healthy and mastitic cows were determined. The types of bacteria isolated had significant effects only on lactose and acidity.

Key words : Cattle, Mastitic milk, Milk composition

The onset of udder infection (mastitis), influence the composition of milk by varying the concentration of it's constituents (Mernyi and Wagner 1986, Vianni and Nader 1991).

The most marked changes, according to Ashworth *et al.* (1967) are in protein, fat, lactose and chloride. Ishikawa *et al.* (1982) found subclinical mastitis to be associated with an increase of the major whey proteins (lactoglobulins and α - lactalbumin). Similarly , O'leary and Leavit (1982) reported a decrease in casein.

The ash levels were also affected, indicating a change in permeability of udder (Munro *et al.* 1984). In most cases, the titratable acidity of mastitic milk was altered (Pedraza *et al.* 1978).

The present study was undertaken to establish the level of changes of some constituents of milk resulting from mastitis infection and to assess the effect of the type of bacteria involved.

MATERIALS AND METHODS

Composite milk samples were taken from 99 mastitis infected and 10 healthy cows. Titratable acidity, fat per cent and lactose were determined immediately followed by the other tests.

Chemical analysis

Total proteins, casein and whey proteins (Rowland 1938); fat % (Martin 1979) ; lactose % (Richards 1959); total solids and ash (Richmond 1925); titratable acidity (Foley *et al.* 1974); chloride (X-ray flourescence techniques X-Rf) (Jenllins *et al.* 1981; Tertian and Claisse 1982) and Kostler value as % of chloride and lactose ; were determined.

Present address : [1,2] Faculty of Animal Production, U. of K. Sudan, P.O. Box 32. [3]Faculty of Veterinary Science, U of K, Sudan PO Box 32.

Table 1. Comparison of healthy and mastitic cows milk

Constituents	Mean±SD	
	Diseased	Healthy
Total	11.98±0.719	12.91±0.328
Protein	3.48±0.529	3.82±0.342
Fat	3.62±0.340	3.87±0.457
Lactose	4 09±0.319	4.62±0.264
Ash	0.75±0.198	0.58±0.153
Chloride	0.173±0.085	0.123±0.041
Casein	2.74±1 210	3.07±0.139
Whey proteins	1.08±0.117	0.73±0.083
Acidity	0 19±0.087	0.170±0.016

The data were arranged into computer format, to facilitate the statistical analysis, using numerical codes for stages of lactation (early , medium and late), types of bacteria isolated (*S. aureus* , *A. pogenes*, Gram negative bacteria and others) and seasons (summer and winter).

Descriptive (frequency) analysis and 1-way and 2-way analysis of co-variance in the randomized complete block design (Gill 1978) were used to analyze the results.

RESULTS

The maximum, minimum, mean and SD of the milk constituents, for healthy and infected cows varied considerably, particularly lactose, chloride, casein, whey proteins, ash and acidity (Table 1).

The milk of mastitic compared to healthy cows (Table 2) had highly significant differences for total solids, casein, ash, chloride, lactose and casein number ($P<0.01$), while fat content decreased ($P<0.01$) and acidity, total protein and Kostler's

Table 2. The effect of disease (mastits) on milk constituents (%) (means squares from ANOVA tables)

Source of variation	Total solids	Protein	Fat	Lactose	Chloride	Ash	Casein	Whey protein	Acidity	Kos. value	Casein No
Covariate	0.043	0.529	0.284	0.093	0.015**	0.000	4.750*	0.788*	0.020	32.433	49.996
Parity number	0.085	0.186	0.220	0.186	0.029**	0.002	9.094**	1.540**	0.040**	42.832	43.478
Stage of lactation	0.02	0.931	0391	0.000	0.001	0.002	0.179	0.007	0.000	25.561	22.850
Main effects (Health)	7.971**	1.059*	0.675**	2.919***	0.373***	0.271***	18.250***	1.044	0.28*	38.651*	379.257***
Explained	2.685***	0.706*	0.415**	1.035***	1.034***	0.92	9.250***	0.540	0.023**	36.164*	159.750***
Residuals	0.490	0.263	0.120	0.147	0.003	0.039	1.312	0.236	0.007	14.215	29.495
Total	0.551	0.275	0.128		0.007	0.040	1.534	0.245	0.007	15.323	33.113

* = P<0.05 ; ** = P<0.01; ***= P<0.001.

value decreased (P<0.05) , types of bacteria isolated (mainly *S. aureus*) has a significant effect only on lactose (P<0.01) and acidity (P<0.05) (Table 3).

DISCUSSION

Total solids results was in agreement with Rogers and Mitchell (1989). Further, King (1978) observed a significant difference for total protein and caseins within infected and non-infected quarters of the same cows, total protein and casein decreased in the infected cows.These findings are similar to those of Merenyi and Wagner (1986) and Sharaby (1990). According to Ishikawa *et al.* (1982) this may be because of both inflammatory damage of secretory tissue and destruction of the blood milk permeability barriers. Moreover, Murphy *et al.* (1989) observed increased proteinase activity during infection. In contrast Kwai *et al.* (1984) reported an increase in total protein , while Toit *et al.* (1984) reported no variation in the total protein resulting from infection.

Casein number decreased in mastitic cows milk in the present study, since the reduction in casein (P<0.001) outweighed the reduction in the total protein.

The reduction in fat % in mastitic cows was similar to those of Vianni and Nader (1991). This reduction may be because of lipolysis which increase during infection (Murphy *et al.* 1989).

The highly significant effect of mastitis on lactose (Table 2) was similar to that reported by Ashworth *et al.* (1967) and Mollet *et al.* (1989). This reduction may be because of lipolysis which increase during infection (Murphy *et al.* 1989).

The highly significant effect of mastitis on lactose (Table 2) was similar to that reported by Ashworth *et al.* (1989). This reduction may be due to the more alkaline constituents of the blood passing into the milk (Turner 1946), a reduced concentration of α-lactalbumin (Sreekumar *et al.* 1975) or to less glucose begin available to the mammary gland due to reduced blood flow in the mastitic state (Kitchen 1981).

Chloride increased in mastitic cows milk (Table 2). This is in agreement with Kitchen *et al.* (1984) who reported that as a failure to synthesize lactose, the level of chloride increased to maintain the somatic pressure.

The higher level of ash observed in the infected cows was also observed by El Deeb and Hassan (1987).

The lower acidity values were similar to those of Pedraza *et al.* (1978). The type of bacteria causing mastitis isolated during this study has a significant effect (Table 3) which may be due to bacterial acidification (Boretius 1982).

Lactose was markedly affected by the types of bacteria isolated. The effect was highly significant (P<0.01) and was also reported by Kitchen (1981) who found that, *Staph. aureus* infection results in severe tissue damage of the secretory cells. Other milk constituents did not change frequently with the type of bacteria isolate.

In conclusion, mastitis induces marked changes in milk

Table 3. The effect of *S. aureus* infection on mastitic milk constituents (means squares from ANOVA tables)

Source of variation	Total solids	Protein	Fat	Lactose	Chloride	Ash	Casein	Whey protein	Acidity	Kos. value	Casein No.
Covariate	0.198	0.292	0.383*	0.268	0.060	0.031	0.327	0.028	0.036	28.945	42.088
Parity number	0.217	0.200	0.326	0.471	0.089*	0.057	0.390	0.023	0.004	42.345	76.251
Stage of lactation	0.200	0.416	0.483*	0.047	0.037	0.002	0.301	0.029	0.070	19.799	10.912
Main effects (Bact.)	0.605	0.150	0.100	0.604**	0.057	0.040	0.164	0.100	0.168*	27.416	79.374
Explained	0.442	0.207	0.213	0.469***	0.058*	0.036	0.229	0.071	0.115	28.435	64.460
Residuals	0.518	0.282	0.108	0.143	0.023	0.039	0.186	0.070	0.053	13.808	31.202

* = P<0.05 ; ** = P<0.01; ***= P<0.001.

constituents. This affect the nutritive value, processing and keeping qualities, because it contains pathogenic bacteria. These changes correlates with the virulence and types of the causative agent involved.

REFERENCES

Ashworth U S, Forster T L and Luedeke L O. 1967. Relationship between CMT reaction and composition of milk from opposite quarters. *Journal of Dairy Science* **49** (7) : 1078-82.

Boretius J. 1952. Occurrence of variations of Soxhelt Henkel values in herd milk. *Dairy Science Abstract* **44** : 2374.

El Deeb S A and Hassan H N. 1987. Changes in the cow's milk composition as affected with mastitis infection. *Alexandria Journal of Agricultural Research* **32** (3) : 163-74.

Foley J , Buckley J and Murphy M F. 1974. *Commercial Testing and Product Control in the Dairy Industry.* 1974, University College Cork.

Gill J L. 1978. *Design and Analysis of Experiments in the Animal and Medical Sciences* . Vol . 1. The Iowa State University Press, Ames. Iowa.

Ishikawa H , Shimuz T, Hirano H, Saito N and Nakano T. 1982. Protein composition of whey from subclinical mastitis and effect of treatment with levamisole. *Journal of Dairy Science* **65** (4) : 653-58.

Jellnins R, Gould R W and Gedcke D. 1981. *Quantitative X-ray Spectrometery.* Marcel Dellker, Inc, New York.

King J O L. 1978. Cell counts and composition of bovine milk. *Veterinary Record* **103** : 397-98.

Kitchen B J C. 1981. Review of the progress of dairy science. bovine mastitis. milk composition changes and related diagnostic tests. *Journal of Dairy Research* **48** : 167-88.

Kitchen B J C, Sergkwee W, Middleton G and Andres R J. 1984. Relationship between the levels of NAGase in bovine milk and the presence of mastitis pathogens. *Journal of Dairy Research* **51** (1) : 11-16.

Kwai N G, Hang K F, Hayes J F, Moxley J E and Monnorades H G. 1984. Variability test day milk production and composition and relation of SCCs with yield and compositional changes of bovine milk. *Journal of Dairy Science* **67** (2) : 361-66.

Martin P G C. 1979. Manuals of food quality control. 3-commodities. Food and Agriculture Organization of the United Nations Rome 1979.

Merenyi I and Wagner A. 1986. Slight changes in milk composition due to subclinical disease as a source of errors in breeding. *Dairy Science Abstract* **48** (8) : 4732.

Mollett T A, Brooks C B and Leighton E A. 1989. Effects of total CMT score and milk quality of the dam up calf wearing weight in beef cattle. *Journal of Dairy Sciences* **72** (Supplements 1) Abstract No. 1127.

Munro G L, Grive P A and Kitchen B J. 1984. Effects of mastitis on milk yield, milk composition , processing properties and yield and quality of milk products. *Australian Journal of Dairy Technology* **39** (1) : 7-16.

Murphy S C, Cranker K, Senyk G F, Brabano D M, Seaman A I and Galton D M. 1989. Influence of bovine mastitis on lipolysis in milk. *Journal of Dairy Sciences* **72** (3) : 620-26.

O'learly J and Leavitt B. 1982. Influence of mastitis on milk production, milk quality and cheese yield. Progress Report. Kentucky Agricultural Experimental Station, 1982, No. 264.

Pedraza G C, Gara E J, Giuda B C, Palma V R, Alegria R and Zurita A L. 1978. Effect of subclinical mastitis on milk quality. *Dairy Sciences Abstract* **40** (6) : 3129.

Richards E L. 1959. The reaction of lactose with anthrone and its application to the estimation of lactose in casein and the dairy products. *Journal of Dairy Research* **26** (1) : 53-57.

Richmnnd H and Drooper F I C C. 1925. *The Laboratory Book of Dairy Analysis.* 3rd edn. Revised in 1925.

Rogers S A and Mitchael. G E. 1989. The relation between SCC, composition and manufacturing properties of bulk milk. *Australian Journal of Dairy Technology* **44** (2) : 53-56.

Rowland S J. 1938. The determination of the nitrogen distribution in milk. *Journal of Dairy Research* **9** (1) : 42-46.

Sharaby M A. 1990. Effect of subclinical mastitis on the concentrations and yields of milk constituents and their interrelations. *Veterinary Bulletin* **60** : 6768.

Sreekumar B, Yadva R K and Rawat R S. 1975. Effect of subclinical mastitis on chemicals composition of cow milk. *Journal of Indian Dairy Science* **28** (3) : 213-17.

Tertian R and Classie F. 1982. Principles of quantitative x-ray fluorescence analysis . *Heyden, London* 1982.

Toit F Du, Villiers P A De and Smith A. 1984. Influence of bacterial infection of quarters on milk production and composition. *Dairy Science Abstract* **46** : 3968.

Turner W C. 1946. Symposium : *Bovine Mastitis* . 1st edn. Second impression (Eds.) Little R B and Plastridge W N. McGraw-Hill Book Co., Inc., New York and London, 1946.

Vianni M C E and Nader F A. 1991. Variations in the physicochemical and cellular characteristics of milk of the cows with subclinical mastitis. *Veterinary Bulletin* **61** (9) : 6320.

Indian Journal of Animal Sciences 68 (4) : 382-385, April 1998

Effects of season and infection on composition of mastitic milk of the Friesian cattle in Sudan

IBTISAM E MOHAMED[1], G E MOHAMED[2] and O A O ELOWNI[3]

Sudan U. of K., Faculty of Animal Production, Sudan, P. 032

Received : 3 January 1997; Accepted : 28 October 1997

ABSTRACT

Clinical mastitis, as measured by California mastitis tests (CMT), has a significant effect on casein, lactose, total solids, chloride, Koestlerm; value and ash. Similarly, mastitis sample that collected during summer showed significant effects on caseins, whey proteins and ash, total proteins and fat. *Staphylococcus aureus*, isolated from clinical mastitis, has a significant effect on chloride and lactose. However, *S. aureus*, isolated during summer, from mastitic cows, showed significant effects on caseins, chloride, whey proteins, acidity and fat per cent.

Key words : Changes in milk constituents, Mastitic cows milk, *Staphylococcus aureus* infection

Mastitis (udder infection) causes several changes in the composition of milk (Dawson *et al.* 1974, Vianni and Nader 1991).

The degree of tissue damage and change of the permeability of blood vessels vary according to the concentration and virulence of the causative agents (Munro *et al.* 1984). *S. aureus* and other organisms cause more severe tissue damage of secretory cells (Kitchn 1981).

The aim of the present study is to evaluate the effect of season on the diseased cows (mastitic) and to assess the effect of CMT, different scores and their interaction with the types of bacteria involved.

MATERIALS AND METHODS

Diseased cows (99) were divided in to summer cases (49 cows) and winter cases (50 cows). Similarly, the degree of infection of the quarter was grouped as normal quarters, subclinical mastitis, clinical mastitis, clinical mastitis with pus and blood secretions.

Chemical analysis

The total proteins , casein and whey proteins were determined with Kjldhal's method (Rowland 1938), the Gerber's methods was used for determination of fat % (Martin 1979). Lactose was done as per Richards (1959) using Anthrone methods. The total solids and ash were done by the methods of Richmond (1925). The titratable acidity was determined b;y the method of Foley *et al.* (1974). The chloride was estimated using the X-ray flourecence techniques (X-RF) described by Jenllins *et al.* (1981) and Tertian and Claisse (1982), and Kostler value was determined as % of chloride and lactose.

Table 1. The effect of summer on mastitic milk constituents (mean squares from ANOVA tables)

Source of variation	Total solids	Protein	Fat	Lactose	Chloride	Ash	Casin	Whey protein	Acidity	Koestler value	Casein No.
Covariate	0.198	0.370	0.269	0.355	0.015	0.014	3.236**	0.819***	0.016**	30.321	82.407*
Parity number	0.002	0.290	0.115	0.496	0.029*	0.022	6.410***	1.571***	0.033***	35.654	6.009**
Stage of lactation	0.391	0.491	0.448*	0.178	0.000	0.006	0.131	0.100	0.000	28.361	0.160
Main effects (seasons)	1.979*	1.151*	0.020	0.493	0.003	0.367***	71.385***	15.656***	0.311***	0.746	11.406***
Explained	0.792	0.631	0.186	0.401*	0.011	0.132**	25.952***	5.765***	0.113***	1.594	5.828***
Residuals	0.509	0.269	0.114	0.148	0.007	0.036	0.684	0.093	0.004	15.029	27.116
Total	0.517	0.280	0.116	0.159	0.007	0.036	1.446	0.268	0.008	15.302	31.124

* = P<0.05, ** = P<0.01, *** = P<0.001.

Present address : [1,3]Faculty of Animal Production, U. of K. Sudan, P.O. Box 32. [2]Faculty of Veterinary Science, U. of K., Sudan, P.O. Box 32.

Statistical analysis

Numerical codes were used for stages of lactation (early, medium and late), CMT score (healthy, subclinical, clinical, haemorrhagic and purulent), types of bacteria isolated (*S.*

Table 2. The effect of degree season on mastitic milk constituents (mean squares from ANOVA tables)

Source of variation	Total solids	Protein	Fat	Lactose	Chloride	Ash	Casin	Whey protein	Acidity	Kos. value	Casein No.
Covariate	0.043	0.529	0.284	0.093	0.052	0.002	0.507*	0.018	0.040	28.945	49.996
Parity number	0.085	0.186	0.220	0.186	0.082*	0.002	0.389	0.000	0.007	42.345	83.478
Stage of lactation	0.002	0.931	0.371	0.000	0.030	0.002	0.701*	0.035	0.075	19.799	22.850
Main effects (CMT)	1.806**	0.408	0.257	0.756***	0.073***	0.106***	0.546**	0.096	0.065	45.458***	83.123**
Explained	1.303**	0.442	0.264**	0.567***	0.076***	0.077	0.535***	0.073	0.057	40.804***	73.659**
Residuals	0.499	0.264	0.119	0.144	0.020	0.038	0.160	0.071	0.057	12.372	30.003
Total	0.551	0.275	0.128	0.172	0.023	0.040	0.185	0.071	0.057	14.214	33.113

* = P<0.01, ** = P<0.05, *** = P<0.001.

aurues, *A. pyogenes*, Gram negative bacteria and others), and seasons (summer and winter); to facilitate statistical analysis.

The data were analyzed using the randomized complete block design (RCBD) using 2-way three-way covariance analysis according to Gill (1978).

RESULTS

Season had effect on total proteins, total solids, casein, casein number, whey proteins and ash (Table 1).

The degree of the disease (CMT score) had a significant effect on lactose, chloride ash and Koestler value.

Interaction between CMT score and types of bacteria isolated showed a highly significant result only for chloride and lactose (Table 3).

Interference between bacterial types and season affected chloride, casein, whey proteins, affected acidity and fat % (Table 4).

DISCUSSION

Although, the season (Table 1) has a nonsignificant effect on total solids (Waitte *et al.* 1956), it shows effect when it is interacted with bacterial types (Table 4). The significant effects of summer on protein, casein and whey proteins and casein number (P<0.001) was in agreement to the findings of Verdi *et al.* (1987). The interaction of summer with bacteria affected caseins, whey proteins, and casein number. This variation was mainly due to summer (Tables 1, 4).

The fat per cent showed a nonsignificant changes (Table 1). The change produced by the interaction of summer and *S.aureus* bacteria might be due to the damaging effects of bacteria on the secretory tissue (Kitchen *et al.* 1984).

The nonsignificant effect of summer on lactose was in agreement with the results of Mitchel *et al.* (1978), who stated that lactose was the least variable of the gross constituents on the percentage basis.

Chloride showed nonsignificant change to seasonal variation. The interaction of summer with *S. aureus* bacteria affected the chloride contents. This change could be attributed to the types of bacteria involved.

The ash content of mastitic milk was both affected by summer and *S. aureus* bacteria. This was agreed with the finding of Mitchel *et al.* (1978). The acidity was significantly affected by both the seasons and its interaction with bacteria.

Effect of clinical mastitis infection on the milk constituents

Table 3. The effect of clinical mastitis *S. aureus* infection on milk (mean squares from ANOVA tables)

Source of variation	Total solids	Protein	Fat	Lactose	Chloride	Ash	Casin	Whey protein	Acidity	Kos. value	Casein No.
Covariate	0.198	0.292	0.384*	0.268	0.060*	0.013	0.327	0.028	0.036	20.860	50.003
Parity number	0.217	0.200	0.326	0.471	0.89**	0.057	0.490	0.023	0.004	32.510	36.980
Stage of lactation	0.200	0.416	0.483*	0.047	0.037	0.002	0.301	0.029	0.070	12.240	54.370
Main effects	0.486	0.254	0.103	0.333*	0.058***	0.054	0.269	0.074	0.096	37.410	41.840
CMT	0.414	1.200	0.105	0.171	0.038	0.062	0.039	0.119	0.052	33.940	44.130
Bacteria	0.221	0.571	0.045	0.269	0.058***	0.015	0.333	0.059	0.165*	38.260	35.260
2-way interaction (CMT × Bact.)	0.240	1.578	0.087	0.074	0.065***	0.043	0.201	0.078	0.045	54.430	53.550
Explained	0.334	0.338	0.132	0.150	0.062***	0.046	0.241	0.072	0.064	40.290	47.980
Residuals	0.560	0.262	0.111	0.111	0.015	0.037	0.174	0.069	0.055	37.730	24.720
Total	0.515	0.278	0.114	0.039	0.025	0.039	0.188	0.070	0.057	38.660	33.180

* = P<0.05, ** = P<0.01, *** = P<0.001.

Table 4. The effect of *S.aureus* infection and summer on mastitic milk (mean squares from ANOVA tables)

Source of variation	Total solids	Protein	Fat	Lactose	Chloride	Ash	Casein	Whey protein	Acidity	Kos. value	Casein No.
Covariate	4.230	0.133	0.317	0.053	0.065	0.006	0.9818	0.129*	0.005	30.462	67.32
Parity number	5.150	0.117	0.134	0.044	0.089	0.008*	1.383*	0.205*	0.010*	35.490	131.49*
Stage of lactation	2.590	0.126	0.451	0.071	0.030	0.005	0.599	0.072	0.001	29.085	6.059
Main effects	2.810	0.296	0.562*	0.186	0.089	0.017***	3.553***	0.741***	0.013**	28.087	131.29**
Season	8.679*	0.177	1.577**	0.000	0.221**	0.032***	6.793***	1.476***	0 .021***	2.083	174.26
Bacteria	2.930	0.378	0.637*	0.230	0.114*	0.002	0.306	0.032	0.003	33.212	30.718
Explained	3.280	0.142	0.480	0.138	0.081	0.014***	2.674***	0.537***	0.010**	21.824	69.981**
Residuals	1.250	0.251	0.137	0.278	0.228	0.002	0.226	0.028	0.002	15.391	28.347
Total	2.180	0.247	0.295	0.213	0.053	0.007	1.356	0.263	0.006	15.939	31.890

* = P<0.05. ** = P<0.01, *** = P<0.001.

as measured by CMT has a significant effect on the total solid (Table 4). It is in accord with Rogers and Mitchel (1989). Moreover, Vianni and Nader (1991) reported a significant difference in solids-not-fat (SNF) even between CMT-negative and CMT traces. Furthermore, King (1978), could demonstrate a significant effect with the same cows having infected and non-infected quarters.

The level of the total proteins in this study, similar to that of Mollet *et al.* (1989), did not vary with the virulence of the disease. The decrease in casein (P<0.01) and casein number (P<0.05) could be due to the fact that the loss in casein was affected by the increase in serum proteins (Ashworth *et al.* 1967), who concluded that, the fat percentage was not affected by CMT score (Table 2). This could be attributed to decreased yield of milk rather than synthesis of fat (Jones and Jones 1986).

The degree of infection had highly significant effect on lactose. This reduction may be due to reduced concentration of α-lactalbumin (Sreekumer *et al.* 1975), or because of less glucose being available to the mammary gland due to reduced blood flow during the diseased state (Kitchen 1981).

The chloride content was increased with the degree of infection, similar to the results of Fernando and Spahr (1983).

The ash content showed a significant increase, as the degree of infection increased, which is in agreement wish El Deeb and Hassan (1987), and differed from that of Muldoom and Liska (1971). The interaction of clinical mastitis and *S. aureus* bacteria isolated had a highly significant effect on chloride which may be because of either bacterial acidification and the degree of irritation or inflammation of the mammary gland (Kitchen *et al.* 1984), as well as CMT score (Vianni and Nader 1991). The interaction of clinical mastitis and *S. aureus* bacteria, isolated, affected the whey proteins (P<0.05).

We concluded that clinical infection and summer have a significant effect on the level of most of milk constituents. Moreover, the level of alteration is more pronounced, in most cases, in samples infected wish *S. aureus* and in clinical samples collected during summer interacted with the virulence of the causative agents.

ACKNOWLEDGEMENTS

This study was financed by German Scientific Exchange Programme (DAAD). The authors wish to thank the staff of Dairy Land Farm, and staff of the Department of Physics (Faculty of Science, U. of K.). Thanks also extended to Dr Abdella Seed Ahmed (Faculty of Animal Production, U. of K.), and Amar Yassin (School of Mathematics, U. of K.) for their help during statistical analysis of the data.

REFERENCES

Ashworth U S Forester, T L and Luedeke L O. 1967. Relationship between CMT reaction and composition of milk from opposite quarters. *Journal of Dairy Science* **49** (7) : 1078-82.

Dawson R R, Book J A F and Wood P D P. 1974. Some factors associated with the late winter decline in the lactose content of bulk milk. *Journal of Dairy Research* **41** : 317-29.

El Deeb S A and Hassan H N. 1987. Changes in the cow's milk composition as affected with mastitis infection. *Alexandria Journal of Agricultural Research* **32** (3) : 163-74.

Fernando R S and Spahr S L. 1983. Effects of milking interval on selected milk constituents from normal and infected quarters. *Journal of Dairy Science* **66** (5) : 1155-61.

Foley J, Buckley J and Murphy M F. 1974. Commercial testing and product control in the dairy industry. University College Cork.

Gill J L. 1978. *Desigen and Analysis of Experiments in the Animal and Medical Sciences*. Vol. 1. The Iowa State University Press. Ames, Iowa.

Jenlins R, Gould R W and Gedcke D. 1981. *Quantitative X-ray Spectrometry*. Marcel Dellker, Inc., New York.

Jones T O and Jones, P C C. 1986. Cow milk yield and composition before development of *E. coli* mastitis. *Veterinary Record* **119** (13) : 319-21.

King J O L. 1978. Cell counts and composition of bovine milk, *Veterinary Record* **103** : (18) : 397-98.

Kitchen B J C. 1981. Review of the progress of dairy science. Bovine mastitis, milk composition changes and related diagnostic tests. *Journal of Dairy Research* **48** : 167-88.

Kitchen B J, Sergkwee W, Middleton G and Adrews R J. 1984. Relationship between the levels of NA-Gase in bovine milk and

the presence of mastitis pathogens. *Journal of Dairy Research* **51** (1) : 11-16.

Martin P G C. 1979. *Manuals of Food Qulity Control.* 3. Commodities. Food and Agriculture Organization of the United Nations-Rome-1979.

Mitchel E G, Lyal A and Shackel K D C. 1978. Milk composition in Queen Land. *Australian Journal of Dairy Technology* **33** (3) : 80-81.

Mollett T A, Brooks C B and Leighton E A. 1989. Effects of total CMT score and milk quality of the dam upon calf wearing weight in beef cattle. *Journal of Dairy Science*. **72** (Supplements 1) 1129.

Muldoon P J and Liska B J. 1971. Chloride ion activity electrode for the detection of abnormal milk. *Journal of Dairy Science* **54** (1) : 117-19.

Munro G L, Grive P A and Kitchen, B J. 1984. Effects of mastitis on milk yield, milk composition, processing properties and yield and quality of milk products. *Australian Journal of Dairy Technology* **39** (1) : 7-16.

Richards E L. 1959. The reaction of lactose with anthrone and its application to the estimation of lactose in casein and the dairy products. *Journal of Dairy Research* **26** (1): 53-57.

Richmond H and Drooper F I C C. 1925. *The Laboratory Book of Dairy Analysis*. 3rd edn, revised 1925.

Rogers S A and Mitchel G E. 1989. The relation between SCC, composition and manufacturing properties of bulk milk. *Australian Journal of Dairy Technology* **55** : 53-56.

Rowland S D. 1938. The determination of the nitrogen distribution in milk. *Journal of Dairy Research* **9** (1): 42-46.

Sreekumar B, Yadva R K and Rawat R S. 1975. Effect of subclinical mastitis on chemical composition of cowmilk. *Journal of Indian Dairy Science* **28** (3) : 213-17.

Tertian R and Claisse F. 1982. Principles of quantitative X-ray fluorescence analysis. Heyden London.

Verdi, R J Barbana D N Dellavalle M E and Senyk G F. 1987. Variability in true protein, casein, N.P.N. and proteolysis in high and low S.C. milks. *Journal of Dairy Science* **70** (2) : 230-42.

Vianni M C E and Nader, M C E and Nader Filhoa A. 1991. Variations in the physicochemical and cellular characteristic of milk of the cows with subclinical mastits. *Veterinary Bulletin* **61** : 6329.

Waite R, White J C D and Alan Robertson. 1959. Variations in the chemical composition of milk witth particular reference to S N F, 1. Effect of stage of lactation season of the year and age of the cow. *Journal of Dairy Research* **23** : 65-81.

Indian Journal of Animal Sciences 68 (4) : 386-387, April 1998

Factors influencing returns in sheep farming

M RAMA RAO[1] and P RAGHU RAM[2]

Agricultural College, Bapatla, Tirupati, Andhra Pradesh 522 101

Received : 26 February 1996; Accepted : 27 October 1997

ABSTRACT

The study was aimed at studying the factors influencing sheep farming in Nellore district of Andhra Pradesh. Cobb-Douglas type of production function was used to achieve the set objective of the study. The flock size exhibited highest contribution in increasing the returns in sheep farming compared to the other variables. Grazing costs consistently influenced negatively on the gross returns on all types of farms. Veterinary expenditure had positive and significant influence on large farms only. The study indicated the scope to reorganize the input factors which were irrationally used.

Key words : Economics of sheep farming, Sheep farming

Sheep farming is catching up greater prominance in the areas ideal for their profitable rearing. The district Nellore, Andhra Pradesh, is occupying fourth place in sheep farming and the Nellore Zodipi sheep has national acclaim for mutton purpose. In such an expanding enterprise, it is of interest to know the effect of various input factors on the returns obtained by the flock owners. As such, there is a need to estimate the factors influencing the economic returns in sheep farming.

MATERIALS AND METHODS

The district Nellore was purposively chosen for the study in view of its importance in sheep rearing in the state. Kaligiri and Dagadarthi mandals of Nellore district were selected as considerable sheep population of the district was found concentrated in these mandals. Six villages were purposively selected from the two mandals based on the criterion of highest population. The farmers were divided into 3 categories, viz. small, medium and large and from the categories so made, 50 farmers from small (below 54 sheep), 27 from medium (55 to 105 sheep) and 21 from large size group (106 sheep and above) were randomly chosen in probability proportion to their number.

The following form of Cobb-Douglas function was used to study the factors influencing sheep production.

$$Y = a\, x_1^{b1}\, x_2^{b2}\, x_3^{b3}\, x_4^{b4}\, x_5^{b5}\, U$$

Y, gross returns per farm in rupees ;x_1 ,flock size;x_2 ,cost of human labour in rupees in all except medium size;x_3 ,cost of grazing in rupees in all categories except pooled; x_4 ,cost of lamb feed in rupees in all except for large and pooled;x_5 ,veterinary expenditure in rupees in all except for medium; and U,error term.

In respect of small farmers all the 5 variables were retained. But regarding medium, large and pooled farmers some variables were dropped because of the presence of multicollinearity. The variables that were retained were x_1, x_3 and x_4 in medium category, x_2, x_3 and x_5 in large category, and x_1, x_2, x_3 and x_5 pooled category.

RESULTS AND DISCUSSION

The results of the functional analysis are presented in Table 1.

Small farms

The factors, viz., flock size, human labour costs, cost of grazing, cost of lamb, feed and veterinary expenditure were selected. The coefficient of multiple determination (R^2) was 0.85 and significant thereby explaining the fact that the variables included in the function explained 85.00 % of the variation in the dependent variable (gross returns). Of the factors included in the function, the variable flock size alone exerted significant influence. The remaining 4 factors were nonsignificant. The elasticity of flock size was 0.75 implying that 1 % increase in flock size would increase the gross income by 0.75 %. The coefficient of the variables, grazing cost, cost of lamb feed, veterinary aid had negative signs. The above results infer that on small farms the gross returns were largely influenced by the flock size. The cost paid by the small farmers for the grazing lands seems to be on the higher side which reflected the irrational use of this input in sheep rearing. Similarly expenditure on lamb feed and veterinary aid also appear to be more than the optimum label.

Present address: [1,2]Department of Agricultural Economics, S V Agricultural College, Tirupati 517 502.

Table 1. Regression coefficients of factors influencing returns in sheep farming

Particulars	Small	Medium	Large	Pooled
No. of observations	50	29	21	100
Constant (log a)	4.7153	5.9075	8.4759	5.5660
	(0.8621)	(0.6478)	(2.0609)	(0.6813)
Flock size (x_1)	0.7525**	0.4068**	-	0.9794**
	(0.1388)	(0.2245)		(0.0867)
Cost of human labour	0.1623	-	-0.1994	-0.0697
in rupees (x_2)	(0.1541)	(0.2544)	(0.1114	
Cost of grazing in	-0.0107	-0.0312	-0.2173**	-0.0157**
rupees (x_3)	(0.0147)	(0.0121)	(0.0617)	(0.0095)
Cost of lamb feed in	-0.0141	0.2395**	-	-
rupees (x_4)	(0.0349)	(0.1345)		
Veterinary expenditure	-0.0158	-	0.2966**	-0.0173
in rupees (x_5)	(0.0122)		(0.1095)	(0.0105)
Coefficient of multiple determination (R^2)	0.8592**	0.5934**	0.5783**	0.9527**

Figures in parentheses indicate standard errors;** significant at 5 % level.

Hence, the small farms need to keep a watch in the use of these 2 factors.

Medium farms

The coefficient of multiple determination was 0.59, implying that the selected variables have contributed for 59.0 % of the variation in the gross returns. Of the 4 variables included in the function, 3 variables explained either positive or negative influence significantly. The variables flock size and cost of lamb feed were associated with positive signs and significant at 10.0 % level. Grazing costs had a negative significant sign. The elasticities obtained in this function suggest that on medium farms also, the flock size had highest coefficient with a positive sign duly explaining that further increase in flock size will contribute for greater increase in the gross returns. The farmers of this group enjoy still a better chance with respect to increased use of lamb feed as its coefficient ensures a positive gain in the gross returns. The case of grazing cost gave an indication that it needs to be curtailed to overcome its negative affect.

Large farms

The estimated coefficient of multiple determination was 0.58 and significant at 1.0 % level. The significant variables in the function were grazing cost and veterinary expenditure and of these, the coefficient of grazing cost showed negative sign. This trend was that the majority of the farms purchase grazing lands in the absence of their owned grazing fields and the costs collected by the owners, seem to be on the higher side. The elasticity of human labour cost was found to bear nonsignificant negative sign. These observations have helped in drawing the following inferences. As observed in the other 2 groups, grazing cost tended to influence the gross income negatively. Large farmers were also no exception in not rationalizing the expenditure under this head. The elasticity of veterinary expenditure made an impression that its further use can increase the gross income significantly. Another variable, the use of which need to be brought down, was the human labour cost as it was already over used.

Pooled farms

The coefficient of multiple determination turned out to be highest in these farms with 0.95, duly revealing that 95.0 % of the variation was explained by the variables included in the function. The variables, with significant signs were flock size and cost of grazing. Padmanabhan (1994) and Prasanna (1994) reported that flock size significantly influenced income in sheep farms. The elasticities of cost of human labour and veterinary expenditure were nonsignificant. The coefficient of flock size was highest of the lot with 0.97, indicating that 1.0 % increase in flock size will raise the gross income by 0.97 %. The elasticity coefficient for cost of grazing was negative, there by showing its negative impact on the pattern of gross returns.

CONCLUSIONS AND POLICY IMPLICATIONS

The functional analysis highlighted that the flock size had the highest contribution, in increasing the gross returns in sheep farming over any of the variables included, either independently or collectively. The contribution of other variables was negligible. However, the other factors in sheep farming like grazing, consistently influenced negatively on the gross returns on all the types of farms. Human labour, barring on small farms, was identified to be excessively used as their productivity was dwindling. Though the results of the large farmers showed the scope of furthering the veterinary expenditure for still better income, pooled farms revealed the cause of anxiety as the veterinary expenditure on sheep health was overused. The sheep farmers need to recognize their expenditure on these cost factors which were irrationally utilized.

Concentrates were not found included in the feeding regime, which is very vital for the growth of sheep. The concentrates in the form of oilcake, horsegram etc. should be supplied to the sheep farmers by the Government agencies at subsidized rates. The sheep farmers need to be trained for scientific methods of sheep farming. Livestock Research Stations should come forward to train the sheep farmers for better returns in the enterprise.

REFERENCES

Padmanabhan N R. 1994. An analysis of sheep farming in Tamil Nadu with particular reference to economics and resource use efficiency. *Indian Journal of Animal Sciences* **64**(6): 639-42

Prasanna T. 1994. 'An economic analysis of farming systems in Guntur district of Andhra Pradesh'. M.Sc (Ag) Thesis, A P Agricultural University, Hyderabad.

Indian Journal of Animal Sciences **68** (4) : 388-389, April 1998

Non-normal distribution of some traits of Sahiwal × Jersey crossbred cows

V K BHARTI[1], A K SHUKLA[2] and S N MAURYA[3]

Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttar Pradesh 263 145

Received : 19 September 1995; Accepted : 28 October 1997

Key words : Crossbred cows, Non-normal distribution of traits, Traits

Generally various routine statistical tests are carried out with the basic assumption that the character under study follows normal distribution in the population (Amble 1975). In cases where distribution of the character does not follow normal distribution, these test procedures cannot be applied. Therefore it is essential that the distribution pattern of the characters in a population is ascertained to decide the statistical techniques to be used for the analysis (Srivastava and Tandon 1984).

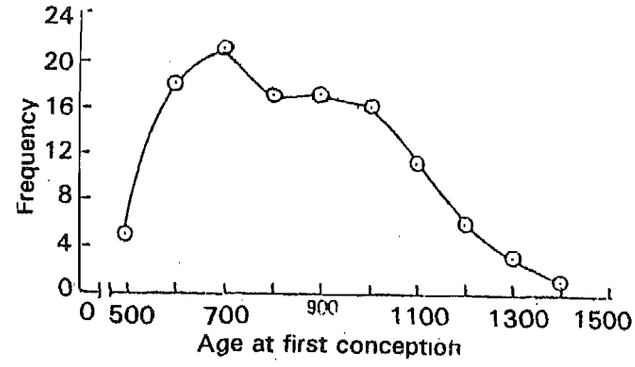
Four important characters having significant effect on milk yield, viz. age at first conception (AFC), first gestation period (FGP), first lactation period (FLP) and interval between first and second calving ((FSC), were taken. The aim was to fit normal distribution and if normal is not fitted, to ascertain the exact distribution.

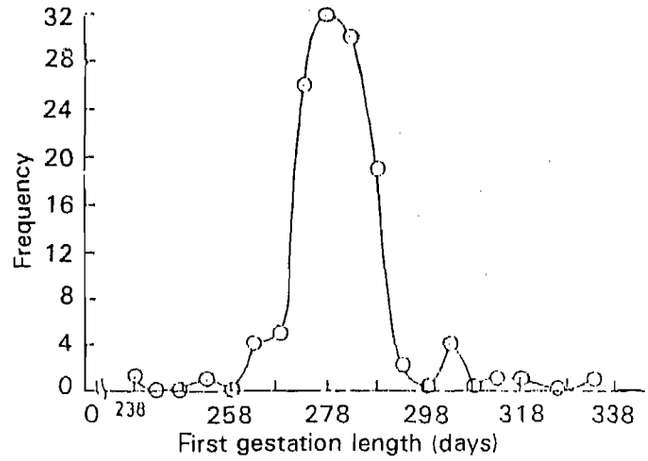
The data were obtained from the Livestock Research Centre of this university for 11 years i.e. from 1971 to 1981. The cows were kept under standard management practices. Depending on the available records for each character under study 114 Sahiwal-Jersey crossbred cows for age at first conception, 127 for first gestation period, 128 for first lactation period, and 146 for interval between first and second calving were considered.

Table 1. Number of observations (n), mean, S E, β_1, β_2, α_2 and κ values for the variables AFC, FGP, FLP and IFSC

Variables	n	mean	SE	β_1	β_2	α^2 cal	κ
Age at first conception	114	847.8	19.504	0.141	2.382	15.059*-	-0.0001
First gestation period	127	280.3	0.965	1.712**	11.850**		-0.031
First lactation period	128	309.1	5.980	0.035	3.641		-0.023
Interval between first and second calving	146	424.3	7.821	2.490**	5.572**		-132.801

** P<0.1 ; ** P<.05.

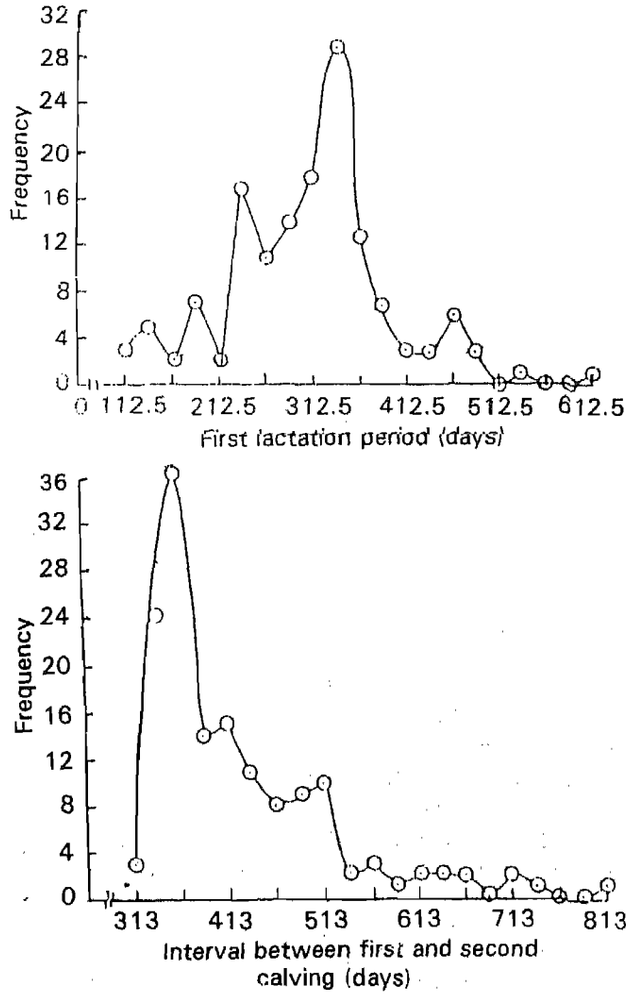




Figs 1-2. **1.** Frequency curve for the variable age at first conception. **2.** Frequency curve for the variable first gestation period.

Frequency curves were drawn for all the variables understudy (Figs 1-4). An essential condition for being a normal

Present address : [1]Statistician, AICRP, Department of Agronomy; [2]Associate Professor, Department of Mathematics and Statistics; [3]Professor and Head, Department of Gynaecology and Obstetrics.



Figs 3-4. 3. Frequency curve for the variable first lactation period. 4. Frequency curve for the variable interval between first and second calving.

curve is that β_1 should be zero and β_2 should be 3 (Madansky 1988); hence β_1 and β_2 were obtained (Table 1) and Z-test was applied to test the hypotheses $\beta_1 = 0$ and $\beta_2 = 3$. If there is no evidence found against both the hypotheses, fitting of normal distribution was done, applying chi-square test goodness of fit (Snedecor and Cochran 1967). If either of the hypotheses or both were rejected, the value of κ ($\kappa=\beta_1^2 / 4b_0\beta_2$) was obtained to know the exact non-normal distribution among the Pearson's family of distributions (Kendall and Stuart 1958).

In case of the variable 'age at first conception ' no evidence was found against both the null hypotheses, i.e. $\beta_1=0$ and $\beta_2=3$. Hence fitting of normal distribution was done. The hypotheses of normality was not rejected at 1% level of significance (LOS P>.01) and rejected at 5% LOS (P<.05) by chi-square test of goodness of fit. Since the hypothesis was not rejected at 1% LOS 'κ, value was obtained. to know the exact probability distribution, which is less than 0 and that falls under the category of Pearson's Type I (Table 1). It was concluded that if LOS is 5%, variable age at first conception has normal distribution and if LOS is reduced to 1% it has Pearson's Type I distribution . Both the hypotheses for β_1 and β_2 of first gestation period' were rejected, 'κ' value was obtained to know the exact Pearson's form of distribution which is lying between 0 to 1 and that belongs to the category of Parson's Type VI (Table 1). For the variable first lactation period sufficient evidence was not found against the hypothesis $\beta_1=0$, hence not rejected . It was just reverse for $\beta_2=3$, hence rejected . Since both the conditions, viz. $\beta_1=0$ and $\beta_2=3$, for fitting of normal distribution were not fulfilled, it was not done but 'κ' was obtained to know the exact probability distribution , which is less than 0 and that falls under the category of Pearson's Type I (Table 1). Sufficient evidence was found to reject the null hypotheses $\beta_1=0$ and $\beta_2=3$ for the variable ' interval between first and second calving , hence fitting of normality was not performed and the value 'κ' was obtained to know the exact probability distribution among the Pearson's set of distribution , which is less than 0. This comes under the category of Pearson's Type I (Table 1).

Overall it was concluded that the variables, age at first conception (at 1% LOS), first gestation period, first lactation period and interval between first and second calvings follows Pearson's Type I, Pearson's Type IV, Pearson's Type I and Pearson's Type I distribution, respectively, which are non-normal distributions. Therefore the statistical techniques requiring the summation of normal distribution cannot be applied for these characters related to cattle reproduction and production. However, other nonparametric tests can be applied in case of these traits (Gibbons 1971).

ACKNOWLEDGEMENTS

We thank the Director, Experiment Station of this University, for providing necessary help during the investigation.

REFERENCES

Amble V N. 1975. *Statistical Methods in Animal Sciences*. Indian Society of Agricultural Statistics, IASRI, Dr Krishnan Marg, New Delhi 110 012.

Kendall M G and Stuart A. 1958. *The Advanced Theory of Statistics (Distribution Theory)*. Vol. 1. Charles Griffin and Co., Ltd, London and High Wycombe.

Madansky A. 1988. *Prescriptions for Working Statisticians*. Springer-Verlag, 175 Fifth Avenue, New York, 10010.

Snedecor G W and Cochran W G. 1967. *Statistical Methods*. 6th edn. Oxford and IBH Pub. Co., Calcutta.

Srivastava R and Tandon O P. 1984. Fitting statistical distributions to incidences of conception under artificial insemination of crossbred cattle. *Indian Journal of Animal Sciences* **54** (11) : 1057.

Gibbons J D. 1971. *Nonparametric Statistical Inference*. McGraw-Hill Kogakusha, Ltd, Tokyo, Japan.

Indian Journal of Animal Sciences 68 (4) : 390-391, April 1998

MOET versus progeny testing for genetic improvement of dairy cattle

R S GANDHI[1], AVTAR SINGH[2] and M GURNANI[3]

National Dairy Research Institute, Karnal, Haryana132 001

Received : 24 August 1995

Key words : Dairy cattle, Genetic improvement, MOET, Progeny testing

Multiple ovulation and embryo transfer (MOET) applied on 2 cow pathways i.e. cow to breed bull and cow to breed cow increases the selection intensity of females leading to higher rates of genetic gain (Smith and Ruane 1987, Taneja *et al.* 1992). In India, MOET can be applied to dairy cattle breeding programmes in two ways : (i) as a supplement to conventional progeny testing, and (ii) by applying the juvenile and adult MOET schemes to nucleus breeding herds. In the present investigation, an attempt was made to compare the expected genetic gains from different MOET breeding schemes with conventional progeny testing in Sahiwal cattle, a representative of indigenous dairy breeds.

Data pertaining to 2374 Sahiwal cattle maintained at 5 organized farms were considered to generate the following parameters:

Average 305-day or less milk yield	=	1788 kg
Phenotypic standard deviation for milk yield	=	644 kg
Genetic standard deviation for milk yield	=	322 kg
Average age at first calving	=	3.438 year
Average generation interval from 4 under progeny testing paths	=	6.881 year
Average generation interval for juvenile MOET	=	3.438 year
Average generation interval for adult MOET	=	5.514 year

The following parameters were assumed for comparing different breeding schemes:

Population size	=	400
Conception rate	=	0.30
Joint effect of survivability and culling up to maturity	=	0.84
Sex ratio	=	1:1
Heritability of milk yield	=	0.25
Repeatability of milk yield	=	340.40
Replacement rate	=	0.25

Present address: [1] Scientist (Senior Scale), [2] Scientist, [3] Principal Scientist and Head, Dairy Cattle Breeding Division.

Expected genetic gain under progeny testing

The expected genetic gain from progeny testing was estimated (Robertson and Rendel 1950).

Expected genetic gain by supplementing progeny testing with MOET

In conventional progeny testing system the proportions selected from 4 paths viz. bull to breed bull (BB), bull to breed cow (BC), cow to breed bull (CB) and cow to breed cow (CC) in general are 0.10,0.20,0.20 and 0.80 respectively. When MOET is applied to CB and CC paths, these proportions can be reduced depending upon the number of progeny/donor. If 2 females/donor are produced, the proportions selected for CB and CC paths can be reduced to 0.10 and 0.40 respectively. Similarly, the proportion selected for 2 bull paths (BB and BC) can further be reduced to 0.05 and 0.10, respectively, to increase the rate of genetic gain.

Expected genetic gain through juvenile MOET

Considering a herd of 400 breedable cows and if 10% elite cows (40 donors) are superovulated and inseminated with semen of 10 sires (assuming 4 flushings/donor/year, 4 transferable embryos/flush and joint effect of conception rate, survivability and culling rate on poor growth rate equals to 0.25), we would have 80 male and 80 female calves per year i.e. 2 males and 2 females/donor/year. These males and females would be selected on the basis of their pedigree assuming average number of 3 lactations of their dams. The corresponding accuracy of selection would be low (0.323), though the generation interval would be lower i.e. 3.438 years.

Expected genetic gain through adult MOET

Considering other parameters to be same as in juvenile MOET, the expected genetic gain under this scheme would be different due to higher generation interval as well as higher accuracy of selection as information on sibs (half- and full-) and individual's record (in females) in addition to pedigree information is included for selecting the animals. Though the generation interval is more (5.514 years), yet the genetic gain is expected to be more as accuracy of selection is high. For each bull to be evaluated there would be information on 2

fullsibs (1 record each), 6 halfsibs (1 record each) and 3 records on dam. Similarily, for each cow information on 1 fullsib, 6 halfsibs, 3 dam's records and cows own record will be available. The accuracy of selection was calculated incorporating the above information in an index (Van Vleck 1981).

To minimize the effect of inbreeding 10 bulls will be selected out of 80 bulls from different fullsib families. The remaining bulls may be supplied to other developmental agencies. Eighty females produced would be retained in the herd and 20 cows would be purchased from outside agencies every year. Hence, 100 females would be sold every year to maintain a herd size of 400 cows. If herd has not to be opened, the replacement rate can be reduced to 20% only. The annual genetic gain was calculated as per Nicholas and Smith (1983).

The approximate rate of inbreeding per year in MOET schemes was calculated (Nicholas and Smith 1983) as under:

$1/8L^2(1/S+1/D)$

Where, S and D are number of sires and donors selected for breeding and L is generation interval.

The average expected genetic gain from conventional progeny testing scheme was 195.79 kg/generation or 28.45 kg/year, which was 1.591% of herd mean (Table 1). This estimate was higher than the value reported by Taneja *et al.* (1992) in crossbred cattle.

When conventional progeny testing was supplemented with MOET the expected genetic gain per year by applying MOET on CB and CC paths was 33.40 kg/year, which was 1.868% of population mean (Table 1). This was attributed to higher intensity of selection for both CB and CC paths. The increase in genetic gain was above 17% over conventional progeny testing programme.

The proportion of males selected could further be reduced for BB and BC paths in the above scheme to 0.05 and 0.10 as on an average 2 males are expected from each donor. This would increase the intensity of selection for these 4 paths (2.064 1.755%, respectively). The expected genetic gain per year from selecting few bulls under progeny testing supplemented with MOET was 37.84 kg, which was 2.116% of herd mean. By selecting few bulls in hybrid MOET the increase in genetic gain per year over conventional progeny testing scheme was 33.02%.

The genetic gain per year was 51.07 kg (2.856% of herd mean), when selection was done on the basis of pedigree for both males and females in juvenile MOET (Table 2). The increase in genetic gain per year was 79.52% more than that of conventional progeny testing programme. In adult MOET, the accuracies of male and female selection based on sib information, individual's record (in females) and dam's records were 0.535 and 0.698 respectively. The expected genetic gain was 61.10 kg/year, which was 3.417% of herd mean. This response was 114.76% of genetic gain expected from progeny testing. The rates of inbreding per year were estimated as 0.1322 and 0.0754%, respectively, from juvenile and adult MOET schemes, which were negligible in magnitude. In small herds rate of inbreeding would be higher than the expected since there would be tendency to select replacement from a small number of superior females.

To sum up, it was concluded that MOET schemes were better than conventional progeny testing scheme. Adult MOET seems to be more effective in augmenting the genetic gain in milk yield followed by juvenile MOET. Hence, wherever, infrastructure for embryo transfer is available, this technology should be applied at organized farms to raise progeny from elite cows for bringing about faster genetic improvement in milk yield.

REFERENCES

Nicholas F W and Smith C. 1983. Increased rates of genetic change in dairy cattle by embryo transfer and splitting. *Animal Production* **36**:341-53.

Robertson A and Rendel J M. 1950 . The use of progeny testing with artificial insemination in dairy cattle. *Journal of Genetics* **50**:21-31.

Smith C and Ruane J . 1987. Use of sibtesting as a supplement to progeny testing to improve the genetic merit of commercial semen in dairy cattle. *Canadian Journal of Animal Sciences* **67**: 985-90.

Taneja V K, Singh A, Bhat P N and Raheja K L. 1992 . Genetic improvement in milk production through embryo transfer. *Indian Journal of Animal Sciences* **62**:977-82.

Van Vleck L D. 1981. Potential genetic impact of artificial insemination, sex determination, embryo transfer, cloning and splitting in dairy cattle. *New Technologies in Animal Breeding*.pp.221-42. (Eds) Brackett B C, Seidel G E (Jr) and Seidel S M. Academic Press, London.

Indian Journal of Animal Sciences 68 (4) : 392-394, April 1998

Genetic architecture of Marwari sheep. 1. Body weight

U PANNU[1] , N K BARHAT[2] ,V K SINGH [3] and K P PANT[4]

Rajasthan Agriculture University, Bikaner, Rajasthan 334 001

Received : 19 August 1996

Key words: Genetic architecture, Marwari sheep, Sheep

Small ruminants play a major role in subsistence of poor peasants and livestock owners in semi - arid conditions of Rajasthan. The Marwari sheep is spread in 6 north west districts of Rajasthan with a population of 4.98 million. Improvement in wool quality and body weight will directly benefit the sheep owners. Therefore, an attempt has been made for overall economic improvement of Marwari sheep through selection for increase in greasy fleece weight and selection against modulation and to observe its correlated responses, if any.

The present investigation has been conducted on body weight of Marwari sheep in 3 selection lines. Data (1309) were obtained from Central Sheep and Wool Research Institute, Bikaner, from 1987 to 1992. Body weight at birth, 3, 6, 9 and 12 month of age were recorded. The data were classified according to selection line, year of birth and sex of lamb. The selection experiments were conducted against medulation and for increase in greasy fleece weight. So the flock was classified in 3 groups, viz. 1, random mating (RM), 2, selected group against medulation (AGM), 3, selected group in favour of greasy fleece weight (GFW).

Since the data were non - orthogonal the least - squares technique of fitting constants (Harvey 1987) was used to estimate the effect of various factors, viz. year of birth, sex of lamb and selection line. Duncon' s multiple range test (Kramer 1957) was used to make pair -wise comparison of the least - squares constant for significant effects. Heritability was estimated from the sire component of variance and its standard error was calculated by the approximate method (Dickerson 1960) and modified by Backer (1984). Genetic and phenotypic correlations were calculated by sire component of variance and covariance and their standard errors were calculated by the approximate method proposed by Robertson (1959).

The overall least -squares means for body weight at birth, 3, 6, 9 and 12 months of age (Table 1) were in agreement with those of Nehra (1991) and Ganai (1992).

Significant effect of selection line was evident only on body weight at birth and 3 months of age. At birth AGM group was significantly heavier than that of other groups. At 3 months of age RM group was significantly heavier as compared to AGM and GFW groups.

Year of birth had significant effect on body weight from birth to 1 year of age. Similar results were also obtained by Kulkarni and Deshpande (1991). The trend over body weight was inconsistent over the year. In general lower body weights were observed in 1991 except weight at birth and 9 months of age. At these 2 stages lower body weight may be due to drought condition (1987 and 1988). The significant effect of year of birth may be because of difference in availability of nutrients to dams for birth weight and weaning weight and to the lambs for higher body weights and other specific and nonspecific environmental conditions.

Sex of lamb also had significant effect on body weight at all the stages except birth weight. Males were heavier as compared to females in all age groups. These findings indicated that males grew at faster rate and gained more weight. Another reason for heavier male might be retention of heavier male lambs for breeding and disposal of slow growing. Negi *et al.* (1987) also reported nonsignificant effect of sex of lamb on birth weight. For other body weights similar results were obtained Ganai (1992).

There was no influence of generation on various body weights. Similar results were also obtained by Amble *et al.* (1967) whereas Singh and Dhillon (1991) reported significant effect of generation on weight at 3 and 6 months of age.

Regression coefficients revealed significant effect of dam's weight at lambing on birth weight and weaning weight. Similar results were obtained for birth weight by Gupta and Reddy (1988) and for weaning weight by Singh and Dhillon (1991).

Possible reason might be that heavier dams provided better nourishment and more space for developing foetus and more milk to the lambs during pre - weaning period. Weaning weight was not influenced by birth weight. Significant effect of weaning weight on 6 months of body weight indicated that lambs which were weaned heavier at 3 months of age had

Present address:[1] Assistant Professor, [2] Associate Professor, [4]Professor, Department of Animal Breeding and Genetics, College of Veterinary and Animal Science.

[3] Principal Scientist, CSWRI, Bikaner 334 001.

Table 1. Least - squares means (SE) for different body weights (kg)

	Body weight at different ages (age in month)									
Class/subclass	No	B wt	No	3	No	6	No	9	No	12
Overall	1319	2.83 ±0.066	1016	10.99 ±0.070	886	15.74 ±0.100	837	18.13 ±0.118	711	19.58 ±0.131
Selection lines		*		**						
RM	333	2.69a ±0.118	225	11.29b ±0.132	199	15.48a ±0.182	182	18.04a ±0.214	157	19.61a ±0.236
AGM	244	3.10b ±0.138	203	10.98a ±0.141	178	16.05a ±0.196	170	18.36a ±0.226	149	19.64a ±0.248
GFW	742	2.70a ±0.079	588	10.71a ±0.083	509	15.69a ±0.118	485	18.00a ±0.137	405	19.51a ±0.153
Year of birth		**		**		**		**		**
1987	224	3.41b ±0.161	*189*	*10.52b* ±0.163	*190*	*18.03c* ±0.253	*172*	*15.77* ±0.250	*113*	*17.54a* ±0.310
1988	157	2.62a ±0.185	92	11.02c ±0.219	62	15.29b ±0.339	52	19.03b ±0.411	43	20.31c ±0.458
1989	282	2.99a ±0.142	191	11.15c ±0.158	158	15.93c ±0.223	155	20.54c ±0.253	120	21.85e ±0.259
1990	233	2.66a ±0.145	187	10.59b ±0.148	142	15.13c ±0.215	134	18.73b ±0.248	124	21.87 ±0.261
1991	226	2.63a ±0.159	198	9.52a ±0.155	186	13.81a ±0.206	183	16.06a ±0.229	175	17.18a ±0.232
1992	197	2.67a ±0.188	159	13.19d ±0.193	148	16.25c ±0.274	141	18.68b ±0.297	136	19.56b ±0.299
Sex of lamb				**		**		**		**
Male	646	2.92a ±0.089	503	11.34b ±0.094	420	16.07b ±0.136	385	19.14b ±0.159	283	20.78b ±0.188
Female	673	2.74a ±0.087	513	10.64a ±0.093	466	15.41a ±0.128	452	17.13a ±0.147	428	18.39a ±0.157

Least-squares means with different letter within the same column differ significantly,wt, weight; B, birth; RM, random mating; AGM, selected against medullation; GFW,selected in favour of greasy fleece weight; * significant at P<0.05; ** significant at P<0.01.

higher body weight at 6 months of age also. Such lambs adjust themselves in all conditions better than lambs with lower weight and also gain more weight at subsequent ages.

Heritability estimates for birth weight and 9 months body weight were very low. Similar finding was observed by Ganai (1992). The heritability estimates for 3, 6 and 12 months of body weight were moderate (0.23 to 0.34). These results are in close agreement with the findings of Ganai (1992). Low heritability estimate of birth weight and 9 months body weight suggested that the weight at birth and 9 months of age might be a poor criterion for selection.

Phenotypic correlation of body weight at birth with weights at subsequent ages was positive and low. These results are in agreement with the findings of Nehra (1991) and Ganai (1992). Phenotypic correlation of body weight at 3, 6 and 9 months of age with body weight at subsequent ages were moderate to high (0.390 to 0.797). These results showed that phenotypic correlation of body weight at higher ages were high with body weight at subsequent ages. Genetic correlation of birth weight with 1 year body weight was positive but very low and with 9 months body weight

Table 2. Estimate of heritability for different body weight with their genetic and phenotypic correlation

	B wt	3 month wt	6 month wt	9 month wt	12 monts wt
B wt	0.07 ±0.087	0.233 ±0.001	0.158 ±0.001	0.145 ±0.001	0.263 ±0.001
3 month wt	0.711 ±0.846	0.34 ±0.119	0.402 ±0.001	0.390 ±0.151	0.397 ±0.156
6 month wt	0.855 ±1.009	0.403 ±0.229	0.34 ±0.110	0.517 ±0.266	0.474 ±0.223
9 month wt	0.247 ±0.051	0.657 ±0.305	0.833 ±0.212	0.12 ±0.094	0.797 ±0.633
12 month wt	0.002 ±0.787	0.313 ±0.274	0.310 ±0.273	0.797 ±0.169	0.23 ±0.157

B, Birth; wt, weight; the values on the diagonal are heritabilities, above the diagonal are phenotypic correlations and below the diagonal are genetic correlations.

was moderate. Whereas rest of the genetic correlations were positive and high.

The estimated heritability for weaning and 6 months

body weight were moderate with high and positive genetic and phenotypic correlation with their subsequent ages suggesting that selection for heavier lamb at these ages may ensure heavier lambs in subsequent generation also. So selection may be practiced at 3 and 6 months of age.

REFERENCES

Amble V N, Khandekar N C and Garg J N. 1967. Statistical studies on breeding date of Deccani and crossbred sheep. *Indian Journal of Veterinary Science and Animal Husbandry* **37** :578-84.

Backer W A. 1984. *Manual of Procedures in Quantitative Genetics*. 4th edn. Washington State University, Washington.

Dickerson G E . 1960. Techniques and Procedures in Animal Production Research. American Society of Animal Production Publication.

Ganai G A. 1992. 'Selection indices for genetic improvement of Marwari Sheep.' M.V.Sc. Thesis, Rajasthan Agricultural University, Bikaner.

Gupta B R and Reddy K K. 1988. Factors affecting birth weight in Nellore and Dorset × Nellore and synthetic lambs. *Indian Journal of Animal Sciences* **58** : 391-93.

Harvey W R. 1987. *User's guide for LSMLWM PC-1 Version Mixed Model Least-squares and Maximum Likelihood Programme*. Iowa State University, USA.

Kramer C Y. 1957. Extension of multiple range test to group correlated adjusted means .*Biometrics* **13** : 13-17.

Kulkarni A P and Deshpande K S. 1991. Birth weight in Deccani sheep and its crosses with exotic breeds. *Indian Veterinary Journal* **68** : 33-35.

Negi P R, Bhat P N and Garg R C 1987. Factors affecting pre-weaning body weight in Gaddi sheep and its crosses. *Indian Journal of Animal Sciences* **57** : 489-92.

Nehra K S 1991.' Study on growth and wool production of Marwari sheep in Arid Zone.'M.V.Sc. Thesis, Rajasthan Agriculture University, Bikaner.

Robertson A 1959. The sampling variance of the genetic correlation. *Biometrics* **15** : 469-85.

Singh G and Dhillon J S 1991. Factors affecting body weights of Avikalin lambs. *Indian Journal of Animal Sciences* **62** : 855-60.

Indian Journal of Animal Sciences **68** (4) : 395-396, April 1998

Index selection in broiler sire and dam lines-correlated responses

B L N REDDY[1], R P SHARMA[2] and B P SINGH[3]

Central Avian Research Institute, Izatnagar, Uttar Pradesh 243 122

Received : 28 April 1997; Accepted : 28 October 1997

Key words: Correlated responses, Dam line, Secondary traits

Continuous selection for certain primary traits will bring appreciable changes in related traits, thereby affecting the over all performance at the final generation. Dunnington and Siegel (1985), Pym *et al.* (1984) reported correlated responses in broiler traits supporting realized genetic correlations. Reproductive traits are influenced by selection for growth rate (Marks 1985, Siegel and Dunnington 1985). The present study was under taken to estimate the correlated responses in secondary traits in purebred broiler lines.

Red Cornish line (IC-3) and White Rock line (IR-3) maintained as pedigreed closed flock at Central Avian Research Institute, Izatnagar, were utilized for the study. IC-3 selection was based on the index including 4 and 6 week body weights, 6 week breast angle and 6 week shank length. Rock line was selected on the basis of Index including 6-week body weight, 40 week egg production and 40 week egg weight. After 4 generations of selection correlated responses were recorded in secondary traits. In each generation on 20

Table 1. Genetic and phenotypic responses for correlated traits in IC-3 and IR-3

Gen	20wk bw (g)				40wk bw (g)				% F		% HT		% HF	
No.	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀						
(n)	$\bar{X}$	$\bar{X}$	$\bar{X}$-C	$\bar{X}$-C	$\bar{X}$	$\bar{X}$	$\bar{X}$-C	$\bar{X}$-C	$\bar{X}$	$\bar{X}$-C	$\bar{X}$	$\bar{X}$-C	$\bar{X}$	$\bar{X}$-C
IC - 3														
G1 (877)	3160	2402	430	316	4530	3483	490	469	85.24	-1.76	65.25	-4.00	73.89	-5.01
G2 (882)	3148	2404	411	339	4564	3710	325	624	90.10	6.65	76.57	-7.20	84.98	15.07
G3 (974)	3293	2312	558	358	4603	3573	881	662	90.53	0.45	69.53	3.34	77.23	2.98
G4 (1137)	3141	2281	850	274	4598	3485	-147	-301	91.43	15.49	17.53	17.09	77.14	6.76
b	8.8	-45.5	141	-10.7	24.7	-13.1	-136	-228	1.90	4.55	0.88	6.71	0.20	1.73
±SE	38.9	12.1	49.2	18.3	6.34	57.7	212.7	187	0.72	1.67	2.48	3.81	2.57	4.94
IR - 3														
G1 (839)	3010	2186	280	100	4450	3391	410	377	90.77	3.00	70.77	1.52	80.05	-6.95
G2 (897)	3148	2404	411	339	4564	3710	325	624	87.70	4.25	87.70	-4.25	85.70	2.25
G3 (1478)	3096	2362	361	408	4475	3633	753	722	90.52	0.44	90.52	0.44	84.92	5.16
G4 (1541)	2733	2280	442	273	4488	3410	-257	-376	88.27	12.33	88.27	-12.33	84.41	-8.47
b	-88	24.0	43.6	58.8	2.5	-2.0	-157	-216	-0.47	2.42	1.52	-0.29	1.23	-0.16
±SE	79.6	49.9	23.3	59.2	26.8	195.7	201	225	0.78	6.05	4.87	3.45	1.08	3.68

n, Population size, $\bar{X}$, population mean , $\bar{X}$ - C, control deviated mean.

Present address : [1] Scientist, Project Directorate on Poultry, Hyderabad 500 030.

[2,3] Senior Scientists.

and 40-week body weight, livability (6-week), feed efficiency (6-week), dressing percentage (6-week), fertility and

hatchability traits were recorded on broiler breeders. Phenotypic responses were obtained by the regression of generation mean on generation number, while genetic responses were obtained by the regression of control deviated means. Since chicks were obtained in more than one hatch in each generation, data was corrected for hatch effects by fitting least square constants (Harvey 1975).

Adult body weights

On phenotypic scale responses for 20-week body weights were positive in IC - 3 males and IR - 3 females (8.8 g and 24 g) and are negative in IC - 3 females (-45.50) and IR - 3 males (-88.00 g). These responses indicated that positive phenotypic gains are likely to occur for 20-week body weight in both the lines. On genotypic scale responses for 20-week body weights are positive in IC-3 males (141 g), IR-3 males (43.60 g) and IR-3 females (58.80 g). Since most of the responses are positive in direction genetic gains in 20-week body weight was expected due to selection for juvenile body weights. On phenotypic scale responses for 40- week body weight are positive in IC-3 males and IR-3 females. Responses on genetic scale are negative in both the lines. The positive correlated responses observed in this study for adult body weights are similar to those reported by Dunnington and Siegel (1985) and Pym *et al.* (1984). These responses explain the high estimated and realized genetic correlations among the growth traits.

Fertility and hatchability traits

Phenotypic response for fertility was positive in IC-3 and was negative in IR-3, while genetic responses are positive in both the lines. Positive responses for this trait may be attributed to low heritability of this trait as selection for body weight likely to reduce the fertility. Short term nature of the index may not have effect on the fertility. Phenotypic responses for hatchability traits are positive in both the lines, while genetic responses are positive in IC-3 and are negative in IR-3. Environmental effects are more in the heritability of fitness traits and this may explain the nature of correlated responses. In a long term selection experiment Marks (1985) and Siegel and Dunnington (1985) reported negative responses for these traits. Negative responses observed in IR-3 for hatchability and fertility traits agree with the literature reports.

Livability, feed efficiency and dressing percentage

On phenotypic scale responses for livability are negative in both the lines. On genotypic scale responses are positive in IC-3 (0.36±0.29) and are negative in IR-3 (-1.91± 3.56). As none of the b values was statistically significant (Table 2). Hence no interference can be drawn. Feed efficiency was positive on phenotypic scale, while genetic responses were revealed that feed requirement to get 1 kg of live weight is reduced. Similarly Pym and Nicholls (1979) and Wilson (1969) opined that selection for growth rate improves the feed efficiency. Phenotypic response for dressing percentage is positive in IC-3 and is negative in IR-3, while genetic responses are positive in both the lines. This explains the effect of selection for growth rate on dressing percentage. Jaap *et al.* (1950) reported that body growth is the major factor increasing the yields of dressed and eviscerated carcasses. Genetic correlation between body weight and dressing percentage are high (rg=0.55, Muir 1963, Goodman 1973).

Table 2. Livability, feed efficiency and dressing percentage in IC-3 and IR-3

Gen. No.	Livability% (6wk)		Feed efficiency (6wk)		Dressing percentage (6wk)	
(n)	$\bar{X}$	$\bar{X}$-C	$\bar{X}$	$\bar{X}$-C	$\bar{X}$	$\bar{X}$-C
IC - 3						
G1	91.92	-0.75	2250	320	67.78	-1.23
G2	95.90	-0.80	2450	-400	69.10	-0.001
G3	94.55	-0.56	2300	-450	67.09	6.29
G4	91.32	-0.59	2440	-670	70.42	0.02
b	-1.58	0.36	102	-110	0.74	1.004
±SE	0.40	0.29	47.11	26.26	0.61	1.01
IR - 3						
G1	96.32	4.30	2630	60	68.24	-1.44
G2	96.75	4.19	2390	720	67.18	-1.00
G3	97.42	-1.90	2710	140	68.96	-0.54
G4	94.91	-0.05	2550	200	66.60	-0.62
b+	-0.36	-1.91	8.0	-500	-0.31	0.29
±SE	0.52	3.56	74.62	347.56	0.54	0.09

n, Population size, $\bar{X}$, population mean, $\bar{X}$ - C, control deviated mean.

REFERENCES

Dunnington E A and Siegel P B. 1985. Long term selection for 8 week-body weight - direct and correlated responses. *Theoretical and Applied Genetics* **77**: 305-13

Goodman B L. 1973. Heritabilities and correlations of body weight and dressing percentage in broilers. *Poultry Science* **52**: 379-80.

Harvey W R. 1975. *Least-Square Analysis of Data with Unequal Subclass Numbers.* Agricultural Research Services. USDA. Washington.

Jaap R G, Renard M M and Buckingham R D. 1950. Dressed and eviscerated meat yields from chickens at twelve weeks of age. *Poultry Science* **29**: 874-80.

Marks H L. 1985. Direct and correlated responses to selection for growth. *Poultry Genetics and Breeding.* (Eds) Hill W G, Manson J M and Hewitt. British Poultry Science Symposium **18**.

Muir F V. 1963. 'Heritabilities and genetic correlations between growth rate, eviscerated yield and breast angle in broilers.' M S Thesis, Southern Illinois University, Carbandale.

Pym R A E, Nicholls P J, Thamson E, Choice A and Farrel D J. 1984. Energy and nitrogen metabolism of broilers selected over ten generations for increased growth rate, food consumption and conversion of food to gain. *British Poultry Science* **25**: 529-39.

Wilson S P. 1969. Genetic aspects of feed efficiency in broilers. *Poultry Science* **48**: 487-95.

Indian Journal of Animal Sciences **68** (4) : 397-399, April 1998

Effect of feeding dried subabul (*Leucaena leucocephala*) leaf-meal in the complete diets of crossbred calves

C SRINIVASULU[1], K V SUBBA REDDY[2] and M PARTHASARATHY[3]

Acharya N G Ranga Agricultural University, Tirupati, Andhra Pradesh 517 502

Received : 20 August 1997; 21 October 1997

Key words : CP replacement, Digestibilities, Feeding crossbred calves, Subabul (*Leucaena leucocephala*), Nutrient utilization

Subabul (*Leucaena leucocephala*) is a promising forage crop for the tropics. The potential value of leucaena as an animal feed is well established. The cost of conventional protein supplements is ever escalating with availability dwindling and there is an impending need to evolve an appropriate alterative for ruminant feeding. Subabul can make a suitable option because of its high nutritive value which is comparable to that of alfalfa forage (National Academy of Sciences 1977). Therefore the present study was undertaken to study and compare the effect of incorporating 50 and 75% of dried subabul leaf-meal in the rations of crossbred calves on nutrient utilization, nitrogen, calcium and phosphorus balances.

Three complete rations containing 0, 50 and 75 % of dried subabul leaf-meal were formulated. CR-1 was a control, containing horse gram hay and other conventional feed ingredients in the ratio of 75:25. In CR-2 and CR-3, 50 and 75 parts of horsegram hay was replaced by dried subabul leaf-meal respectively. The complete rations were made isonitrogenous by adjusting the proportions of groundnut-cake (deoiled) and maize.

The crossbred bull calves (weighing 201.77 ± 6.01 kg) for each experiment were used in a 3 × 3 latin square arrangement to evaluate the rations for nutrient utilization, nitrogen, calcium and phosphorus balances. Each period of latin square consisted of a 14-day preliminary and a 7-day collection period.

Samples of complete rations, feed ingredients, leftovers, faeces were analyzed for proximate analysis as per AOAC (1980) method and cell wall constituents according to Goering and VanSoest (1970). Calcium and phosphorus were determined according to Ferro and Ham (1957) and Fiske and Subba Row (1975) respectively.

The data were subjected to analysis of variance (Snedecor and Cochran 1968). Differences among treatment means were tested for significance by Duncan's new multiple range test (Duncan 1955).

Chemical composition and cell wall constituents of subabul leaf-meal, was 89.79, 24.00, 4.00, 14.50, 46.05, 11.45, 0.42; 2.81; 0.31, 42.15, 16.10, 26.05, 10.80 and 5.84 for DM, CP, EE, CF, NFE, ash, AIA, Ca, P, NDF, ADF. Hemicellulose cellulose and permangnate lignin, respectively, on per cent drymatter basis. Ingredient and chemical composition of complete rations were presented in Table 1.

Table 1. Ingredient and chemical composition of complete rations containing dried subabul leaves on per cent dry matter basis

	CR-1	CR-2	CR-3
Ingredient composition			
Subabul leaf-meal	0	50	75
Horse gram	75	25	0
Groundnut-cake (deoiled)	18	6	0
Maize	4	16	22
Mineral mixture[a]	1	1	1
Salt	2	2	2
Vitablend[b]		25 g	
Chemical composition			
Dry matter	95.56	95.54	94.96
Organic matter	93.16	91.53	88.33
Non protein organic matter	77.47	75.58	72.79
Crude protein	19.98	20.05	20.09
Ether extract	4.36	3.75	3.25
Crude fibre	27.26	14.09	9.58
Nitrogen free extract	42.02	53.65	55.42
Total ash	6.83	8.46	11.66
Acid insoluble ash	0.62	0.73	0.93
Calcium	1.46	1.80	3.20
Phosphorus	0.81	0.69	0.38

[a] Aries brand of mineral mixture

[b] Vitablend (AD_3) each gram contained vitamin A 50,000 IU and vitamin D_3 5000 IU.

Present address : [1]Assistant Research Officer, Feed and Fodder Technology Division, Regional Agricultural Research Station, Tirupati 517 502.

[2]Professor and Head, Department of Biochemistry, Rajiv Gandhi College of Veterinary and Animal Sciences, Pondicherry 605 009.

[3]Research Officer, Livestock Research Station, Garividi 532 101.

Apparent digestibility coefficients, plane of nutrition and balance of nutrients of rations containing different levels of subabul leaf-meal were present in Table 2. The DM digestibilities were not different between CR-2 and CR-3. However, significant increase in digestibility of control ration was attributed to better quality of horsegram. Similar results were obtained by Gupta *et al.* (1989) when subabul hay was fed at 0, 50 and 80 % in the rations of buffalo bulls. OM digestibilities decreased gradually from ration 1 to 3 as subabul level increased. CP digestibilities were comparable among the experimental rations. Similar results were observed in buffalo bulls (Gupta *et al.* 1989) in bulls (Reddy *et al.* 1994) and in crossbred calves (Reddy *et al.* 1993). EE digestibility of rations 2 and 3 were significantly lower ($P < 0.01$) than ration 1 and no significant difference was observed between rations 2 and 3. These results confirmed the reports Sudhakar and Rama Rao (1987) and Gupta *et al.* (1989). The variation in the present study is attributable to the presence of more pseudofats like xanthophills, chlorophills and carotenoids present in subabul leaf-meal which are estimated as crude fat (EE). CF digestibility results were comparable to the results of Gupta *et al.* (1989). NFE digestibilities were not significantly different among treatments. Sudhakar and RamaRao (1987) and Gupta *et al.* (1989) also reported non-significant differences among NFE digestibilities of rations containing subabul.

The differences in the digestibilities of cell wall constituents (Table 2) were in accordance with the cell wall constituent composition of complete rations and comparable to CF digestibility.

The DMI of calves fed experimental rations were 3.40, 3.71 and 2.67 for rations 1 to 3 respectively. DM intake in ration 3 was significantly lower ($P<0.05$) than that of rations 1 and 2. However, there was no significant difference between rations 1 and 2. These results were comparable with the reports of Lawar and Patel (1986) and Muniga *et al.* (1992).

The DCP and TDN contents were 14.06, 61.45, 13.65, 59.64 and 14.78, 62.18 % for rations 1 to 3 respectively. The DCP and TDN intakes of the calves were comparable to the nutrient requirements of growing calves at 200 kg body weight with an expected growth rate of 500 g (Kearl 1982, NRC 1988). The intake of DCP and TDN of the calves in the present study are similar to the observations of Gupta *et al.* (1986b), Chakraborthy and Ghosh (1988), Reddy *et al.* (1993) and Reddy *et al.* (1994).

Nitrogen intake was not significantly different among the rations. Similar results were reported by Sudhakar and Rama Rao (1987), Gupta *et al.* (1989), and Reddy *et al.* (1993). Nitrogen retention expressed as g/day, N retention expressed as per cent of intake or per cent of absorbed i.e apparent BV (%) were comparable among calves fed rations 1 to 3.

Calcium intake was higher in calves fed ration 3. Calcium retention expressed as g/day was not significantly different among calves fed different rations. However, calcium retention when expressed as per cent of intake, was significant different among the treatments. Bhaskar *et al.* (1987) and Reddy *et al.* (1994) reported positive calcium balances in animals fed subabul leaf-meal.

Intake of phosphorus decreased with increased level of subabul in the rations 2 and 3 compared to the control ration. The retention of phosphorus expressed as g/day was significantly lower in calves fed ration 3. Phosphorus retention expressed as per cent of intake was significantly lower in calves fed ration 2 ($P>0.05$). The present observations

Table 2. Digestibility coefficients, plane of nutrition and balance of nutrients of rations containing different levels of subabul leaf-meal

Parameters	CR-1	CR-2	CR-3	SE± Means
Digestibility coefficients				
DM*	62.78^{b}±0.43	59.14±0.51^{c}	60.89^{bc}±0.27	0.40
OM*	63.14^{b}±0.34	59.53^{c}±0.27	57.36^{c}±0.45	0.57
CPa	70.39±0.34	68.01±0.61	73.58±1.02	0.82
EE**	63.45^{b}±0.23	55.51^{c}±0.36	50.47^{c}±0.33	0.46
CF*	48.87^{b}±0.22	45.09^{c}±0.44	40.46^{d}±0.25	0.52
NFE^{a}	63.93±0.39	61.77±0.79	64.88±0.57	0.84
NDF*	53.61^{b}±0.62	44.24^{c}±1.81	36.69^{c}±0.85	1.44
ADF**	39.02^{b}±0.32	33.26^{c}±0.39	27.46^{d}±0.41	0.14
Hemi-cellulose**	71.25^{b}±0.63	72.06^{b}±0.30	60.82^{c}±0.18	0.26
Cellulose**	51.39^{b}±0.30	46.90^{b}±0.46	41.76^{c}±0.21	0.33
Plane of nutrition				
DMI (kg / 100 kg B.Wt)*	3.40^{b}±0.11	3.71^{b}±0.17	2.67^{c}±0.24	
DCP %^{a}	14.06±2.11	13.65±0.86	14.78±1.12	
TDN%^{a}	61.45±2.89	59.64±3.12	62.18±2.62	
Nitrogen balance (g/day)				
Intake^{a}	174.83±19.23	210.95±18.97	151.67±32.11	7.57
Retained^{a}	105.83±11.22	123.39±10.61	95.98±20.12	5.83
Retained as % of intake^{a}	60.57±0.29	58.52±0.53	63.32±0.88	0.71
Apparent BV (%)^{a}	78.54±0.48	71.76±0.80	80.24±0.97	1.16
Calcium balance (g/day)				
Intake^{a}	82.30±8.91	113.18±11.69	140.40±28.93	11.13
Retained^{a}	45.64±5.11	56.60±5.88	62.81±13.16	7.23
Retained as % intake**	55.41^{b}±0.29	50.00^{c}±0.03	44.68^{d}±0.31	0.31
Phosphorus balance (g/day)				
Intake**	44.99^{b}±4.41	44.13^{b}±5.34	14.93^{c}±3.15	1.36
Retained**	27.60^{b}±2.79	24.96^{b}±3.05	9.40^{c}±1.98	0.71
Retained as % intake**	62.72^{b}±0.05	56.54^{c}±0.08	62.96^{b}±0.14	0.06

^{a}values in the rows are not significantly different ($P>0.05$); ^{bcd}values in the rows bearing different superscripts differ significantly; * $P(<0.05)$; ** $P(<0.01)$.

are similar to the results reported by Bhaskar *et al.* (1987) and Reddy *et al.* (1994).

The present study revealed that subabul can replace up to 90 % of CP of rations in crossbred bull calves without affecting the nutrient digestibility, nitrogen, calcium and phosphorus balances.

REFERENCES

AOAC. 1980. *Official Methods of Analysis.* 13th edn. Association of Official Analytical Chemists. Washington, DC.

Bhaskar B V, Prabhu V H, Gawali S R and Sampath S R. 1987. Chemical composition and nutritive value of subabul leafmeal (*Leucaena leucocephala*), Hawaiian giant, K-8. *Indian Journal of Dairy Science* **40:** 139-41.

Duncan D B. 1955. Multiple range and Multiple F tests. *Biometrics* **11:** 1-9.

Ferro P V and Ham A B. 1957. A simple spectrophotometric method for the determination of calcium. *American Journal of Clinical Pathology* **28:** 208.

Fiske C H and Subba Row. 1925. A colorimetric method for determination of phosphorus. *Journal of Biological Chemistry* **66:** 375-400.

Goering H K and Van Soest P J. 1970. *Forage Fibre Analysis.* ARS, USDA, Agricultural Hand Book No. 379 Washington, DC.

Gupta P C, Virk A S, Sagar V and Lohan I S. 1989. Effect of feeding subabul hay at two levels on the performance of buffalo bulls. *The Indian Journal of Dairy Science* **42:** 108-09.

Gupta P C, Virk A S. Khatta V K and Kumar N. 1986 b. Effect of feeding *Leucaena leucocephala* hay on the nutrient utilization and growth performance in buffalo calves. *Indian Journal of Animal Sciences* **56:** 147-48.

Kearl L C. 1982. *Nutrient Requirements of Ruminants in Developing Countries.* International Feed Stuffs Institute, Utah Agricultural Experimental Station, Utah State University, Logan, Utah, 84324, USA.

Lawar V S and Patel P M. 1986. Nutrient utilization of subabul (*Leucaena leucocephala*) fodder by replacement of crude protein in the ration of crossbred calves. *Livestock Advisor* **4:** 21-25.

Muniga R W, Thorpe W and Topps J H. 1992. Voluntary food intake, live weight change and lactation performance of crossbred dairy cows given *ad lib. Pennisetum purpureum* (Napier grass var. Bana) supplemented with leucaena forage in the low land semi humid tropics. *Animal Production* **53:** (3) 331-37.

National Academy of Sciences. 1977. *Leucaena, Promising Forage and Tree Crop for the Tropics. National Academy of Sciences,* Washington, D C, USA.

NRC. 1988. *Nutrient Requirements of Dairy Cattle.* 6th edn. Washington DC.

Reddy N M, Reddy G V N and Reddy M R. 1994. Utilization of fodder based complete diets in cross bred bulls. *Indian Journal of Animal Sciences* **64:** (6) 631-35.

Reddy D V, Krishna N, Naidu K V and Reddy R R. 1993. Effect of substituting conventional concentrate mixture by *Leucaena leucocephala* leaf fodder in crossbred calves fed rice straw based rations. *Indian Journal of Animal Nutrition* **10:**(1) 21-25.

Snedecor G W and Cochran W G. 1968. *Statistical Methods.* 6th edn. Oxford & IBH Publishing Co., Calcutta.

Sudhakar K and Rama Rao M. 1987. Feeding subabul leafmeal to crossbred dairy heifers. *Indian Journal of Animal Sciences* **57:** 222-23.

Indian Journal of Animal Sciences **68** (4) : 400-401, April 1998

Effect of feeding aflatoxin B_1 on feed consumption through naturally contaminated feeds

P L CHOUDHARY[1], R S SHARMA[2], V N BORKHATARIA[3] and M C DESAI[4]

Gujarat Agricultural University, Anand campus, Anand ,Gujrat 388 110

Received : 24 February 1997 ; Accepted : 28 October 1997

Key wards : Aflatoxin B_1, *Aspergillus* spp., Cows, Feed consumption, Feed level, Rations

Aflatoxins are the secondary metabolites produced by some strains of moulds namely *Aspergillus flavus* and *A. parasiticus* during their growth on feeds, foods and variety of other biological materials. Four naturally occurring aflatoxins, viz. B_1, B_2, G_1 and G_2-are produced exclusively by the moulds. Aflatoxin B_1 is found in the highest concentration and the most potent toxin (Wogan *et al.* 1971). Exposure of domestic animals to aflatoxins may lead to growth retardation, reduced feed consumption, lower reproductive rate, impaired resistance to infectious disease, reduced vaccination efficiency, low-milk yield and enhanced pathological damage to liver and other organs. No systematic studies have been reported on response of Indian dairy cow to the ingestion of aflatoxin B_1 through naturally contaminated rations on feed consumption of cow and buffalo and other species of milch animals under Indian conditions. Therefore, study was planned to evaluate the effect of aflatoxin B_1 on feed consumption.

Indian cows (10; Kankrege) of 5 to 10 year of age and at different stages of lactation, were selected randomly from the herd of the Gujarat College of Veterinary Science and Animal Husbandry, Gujarat Agricultural University, Anand campus, Anand.They were divided into 5 groups of 2 cows each. The cows in each group were allotted as one of the cow yielded relatively lower milk than the other cow in same group and uniformities were maintained in the groups. Various lots of compound cattle feeds was used as basic ration and deoiled groundnut-cake having high level of aflatoxin B_1 was used to raised the level of aflatoxin B_1 in various treatments. The other ingredients, viz. rice bran, rice polish, molasses, mineral mixture and salt were added as per the standard practice to make the rations complete. The various feed ingredients and their proportions used in formulation of different rations (Table 1).

Table 1. Proportions of different ingredients in various rations formulated for feeding trials

Feed ingredient	Quantity taken for formulation of rations (kg)				
	Control	T_1	T_2	T_3	T_4
Compound cattle *Feed (Amuldan)*	80.00	45.00	35.00	5.00	5.00
Rice polish	20.00	30.00	20.00	20.00	-
Deoiled rice bran	-	-	15.00	35.00	50.00
Deoiled-groundnut-cake DOC-LL	-	8.00	6.00	10.00	9.00
Deoiled-groundnut-cake DOC-HL	-	4.00	11.00	17.00	23.00
Molasses	-	10.00	10.00	10.00	10.00
Salt	-	1.00	1.00	1.00	1.00
Mineral mix	-	2.00	2.00	2.00	2.00
Aflatoxin B_1 (µ\kg) calculated	10.00	26.00	56.40	81.10	108.50
Analyzed	12.50	25.00	52.53	77.90	108.40

Analysis of feed and feed ingredients for aflatoxin B_1

Aflatoxin B_1 was analyzed as per the method described by the Bureau of Indian Standards (IS:13427:1992). The formulated concentrate rations having aflatoxin B_1 content of 12.50, 25.00, 52.53, 77.90 and 108.45 µg/ kg were designated as control (C) ,treatment $_1$(T_1), treatment $_2$ (T_2), treatment $_3$ (T_3) and treatment $_4$ (T_4) respectively (Table 1).

Feeding schedule of lactating cows under different treatments

Experimental rations (concentrate mixture) were fed to the selected cows during the study as per the feeding schedule proposed by Sen *et al.* (1978).All the 10 lactating cows in the 5 different groups, viz. control, T_1, T_2, T_3 and T_4 were first put on the control ration for 15 days and thereafter, the cow were put on rations formulated for different treatments. The entire feeding experiment was divided into 3 phases,

Present address : [1]Dairy Chemistry, College of Dairy Technology, IGKVV, Krishak Nagar, Raipur.

[2]Principal. [3] Senior Research Assistant, [4]Research Scientist.Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, Anand, Gujarat.

Table 2 . Effect of aflatoxin B_1 levels in feed on the feed consumptions by cows in 3 phases of the experiment

Treatment levels	Feed consumption (kg/day)			
	Phase 1	Phase 2	Phase 3	Pooled
Control	3.80	3.91	3.58	3.763
T_1	4.23	3.85	3.35	3.808
T_2	4.00	4.00	3.50	3.835
T_3	4.00	3.49	3.04	3.512
T_4	3.43	3.18	2.85	3.152
SEm (±)	1.015	0.942	0.777	0.095
CD	NS	NS	NS	0.267*
CV (%)	16.69	14.24	6.43	11.75

* Significant at 5% level.
NS, Nonsignificant values are the average of 8 days.

viz. phase 1, phase 2 and phase 3. During phase I, the cows in the respective groups were fed rations control, T_1, T_2, T_3 and T_4 for 7 days (treatment period) and on the eighth day, the cows in all the 5 groups were fed the control ration for next 7 days, i.e. residual period (Table 2). Thus, each feeding schedule was repeated thrice on the same group of cows which gave 6 observations under each treatment. Observations recorded during the experiment group in split-split plot design proposed by Steel and Torrie (1980).

Cows on control feeding showed no apparent effect on the feed consumption (Table 2). However, the feed intake data elucidate that in cows under T_1, T_2, T_3 and T_4 there is a gradual reduction of feed intake when the animals moved on from phase 1 to phase 3. Similarly, when the results of the pooled averages of 3 phases are analysed, significant ($P < 0.05$) reduction in feed intake observed. The results, thus, suggest that a long-term feeding schedule of increasing levels of aflatoxin B_1 through feed gradually reduce the feed intake by the animals.

Studies reported on the effects of intake of various levels of aflatoxin B_1 in relation to feed consumption are only few. Jones and Ewart (1979), while studying the effect on milk production associated with consumption of decorticated and extracted groundnut meal contaminated with aflatoxin B_1 (0.02 mg/kg), observed that feed intake in a herd of 40 Friesian cows reduced due to the consumption of contaminated ration. Approximation of this value as of total aflatoxin B_1 ingested per day by an animal was 360 µg. In a study to determine the effect of mycotoxins upon rumen functions of lactating dairy cows, Mertens (1979) reported that when cows were given 110 or 330 µg of aflatoxin B_1 /kg of body weight daily for 5 days, a significant drop in feed intake was observed as compared to the cows under control group. Similarly Applebaum *et al.* (1982) studied responses of dairy cows to dietary aflatoxin and observed that fluctuations in feed intake occurred during treatment period in those cows receiving 13 mg of impure aflatoxin B_1/day. Similarly, Polan *et al.* (1974) fed approximately 20 µg aflatoxin B_1/kg of body weight through feed concentrate and observed that there was no change in the appetite of the cows. Thus it is concluded that aflatoxin B_1 ingested through feed significantly ($P < 0.05$) reduced the feed consumption. This suggests that possibly feeding animals with increasing levels of aflatoxin B_1 gradually reduced the total feed consumption.

ACKNOWLEDGEMENTS

Authors are grateful to NDBB for providing feeds and fodder to conduct animal experiment, and other staff members of the animal nutrition department .

REFERENCES

Applebaum R S, Brackett R E, Wiseman D W and Marth E H. 1982. Responses of dairy cows to dietary aflatoxin : Feed intake and yield, toxin content and quality of milk of cows treated with pure and impure aflatoxin. *Journal of Dairy Science* **65** : 1503 -08.

Jones M G S and Ewart J M. 1979. Effects on milk production associated with consumption of decorticated extracted groundnut meal contaminated with aflatoxin. *Veterinary Record* **105** : 492 -39.

Mertens D R.1979. Biological effects of mycotoxins upon rumen function and lactating dairy cows. *Interaction of Mycotoxinsin Animal Production* .Proceeding. Symposium on American Society of Animal Sciences. American Dairy Science Association ,Committee on Animal Production, National. Research Council. National Academy Sciences, Washington D C.118.

Polan C E, Hayes J R and Campbell T C. 1974. Consumption and fate of aflatoxin B_1 by lactating cows. *Journal of Agricultural Food Chemistry* **22** : 635-38.

Sen K C, Ray S C and Ranjan S K.1978. *Nutritive Value of Indian Cattle Feed and Feeding of Animals*. ICAR , New Delhi.

Steel R G D and Torrie J H.1980.*Principal and Procedure of Statistics : A Biometrical Approach*. 2nd edn , pp. 393.McGrew Hall, Kangakusha Ltd., New Delhi.

Wogan G N, Edwards G S and Newberne P M.1971. Structure-activity relationship in toxicity and carcinogenecity of aflatoxins and analogs.*Cancer Research* **31**:1936-42.

Indian Journal of Animal Sciences **68** (4) : 402-404, April 1998

Phenotypic alterations in goats fed subabul (*Leucaena leucocephala*)*

G V P P S RAVI KUMAR[1] and ASHWANI SHARMA[2]

National Dairy Research Institute, Karnal, Haryana 132 001

Received : 18 August 1997; Accepted :28 October 1997

Key words : Phenotypic alterations, Subabul feeding

In India, due to crossbreeding programmes and better animal management practices, there is an increasing demand for proteins in animal feed. The annual requirements as projected are 25.4 million tonnes of concentrates, 353.0 million tonnes of dry fodder, and 308.1 million tonnes of green fodder. However, only 16.5 million tonnes of concentrate, 300.5 million tonnes of dry fodder and 261.0 million tonnes of green fodder are available (FAO 1994). Further, National Commission of Agriculture (1976) declared that there will be a considerable deficit of green fodder and other good proteinaceous sources by 2000 AD. Therefore, it is logical to think that there is an urgent need for building up animal feed resources. *Leucaena leucocephala* is a multipurpose tree species which may contribute towards additional feed and fodder availability in India. It is vigourous, rapidly growing, drought tolerant, palatable, high yielding and its leaves contain 20-30 % of CP. It can increase the rate of productivity per unit of land in terms of variety of products including milk, meat, fuel, food and timber. Since it is a perennial crop, it can be chopped 8 times a year and this can be used as feed supplement during lean periods of the year when there is scarcity of green forage.

Alopecia, loss of appetite, excessive salivation, incoordination of gait and enlarged thyroid were observed in buffalo calves fed on leucaena (Letts 1963). Decreased dry-matter intake, reduced weight gain, alopecia and in coordination of gait were also observed in buffalo calves fed on a diet containing 40 % leucaena (Gupta *et al.* 1992). Lohan *et al.* (1988) reported adverse effects on sperm motility and growth in breeding bulls.

Keeping the above facts in view, the present investigation was undertaken.

Growing male kids (15; Alpine × Beetal), 3-month-old, weighing 7.5 to 21 kg, maintained at the institute, were randomly divided into groups A, B and C, so that the average body weight of all the groups was approximately same. The *Leucaena leucocephala* seed used for this study was procured from BAIF Development Research Foundation, Urlikanchan.

Three concentrate mixtures were prepared for group A (control) and experimental groups B and C. All the 3 concentrate mixtures were isonitrogenous (Table 1). Prior to the commencement of feeding trial, the mimosine content of the 3 concentrate mixtures was estimated by colorimetric method.

Animals of all the 3 groups were fed with the respective concentrate mixtures and berseem fodder as per NRC requirements for 2 months. Body weights of the animals were taken at the commencement of the experiment and then

Table 1. Formulation of the concentrate mixtures

Ingredients of concentrate	Group A (control parts)	Group B* (parts)	Group C** (parts)
Maize	50	27.5	22
GNC	38	19	0
Leucaena seed	0	25.5	55
Wheat bran	9	25	20
Mineral mixture	2	2	2
Salt	1	1	1
Total	100	100	100
CP on DM basis	21.735 %	21.7075 %	21.73 %

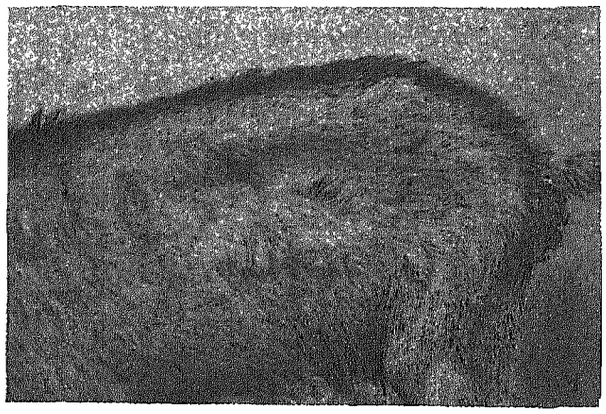
* 50 % of the protein supplied by GNC in the concentrate mixture of group A (control) was replaced by *Leucaena leucocephala* seed in group B;** 100 % of the protein supplied by GNC in the concentrate mixture of group A (control) was replaced by *Leucaena leucocephala* seed in group C.

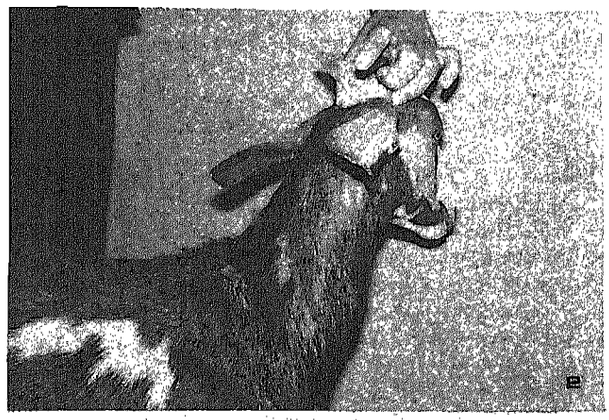
Table 2.Crude protein of various feed ingredients used in formulation of concentrate mixture (on DM basis)

Feed ingredients	Crude protein
Maize	9.70 %
GNC	41.25 %
Leucaena seed	30.72 %
Wheat bran	13.50 %

*Part of M.Sc. (Animal Genetics and Breeding) Thesis of the First Author.

Present address: [1] Ph.D. Scholar, Genetics Division; [2]Scientist (SS), Dairy Cattle Breeding Division.





Figs 1-2. **1.** Rough coat in goat of group C. **2.** Alopecia at the neck region in goat of group C.

at 15, 30, 45 and 60th day of feeding. Weighing was done in the morning before offering feed and water. Animals were also observed for alopecia, frothing and ulceration in mouth, convulsions, rough coat etc.

The data were analyzed to study the effect of time, i.e., duration of feeding and level of mimosine on the body weight of animals of the 3 groups (Sarders 1990).

The CP estimated in maize, GNC, wheat bran was 9.70, 41.25, 13.50 % on dry-matter basis respectively. CP in leucaena the seed was 30.72 % on dry matter basis (Table 2).

Mimosine content in the concentrate mixtures of groups B and C was 0.908 and 1.6 % respectively (Table 3). The mimosine was between 2.9 to 3.6 % in the seed. This finding

Table 3.Absorbance and mimosine content (%) in the standard solution and concentrate mixtures

Sample	Absorbance (535 nm)	Mimosine content (%)
Standard mimosine	0.2235	100
Concentrate mixture B	0.07805	0.908
Concentrate mixture C	0.14235	1.600

Table 4. Body weights of the animals of different groups at fortnightly intervals

Animal No.	0th day	15th day	30th day	45th day	60th day
Group A					
AB 415	7.5	Died	-	-	-
AB 417	14.5	14.8	15.0	15.5	16.0
AB 411	16.0	16.3	17.5	17.5	18.0
AB 413	17.5	17.8	18.0	18.5	19.5
AB 404	21.5	22.0	22.5	22.5	22.0
Mean ± SE	15.4 ± 2.04	17.7 ± 1.34	18.2 ± 1.35	18.5 ± 1.27	18.9 ± 1.09
Group B					
AB 408	13.5	13.0	11.0	14.0	13.5
AB 409	14.0	13.6	14.5	14.3	13.5
AB 400	15.0	14.0	14.5	14.7	15.5
AB 418	16.5	16.5	16.0	16 5	16 0
AB 405	19.5	19.0	19 0	19 2	18 5
Mean ± SE	15.7 ± 0.96	15.2 ± 1.00	15.0 ± 1.15	15 7 ± 0 86	15.4 ± 0.82
Group C					
AB 414	12.0	12.5	11.0	11.0	11.5
AB 412	13.5	13.0	15.0	14.5	14 5
AB 410	15.0	15.5	16.0	15.5	12 0
AB 396	17.5	16.0	17.0	16.0	16.5
AB 401	21.0	20.0	19.0	18.5	17.5
Mean ± S.E.	15.8 ± 1.41	15.4 ± 1.19	15.6 ± 1.18	15 1 ± 1.08	14.4 ± 1 06

was similar to the reports of Gupta and Raheja (1986), Chakraborty and Chhabra (1988), Gampawar *et al.* (1988).

There was an increase in body weight @ 0.02475 kg/day, in group A and decrease in body weight in groups B and C @ 0.00499 kg/day and 0.0233 kg/day respectively (Table 4). The change was not significant in all the groups when compared between days within groups. When compared between groups change was significant between A and C at 10 % level of significance (Table 5).

The decline in body weight in groups B and C though not significant is similar to the reports of Gupta *et al.* (1983) in sheep; Thirmalai (1991) in cattle heifer; Girdhar *et al.* (1991) in Black Bengal goats; Ram *et al.* (1994) in calves.

In the present study, clinical symptoms like rough coat (Fig. 1) and alopecia (Fig. 2) were observed on 30th day of feeding in group C as reported earlier by Joshi and Upadhyaya (1976) in sheep; Pauchari and Pathak (1989) in calves. Frothing and ulceration in the mouth and convulsions were not observed as reported by Prasad (1989) and Pachauri and Pathak (1989).

However, the alopecia disappeared within 7-8 weeks of feeding which indicated that the animals were adapted to mimosine and are capable of degrading mimosine and 3,4-dihydroxy pyridone. This is in conformity with the report

Table 5. Mann-Whitney's test to find out the difference in body weight between groups within days

Days	Group combinations	10% level of significance	5% level of significance
0th	AB	NS	NS
	BC	NS	NS
	AC	NS	NS
15th	AB	NS	NS
	BC	NS	NS
	AC	NS	NS
30th	AB	NS	NS
	BC	NS	NS
	AC	NS	NS
45th	AB	NS	NS
	BC	NS	NS
	AC	NS	NS
60th	AB	NS	NS
	BC	NS	NS
	AC	S	NS

NS, Nonsignificant; S, significant.

of Gupta (1995), where he concluded that cattle would acquire substantial DHP degrading ability by 21st day of gradual adaptation of *Leucaena leucocephala*.

Animals of group A (control) suffered with coccidiosis in the early days of the trial which may be one of the causes for lower rate of increase in body weight of animals. Animal No. AB 415 died due to coccidiosis and pneumonia on 14th day of trial (Table 4). Coccidiosis was treated .

Further, no toxicity was reported in goats (Kraneveld and Djaemoedin 1947), sheep (Owen 1958), Hawaiian goats (Jones and Megarrity 1983), buffaloes (Kapoor *et al.* 1983) and Black Bengal goats (Chakraborty and Ghosh 1988) on mimosine feeding. Hence, *Leucaena leucocephala* could be fed to goats to increase the rate of productivity per unit of land in terms of products including milk and meat.

REFERENCES

Chakraborty R P. and Chhabra A. 1988. Chemical composition of *Leucaena leucocephala* seeds and effects of its feeding on growth and feed conversion efficiency in goats. *Indian Journal of Animal Nutrition* **5**: 244-47.

Chakraborty T and Ghosh T K. 1988. Chemical composition and nutritive value of subabul (*Leucaena leucocephala*) foliage in Black Bengal goats. *Indian Journal of Animal Nutrition* **5**: 237-39.

FAO. 1994. *Yearbook.* Vol. 48.

Gampawar A S, Gorantiwar V M and Bhaiswar S S. 1988. Effect of feeding subabul (*Leucaena leucocephala*) seed as a part of protein supplement on growth in Sahiwal x Jersey calves. *Indian Journal of Animal Nutrition* **5**: 240-43.

Girdhar N, Lall D and Pathak N N. 1991. Effect of feeding *Leucaena leucocephala* as the sole ration on nutrient utilization and body weight in goats. *Journal of Agricultural of Science (Cambridge)* **116**: 303-07.

Gupta B K and Raheja R K. 1986. Lipid, mineral and other nutritional components of Subabul (*Leucaena leucocephala*) seed. *Indian Journal of Animal Nutrition* **3**: 233-37.

Gupta B K, Ahuja A K and Malik N S. 1992. Leucaena leaves as a high protein substitute for de-oiled seed-cake in concentrates for dairy cattle in India. *Leucaena-Research-Reports* **13**: 24-25.

Gupta H K. 1995. 'Mimosine degradation, its residual effect on milk and meat in animals on *Leucaena leucocephala* diet'. Ph.D. Thesis ,NDRI Deemed University, Karnal .

Gupta P C, Khirwar S S and Singh K. 1983. *Koo*-babul: a promising fodder tree. *Indian Farming* **32**: 21-22.

Jones R J and Megarrity R G. 1983. Comparative toxicity responses of goats fed on *Leucaena leucocephala* in Australia and Hawaii. *Australian Journal of Agricultural Research* **34**: 781-90.

Joshi D C and Upadhyaya R B. 1976. *Leucaena leucocephala*, an evergreen protein-rich tree fodder and the possibility of using it in the diet of animals. 1. Sheep. *Indian Veterinary Journal* **53**: 606-8.

Kapoor P D, Puri J P and Dwarakanath. 1983. Effect of supplementation of *Leucaena leucocephala* on the rumen metabolism in buffaloes. *Indian Journal of Animal Sciences* **53**: 461-64.

Kraneveld F C and Djaemoedin R. 1947. Haaruitval bij dieren na Voedering met lamtoro (*Leucaena glauca*). (*fide* Jones R J. 1979. *World Animal Review* **31**: 13-23.)

Letts G A. 1963. *Leucaena glauca* and ruminants. *Australian Veterinary Journal* **39**: 287-88.

Lohan I S, Singh S P and Gupta P C. 1988. Effect of Subabul (*Leucaena leucocephala*) feeding on reproductive performance and hormonal profile in Murrah males. *Indian Journal of Dairy Science* **31** : 404-05.

National Commission on Agriculture. 1976. Report on Animal Husbandry, Part 7, Ministry of Agriculture and Irrigation, Govt. of India, New Delhi.

Owen L N. 1958. Hair loss and other toxic effects of *Leucaena glauca* (jumbey). *Veterinary Record* **70**: 454-57.

Pauchari V C and Pathak P S. 1989. Effect of feeding *Leucaena leucocephala* in combination with hybrid napier on growth and nutrient utilization in crossbred calves. *Indian Journal of Animal Nutrition* **6**: 151-61.

Prasad J. 1989. Toxic syndromes in calves given a leucaena mixed forage diet. *Indian Journal of Veterinary Medicine* **9**: 149-50.

Ram J J, Atreja P P, Chhabra A and Chopra R C. 1994. Mimosine degradation in calves fed sole diet of *Leucaena leucocephala* in India. *Tropical Health Production* **26**: 199-206.

Sarders D H. 1990. *Statistics : A Fresh Approach.* McGraw-Hills Book Co., Singapore.

Thirmalai S. 1991. Effect of feeding subabul leaf-meal (*Leucaena leucocephala*) on growth of crossbred dairy heifers. *Indian Veterinary Journal* **68**: 391-93.

Indian Journal of Animal Sciences 68 (4) : 405-406, April 1998

Nutritional evaluation of gliricidia (*Gliricidia maculata*) leaves in goats

C SRINIVASULU[1], M RAJ REDDY[2] and D NARASIMHA REDDY[3]

Acharya NG Ranga Agricultural University, Hyderabad, Andhra Pradesh 500 030

Received : 29 August 1997; Accepted : 28 October 1997

Key words : *Gliricidia maculata* ,Goats, Nutritive value

Gliricidia (*Gliricidia maculata*), a medium sized, quick growing and deep rooted tree legume, mostly grown as livefence support plant for vines of black pepper and shady tree for the population in tropics and subtropics. The leaves can be harvested at every 3 to 4 months interval which is a palatable fodder for ruminants and could be used as a protein supplement (Ranjhan 1980). Smith and Van Houtert (1987) reviewed the feeding value of *Gliricidia sepium* as a live-stock feed and concluded that it has a high nutritive value for ruminants. The potential of gliricidia leaf-meal as a fodder has not been fully exploited in India except in some hilly areas hence the present study was carried out to determine the nutritive value of gliricidia leaves for goats.

Shade-dried gliricidia leaves were fed as sole diet to 6 adult local goats in an attempt to assess its nutritive value. Since the DMI with gliricidia leaves as sole diet was quite low, they were fed along with chopped sorghum straw in 1:1 ratio on dry-matter basis and the nutritive value of gliricidia leaves was calculated by difference method. Feed and faecal samples were analyzed for proximate composition (Table 1) and phosphorus using AOAC (1985) procedure. Calcium was estimated according to the method of Talapatra *et al.* (1940).

Nutritive value of sorghum straw was taken from another experiment conducted in the same laboratory (Sriramamurthy 1992) on same bucks. The apparent digestibility coefficient values of sorghum straw in goats fed as sole diet for DM, OM, CP, CF, EE and NFE were 56.45 ±2.86, 57.22 ± 2.88, 25.39 ± 3.03, 69.89 ± 9.99, 48.75 ± 5.95 and 48.03± 4.45 % respectively. Digestibility coefficients and nutritive value of combined ration was estimated first and by difference method nutritive value of gliricidia leaves for goats was calculated.

The proximate composition of gliricidia leaves recorded in the study was comparable with the results reported by Murugan *et al.* (1985), Dharia *et al.* (1987), Majgoankar *et al.* (1990), Rao *et al.* (1993) and Perera *et al.* (1994).

The average digestibility coefficients and balance of various nutrients in goats fed with combined ration (*Gliricidia* leaves + sorghum straw in 1:1 ratio) was presented in Table 2. DMI of experimental animals and digestibility coefficient of DM, OM, CP, CF, EE and NFE of combined ration were comparable to the reported values of Kantharaju and Chadhokar (1981a), Murugan *et al.* (1985), Ash (1990), Ifut *et al.* (1992), Bosman *et al.* (1995) and Richards *et al.* (1994a). All the experimental animal, were on positive nitrogen, calcium and phosphorus balance. The DCP and TDN content of the combined ration was 8.95 and 51.33 % for goats. The intakes and content of DCP and TDN on this ration were higher than the recommended values of ICAR (1985).

Digestibility and nutritive value of gliricidia leaves calculated by difference method (Table 2) were 53.51 ± 2.01, 59.87 ± 1.80, 79.32 ± 3.78, 58.81 ± 2.79, 31.57 ± 2.69 53.58 ± 3.31, 16.99 ± 0.80 and 56.91 ± 2.15 for DM, OM, CP, CF, EE, NFE, DCP and TDN respectively. Gliricidia having 16.99 % DCP for goats was excellent source of protein supplement, but moderate in TDN content (56.91 %), hence it requires supplementation of energy source while feeding, especially when used along with low quality crop residues.

Table 1. Chemical composition of gliricidia leaves and sorghum straw, per cent on dry-matter basis

	DM	OM	CP	CF	EE	NFE	Ash	AIA	Ca	P
Gliricidia leaves	90.34	89.68	21.42	24.59	11.58	32.09	10.32	1.93	4.21	0.94
Sorghum straw	94.80	81.54	3.56	35.86	0.88	41.24	18.46	5.32	0.66	0.13

Present address : [1]Assistant Research Officer, Feed and Fodder Technology Division, Regional Agricultural Research Station, Tirupati 517 502.

[2]Professor and Head, [3]Associate Professor, Department of Feed and Fodder Technology, College of Veterinary Science.

Table 2. Average digestibility coefficients and balance of various nutrients in goats fed gliricidia leaves and Sorghum straw (combined ration) and gliricidia leaf-meal (by difference method)

Parameters	Gliricidia leaf-meal + sorghum straw	Gliricidia leaf-meal
DM intake / 100 kg body weight (kg)	2.92±0.11	
Water intake (L/kg DMI)	2.96±0.36	
Nutrient digestibility (%)		
DM	53.26±3.87	53.51±2.01
OM	58.60±0.94	59.87±1.80
CP	71.64±2.95	79.32±3.78
CF	65.38±1.12	58.81±2.79
EE	33.18±2.46	31.57±2.69
NFE	50.47±3.17	53.58±3.31
Nutritive value (%)		
DCP	8.95±0.40	16.99±0.80
TDN	51.33±1.39	56.91±2.15
Balances (g/day)		
N	8.52±0.80	
Ca	7.77±0.66	
P	1.76±0.21	

REFERENCES

AOAC. 1985. *Official Methods of Analysis.* 14th edn, pp. 129-38. Association of Official Analytical Chemists, Washington DC.

Ash A J. 1990. The effect of supplementation with leaves from the leguminous tree *Sesbania grandiflora*, *Albizia chinensis* and *Gliricidia sepium* on the intake and digestibility of guinea grass hay by goats. *Animal Feed Science and Technology* **28**: 225-32.

Bosman H H, Versteegden C J G M, Odeyinka S M and Tolkamp B J. 1995. Effect of amount offered on intake, digestibility and value of *Gliricidia sepium* and *Leucaena leucocephala* for West African Dwarf goats. *Small Ruminant Research* **15**(3): 247-56.

Dharia S K, Majgoankar S V and Toro V A. 1987. Effect of feeding *Gliricidia maculata* leaves on growth of crossbred heifers. *Indian Journal of Animal Nutrition* **4**(4): 260-63.

ICAR. 1985. *Nutrient Requirements of Livestock and Poultry.* Publication and Information Division. Indian Council of Agricultural Research, New Delhi.

Ifut O J, Hahu S K (Ed.), Reynolds (Ed.) and Egbunike. 1992. The potential of cassava peel for feeding goats in Nigeria. Cassava as livestock feed in Africa: Proceedings of the IITA-ILCA University of Ibaden workshop on potential utilization of cassava as Livestock feed in Africa. pp. 72-81.14-18 November 1988, Ibaden - Nigeria

Majgoankar S V, Bhavsar G C and Toro U A. 1990. Utilization of *Gliricidia maculata* leaves in the diet of lactating cows. *Indian Journal of Animal Nutrition* 7(1): 67-70.

Murugan M, Katha Perumal V and Jothiraj S. 1985. Preliminary Studies on the proximate composition and nutritive value of *Gliricidia maculata* leaves for goats. *Cherion* **14**(1): 217-20.

Perera A N F, Perera E R K, Djajanegara and Sukmawati A. 1994. Potential nutrient livestock production with natural pastures under Pinus. Sustainable animal production and the environment. Proceedings of the 7th AAAP Animal Science Congress, Bali, Indonesia, 11-16 July, Volume 2: 491-92.

Ranjhan S K. 1980. *Animal Nutrition in Tropics.* Vikas Publishing House, New Delhi.

Rao B V, Parthasarathy M and Krishna N. 1993. Effect of supplementation with tree leaves on intake and digestibility of hybrid napier (NB-21) grass in Nellore Brown sheep. *Animal Feed Science and Technology* **44**: 265-74.

Richards D E, Brown W F, Ruegsegger G and Bates D B. 1994a. Replacement value of *Gliricidia sepium* for growing goats. *Animal Feed Science and Technology* **46**: 1-2, 37-51.

Smith G W and Van Houtert M F J. 1987. The feeding value of *Gliricidia sepium*. *World Animal Review* **62**: 57-68.

Sriramamurthy K. 1992. 'Processing of low cost concentrate supplements utilizing poultry litter/ poultry droppings and their nutritive evaluation among goats and sheep.' Ph.D Thesis, Andhra Pradesh Agricultural University, Hyderabad.

Talapatra S K, Day S C and Sen K C. 1940. The analysis of mineral constituents in biological material. 1. Estimation of phosphorous, chlorine, calcium, magnesium, sodium and potassium in food stuffs. *Indian Journal of Veterinary Science and Animal Husbandry* **10**: 243-58.

Indian Journal of Animal Sciences 68 (4) : 407-409, April 1998

Status of *Pampus argenteus* fisheries in Khoozestan waters (North-West Persian Gulf), I.R.Iran

A PARSAMANESH[1] , M SHALBAF[2] and N NAJAFPOUR[3]

Khoozestan Fisheries Research Center , P.O.Box 61335416, Ahwaz , Iran

Received : 10 January 1997; Accepted : 27 October 1997

ABSTRACT

Length frequency samples collected from October 1992 to March 1996, on major landing centres of Khoozestan province have been analyzed, using ELEFAN and other softwares. *Pampus argenteus* is a high-priced fish in the local and neighbouring markets. Biological parameters have been estimated for available data. Relative yield per recruit analysis and Thompson and Bell length-based predictive model have been applied to the data. Growth parameter (K) for the available data is estimated to be 1 and asymptomatic (fork) length 35.6 cm. Natural and total mortality rates were estimated to be 1.5 and 3.3, respectively, for the period of study. The results showed that at present, this species is fished at or near its MSY level and there should not be any further increase in the effort to catch *P. argenteus*.

Key words: Fisheries, *Pampus argenteus*, Persian Gulf, Silver pomfret

The fishery is mainly a gillnet fishery with the most valuable fish in demand in Khoozestan Province *Pampus argenteus* . Because of the high demand for this fish and its high market price in Khoozestan and neighbouring countries (Kuwait and U A E), there has been an increase in the level of effort to catch *P.argenteus*. Mean annual landing of *P.argenteus* has been estimated to be about 780 metric tonnes in recent years . The length-based method seems to be the best applicable way to assess *P. argenteus* stocks , although some authors have noted the possibility of a right-hand-selection in *P. argenteus* samples from commercial catch (Mathews C P). Since 1994 a 45-day ban period has been implemented for *P. argenteus* in northern coasts of the Persian Gulf including Khoozestan waters. The ban period starts from early May and ends on 21 June.

MATERIALS AND METHODS

Length frequency data from October 1992 to March 1996 were collected on a monthly basis from the landed gillnet catch at major commercial landing centres in Khoozestan Province . The lengths were measured from the tip of the snout to the point where the caudal fin divides (fork length), to the nearest cm below. Length data were grouped in 1 cm classes and were arranged in ELEFAN and LFDA package. Since the L/F data used in this study have mostly come from catches done by gillnets, they were corrected for selectivity of gillnets. The data were also smoothed for the probable noises in the length frequencies (5 point running average method).

Using available computer softwares, von Bertalanffy model [$Lt=L_\infty (1-e-k(t-t_0))$] was fitted to the available data. The species studied, considered to have no seasonal oscillations in its growth pattern. For finding the best set of parameters, ELEFAN , SLCA and Projection Matrix methods were used

Table 1. Estimated population parameters for *P.argenteus*

	Ll	K	t_0	Z Catch curve	Z Pooled catch curve	M (Pauly)	F	E	$F_{0.1}$	θ
Oct.92-Mar.96 (raw data)	36	0.9	-	3.2	2.8	1.45	1.75	0.55	1.1	3.06
Oct.92-Mar.96 (smoothed and corrected data)	35.6	1	-0.33	3.3	3.3	1.5	1.8	0.55	1.1	3.10

Length-weight parameters used (Parsamanesh *et al.* 1995) in calculations: a = 0.020056 , b = 3.162 (W=a L b).

Present address: [1,2,3] Research Scientists, Khoozestan Fisheries Research Centre.

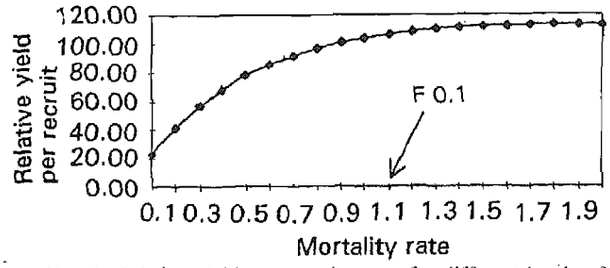


Fig. 1. Relative yield per recruit curve for different levels of mortality rates for corrected and smoothed data from October 1992 to March 1996.

(Gulland and Rosenberg 1992). Separate runs of the programme were used to estimate the best set of parameters for different sets of data. Each set of calculations were run using raw and corrected-smoothed data, and in each case, according to the scores (such as ESP/ASP in ELEFAN) obtained, best set of parameters was chosen.

Mortality rates were estimated by using ELEFAN II subroutine. Total mortality (Z) was estimated by using descending right hand limb of length-converted catch curve. The mortality estimation calculations were also made by the use of pooled L/F samples. Natural mortality (M) was estimated by Pauly formula (Pauly 1980) (mean annual temperature was calculated to be 23 °C for Khoozestan waters). Recruitment pattern was also determined by this subroutine. Fishing mortality (F) was estimated from the equation: F=Z - M and exploitation ratio from: E= F / Z (Ingles and Pauly 1984).

The φ value (Sparre and Venema 1992) was also calculated for each set of parameters (φ / = log K + 2log L ∞).

Length-based relative yield per recruit analysis was carried out and F 0.1 was calculated for each set of data. Where a yield per recruit curve continues to increase , or approaches a broad, flat-topped maximum, the optimum fishing mortality is sometimes taken to be the value at which an increase in one unit of F increases the catch by one-tenth (0.1) of the amount caught by the first unit of F. In other words, the optimum level of F occurs at the point where the slope of the yield curve is 0.1 of the value of the slope at low levels of fishing mortality. This value is $F_{0.1}$. The total allowable fishing efforts are based on $F_{0.1}$ as this is believed to be a more robust and conservative replacement for Fmax (King 1995).

Length-based Thompson and Bell model was used for determining MSY and FMSY values.

Table 2 . Results of applying Thompson and Bell Model for *P. argenteus* data
(L50%=12.5 cm , L75%=13.77 cm , Fmax.=1.8)

X	Yield	Mean biomass
0.0	0.00	4739.38
0.2	1113.70	3240.27
0.4	1601.85	2369.73
0.6	1812.84	1821.86
0.8	1892.06	1455.65
1.0	1906.01	1199.09
1.2	1887.19	1012.43
1.4	1852.24	872.34
1.6	1809.97	764.42
1.8	1765.10	679.42
2.0	1720.20	611.19

RESULTS AND DISCUSSION

Best set of parameters estimated for each set of L/F data are listed in Table 1. Table 2 summarizes the results of applying Thompson and Bell model to all available L/F data. Separate estimates of growth and mortality rates for each year are also presented in Table 3 .

Range of values for K is 0.9 to 1 / year for different data sets (Table 1), which shows that *P.argenteus* is a relatively fast-growing species. These values are higher, compared to the growth rates reported by Herianti *et al.* (1986) , Lee *et al.* (1992) . Khoozestan shallow coasts seem to be a nursery ground for *P.argenteus* and this might be the reason of the high K value obtained in this study. The exploitation ratio is about 0.5 which is a reasonable value and the value of $F_{0.1}$ is about 1.1 . Fig. 1 shows the relative Y/R curve for different levels of fishing mortality for the present length at first capture (Lc= 12.5 cm F.L.) for the gillnets used in the area to catch *P. argenteus*.

Table 3 . Annual estimates of growth and mortality rates for the available L/F data of *P. argenteus*

	K	L¥	t_0	F	M	Z	E
1993	1.06	38	-0.52	3.5	1.5	5.36	0.7
1994	1.12	42	—	2.6	1.6	4.2	0.62
1995	0.85	35	-0.8	1.2	1.4	2.6	0.46

The results of applying Thompson and Bell model to the available data also shows that the FMSY is on the present effort level and the exploitation ratio also indicates the same point. Relative yield per recruit analysis shows that F 0.1 is on the point where the fishing mortality rate is 1.1 .

Although automatic analysis of the recruitment pattern (ELEFAN II) showed 2 peaks (in October with 27.0% and in March with 5.6% of the recruitment), L/F data show the existence of small fishes (less than 10 cm F.L.) nearly year-round. The recruitment peak in October is almost synchronous with the period of high catches reported by the fishermen. Morgan (1982) in the same way suggested that *P.argenteus* spawns throughout the year, which is in accordance with our L/F data.

Results revealed here show that *P. argenteus* is presently fished at or near its MSY and there should not be any increase in effort to catch it. Implementation of the 45-day ban period from 1994 has decreased fishing mortality to the level of 1.2 which is near the F 0.1 value obtained from this analysis. Thompson and Bell model also implies the same, therefore

it could be concluded that *P. argenteus* catch is at or near its MSY level. Results of automatic analysis of the recruitment pattern (two annual peaks of recruitment) are apparently not in line with the presence of small individuals year-round, which needs more studies to be carried out in this field.

ACKNOWLEDGMENTS

The results obtained in this article were a part of a three-year project granted by the Iranian Fisheries Research and Training Institute.

Special thanks should be given to Dr C Amirinia and Dr Taghavy for reviewing this article and providing important guidelines .

REFERENCES

Gulland J A and Rosenberg A A .1992 . A review of length-based approaches to assessing fish stocks. *FAO Fisheries Technical Paper*. No.323. Rome, FAO

Herianti I , Wahyuono H and Subani W .1986. The estimation of growth parameters of Silver Pomfret (*Pampus argenteus*) in north coast of Gresik, East Java. *Journal of Marine Fisheries Research* 37: 23-30

Ingles J and Pauly D. 1984 . *An Atlas of the Growth, Mortality and Recruitment of Philippine Fishes*. ICLARM technical reports pp., 13, 127 Institute of Fisheries Development and Research. College of Fisheries, University of the Philippines in the Visayas

King M. 1995. *Fisheries Biology, Assessment and Management* Fishing news books

Lee D W, Kim Y M and Hong B Q. 1992 . *Age and Growth of Silver Pomfret (Pampus argenteus) in Korean Waters*. Bulletin of Natural Fisheries Resources Division Agency. Korea. No.46, pp.31-34

Morgan G R. 1982 . Preliminary stock assessment of Zobaidy. *Pampus argenteus. Proceedings, Third Shrimp and Fin Fisheries Management Workshop: Fin Fisheries Session*. Vol. 2. (Ed.) Mathews C P. Kuwait Institute for Scientific Research.

Parsamanesh A , Najafpour N , Khodadady M , Davoodi F and Sabzalizadeh S. 1994 . *Preliminary Hydrobiologic Study of Khoozestan Creeks*. Final report, Iranian Fisheries Research and Training Institute.

Parsamanesh A , Shalbaf M and Kashi M .1995 . *Stock Assessment of Khoozestan Province Fishes*. Final Report, Iranian Fisheries Research and Training Institute.

Pauly D. 1980 . On the interrelationships between natural mortality, growth parameters, and mean environmental temperature in 175 fish stocks. *Journal du Conseil* 39(2):175-92

Sparre P and Venema S C. 1992 . Introduction to tropical fish stock assessment. 1. Manual. *FAO Fisheries Technical Paper* No. 306.1, Rev. 1. Rome, FAO.

Indian Journal of Animal Sciences 68 (4) : 410-416, April 1998

Preliminary feeding trials on juveniles of golden mahseer, *Tor putitora* (Ham.) at different stocking densities with artificial dry pellet feeds

SHYAM SUNDER[1], H S RAINA[2] and UMA NAULIA[3]

National Research Centre on Coldwater Fisheries, Bhimtal, Uttar Pradesh 263 136

Received: 10 November 1996; Accepted : 12 August 1997

ABSTRACT

For scientifically managed culture techniques and maximizing the production of healthy stocking material of depleting Himalayan mahseer, 6 artificial feeds were formulated and compounded. The feeding experiments were conducted on hatchery reared hatchlings, advance fry and fingerlings and also on the juveniles of this species collected from wild waters. Artificial feeds of 21.4-50.2% protein levels revealed a better growth and high survival having 45.4% crude protein. A positive correlation was observed between the dietary protein content and the overall performance of the conversion ratio. Impact of different densities for the better culture performance in various juvenile stages have also been worked out. The present communication reviews the practice of conventional diets and scope of nutritional manipulation of present tested practical diets to evolve cost-effective strategies for taking mahseer for aquaculture.

Key words : Agriculture dry pellet, Golden mahseer, Stocking densities, *Tor putitora*

There is hardly any information available on the artificial feeding of golden mahseer (*Tor putitora*) barring preliminary works by Joshi *et al.*(1989), Raina *et al.* (1993) and Sunder *et al.* (1993) Since *Tor putitora* is a slow growing fish in Himalayan uplands, the nutritive diets can play a vital role at nursery rearing stages of the species to promote the growth in captivity for production of suitable sized stocking material and for further aquaculture.

Experimental feeding trials with 6 different laboratory prepared feed formulations *vis-a-vis* wet and conventional diets on young stages of golden mahseer have been conducted to study the overall fish survival, feed efficiency, conversion rate, growth performance etc.

MATERIALS AND METHODS

Test diets (Nos 1-6) were formulated using various ingredients in different ratios and combinations as follows: Soy-meal (35-60%), rice/wheat starch (0-15%), silk worm pupae (0-20%), casein (0-50%), gelatin (0-4%), yeast (0-5%), cod liver oil (5-10%), mineral and vitamin pre-mix (5-6%).

The raw feed ingredients were crushed, sieved and mixed in required proportions adding the fish oil, vitamins and minerals. The whole mixture was doughed by using some water. The conditioned dough was utilized for making crumbles by a hand operating noodle making machine which were dried till moisture content reduced to less than 10%. The dried crumbles were then broken, crushed and sieved to get mash of required size for mahseer young fry, advance fry and fingerling feeding. The chemical composition (Table 1) of 6 feed formulations was estimated as per AOAC (1984).

Table 1. Proximate composition (%) of 6 laboratory feed formulations (1-6)

Particulars	Feeds					
	1	2	3	4	5	6
Crude protein	21.45	27.62	31.94	36.66	45.38	50.21
Ether extract	24.14	16.44	12.47	17.13	14.12	13.69
Nitrogen free extract	39.90	37.11	38.59	24.78	22.35	20.36
Crude fibre	5.15	5.28	5.19	4.33	3.93	2.40
Ash	2.60	2.91	3.25	7.44	5.98	3.04
Moisture	7.26	10.64	8.56	9.66	8.24	10.30

Feeding trials were also conducted with 2 wet (minced sheep/goat's liver and hen's egg yolk emulsion) and 1 conventional feed (mustard oil-cake + rice bran mixed in equal proportions).

Trials for feeding experiments were conducted in GI sheet tanks (1 m^2 dimensions) having water flow through facilities. Mahseer fry produced at Bhimtal hatchery were used for this experiment.

Present address: [1]Senior Scientist (Fisheries),[2] Director.

[3]Ph.D.Scholar, Poultry Nutrition, Department of Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar 263 145.

Table 2. Results of feeding trials in golden mahseer swim-up fry stocked @ 300/m² with various feeds for a rearing period of 30 days

Particulars of experiment	Formulated feeds						Wet Feeds		Conventional Feed
	1	2	3	4	5	6	Egg-yolk	Liver	MOC + RB (1:1)
No.of fish stocked	300	300	300	300	300	300	300	300	300
Initial length (mm)	10-12	10-12	10-12	10-12	10-12	10-12	10-12	10-12	10-12
Av.initial weight (g)	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008
Total initial weight (g)	2.400	2.400	2.400	2.400	2.400	2.400	2.400	2.400	2.400
No.of fish harvested	125	135	160	169	197	202	170	220	100
Rate of survival (%)	41.5	45.0	53.6	56.3	66.2	67.4	56.6	73.3	30.0
Final length (m)	13-18	13-19	15-21	14-20	15-21	13-19	15-18	16-21	13-16
Av.final weight (g)	0.058	0.059	0.068	0.066	0.071	0.064	0.069	0.074	0.058
Total final weight (g)	7.750	7.565	10.880	11.154	13.987	12.028	11.730	16.280	8.900
Gain in weight (g)	4.850	5.565	8.480	8.754	11.597	10.530	9.330	13.880	3.500
Increasing rate of body weight (g)	7.2	7.3	8.5	8.2	8.2	8.9	8.6	9.2	7.3
Av.increase in body weight/day (g)	0.24	0.24	0.28	0.27	0.29	0.26	0.29	0.31	0.24
Total feed given (g)	16.490	17.250	22.890	23.635	27.800	29.485	21 460	29.150	13.300
Conversion ratio	3.4	3.1	2.7	2.7	2.4	2 8	2.3	2.1	3.8
Feed efficiency (%)	29.4	32.3	37.0	37.0	41.7	35.7	43 5	47.6	26.3

Table 3. Results of feeding trials in golden mahseer swim-up stocked @ 500/m² with various feeds for a rearing period of 30 days

Particulars of experiment	Formulated feeds						Wet Feeds		Conventional Feed
	1	2	3	4	5	6	Egg-yolk	Liver	MOC + RB (1:1)
No.of fish stocked	500	500	500	500	500	500	500	500	500
Initial length (mm)	10-12	10-12	10-12	10-12	10-12	10-12	10-12	10-12	10-12
Av.initial wt.(g)	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008
Total initial wt.(g)	4.000	4.000	4.000	4.000	4.000	4.000	4.000	4.000	4.000
No.of fish harvested	198	237	253	281	328	320	269	351	162
Rate of survival (%)	39.6	47.4	50.6	56.2	65.6	63.9	53.8	70.2	32.4
Final length (mm)	14-19	13-19	14-20	13-18	13-20	14-20	15-20	15-21	13-17
Av.final wt. (g)	0.056	0.059	0.067	0.064	0.070	0.061	0.071	0.076	0.055
Total final wt.(g)	11.090	13.980	16.950	17.980	22.960	19.520	19.100	26.680	8.010
Gain in wt. (g)	7.090	9.980	12.950	17.980	22.960	19.520	19.100	26.680	8.910
Increasing rate of body weight (g)	7.0	7.4	8.4	8.0	8.7	7.6	8.9	9.5	6.9
Av.increase in body wt./day (g)	0.23	0.25	0.26	0.26	0.29	0.25	0.29	0.32	0.23
Total feed given(g)	24.106	32.930	36.260	36.350	45.500	45.500	36.240	49.900	18.168
Conversion ratio	3.4	3.3	2.8	2.6	2.4	2.9	2.4	2 2	3.7
Feed efficiency (%)	33.6	30.3	35.7	38.5	41.7	34.5	41.7	45.5	27.0

The feeding schedule was calculated in accordance with water temperature and fish weight and generally amounted to 8-12%. The feed was often broadcast near the water inlet or placed in small dishes by making a dough and lowered carefully into the experimental tanks. The feeding was done every 2 hr from sun-rise till sunset in the early stages of mahseer rearing; however, at advance fry stage, the frequency between 2 feedings was decreased. Periodically the fish guts in each experiment were opened to assess whether the feed has actually been consumed by the test fish.

Six experiments with artificially bred mahseer fry and 1 with natural stock were done, each spreading over a span of 30 to 60 days. In each set of experiment, 3-4 replicates were kept and the data presented here is computed and averaged.

During the experimentation period, the physico-chemical parameters of the hatchery water were: water temperature 13.0-25.0°C; pH 6.9-9.3, dissolved oxygen 6.8-8.5 mg/litre,

Table 4. Results of feeding trials in golden mahseer fry stocked @ 300/m² with different feeds for a rearing period of 30 days

Particulars of experiment	Formulated feeds						Wet Feeds		Conventional Feed
	1	2	3	4	5	6	Egg-yolk	Liver	MOC + RB (1:1)
No.of fish stocked	300	300	300	300	300	300	300	300	300
Initial length (mm)	15-20	15-20	15-20	15-20	15-20	15-20	15-20	15-20	15-20
Av.initial wt.(g)	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060
Total initial wt.(g)	18.000	18.000	18.000	18.000	18.000	18.000	18.000	18.000	18.000
No.of fish harvested	140	142	172	18	221	210	178	238	121
Rate of survival (%)	46.8	47.3	57.2	60.4	72.8	70.1	59.4	79.2	40.3
Final length (mm)	24-30	25-31	24-33	26-32	25-35	25-33	25-33	28-38	23-34
Av.final wt.(g)	0.150	0.162	0.179	0.175	0.183	0.172	0.190	0.195	0.144
Total final wt.(g)	21.000	23.000	30.790	31.675	40.440	36.120	33.820	46.410	17.425
Gain in wt.(g)	3.000	5.000	12.790	13.675	22.440	18.120	15.820	29.410	9.425
Increasing rate of body weight (g)	2.5	2.7	3.0	2.9	3.1	2.9	3.2	3.3	2.4
Av.increase in body wt./day (g)	0.08	0.09	0.10	0.10	0.10	0.10	0.11	0.11	0.08
Total feed given (g)	8.400	13.500	31.975	34.190	51.610	43.220	33.220	53.980	32.990
Conversion ratio	2.8	2.7	2.5	2.5	2.3	2.4	2.1	1.9	3.5
Feed efficiency (%)	35.7	37.0	40.0	40.0	40.0	41.6	47.6	52.6	28.6

free carbondioxide 0.2-1.8 mg/litre, total alkalinity 62.0-78.0 mg/litre, chloride 14.0-23.0 mg/litre; specific conductivity 83.6-170.2 µmhos at 25.0°C and total dissolved solids 41.5-85.0 mg/litre.

RESULTS

Experiment 1

High survival range of 66.2-67.4% was recorded with diets 5 and 6 (Table 2). The young fry had better feed efficiency (41.7%), survival rate (66.2%) and conversion ratio (2.4) with feed 5. These values were poor in the diets having low protein contents and ranged as 29.4-37.0%, 41.5-56.3% and 2.7-3.4 respectively. Of the 2 wet and 1 conventional diets, minced liver feed exhibited best survival (73.3%), conversion ratio (2.1) and feed efficiency (47.6%) followed by egg yolk emulsion. However, the performance of conventional feed was quite poor.

Experiment 2

Within 30 days, fish fed on formulated diets revealed that the survival (65.5%), conversion rate (2.4) and feed efficiency (41.7%) was maximum in feed No.5 compared to others (Table 3). Among wet feeds, liver fed stock recorded these parameters to be highest with their respective values of 70.2%, 2.2 and 45.5% followed by egg yolk diet. However, the poorest performance was observed with conventional diet having survival rate as 32.4%, conversion factor as 3.7 and feed efficiency as 27.0%.

Experiment 3

Feed 5 gave better feed efficiency (43.5%), survival (73.8%) and a conversion factor (2.3), whereas these values were comparatively on the lower side in other compounded feeds (Table 4). The fry fed with minced liver showed best feed efficiency (52.6%), survival (79.2%) and feed quotient (1.9) followed by egg yolk emulsion diet. MOC and RB mix feed recorded the poorest performance with 28.6% feed efficiency, 40.3% survival rate and 3:5 conversion factor.

Experiment 4

Length increment did not exhibit significant variation with different feeds (Table 5). Rate of survival was maximum (83.6%) in the fry fed with minced liver followed by artificial feed 5 (76.6%) and egg yolk (68.4%). Again the feed conversion and efficiency among all the diets was best as reflected by liver diet (1.9 and 52.6%) followed by egg yolk emulsion (2.2 and 45.5%). These parameters amongst the artificial test feeds recorded better performance in feed 5 (2.3 and 43.5%) followed by feed 3 (2.3 and 41.7%). The highest feed quotient (3.4), lowest feed efficiency (47.6%) and survival rate (49.0%) were seen in conventional diet.

Experiment 5

The rate of survival, conversion factor and feed efficiency was best recorded in liver fed stocks @ 89.0%, 1.8 and 55.6%, respectively, against the MOC and RB mixture fed mahseer fishes having the values of 54.0%, 3.3 and 30.3% (Table 6). Feed 5 gave the best results in terms of overall survival (83.6%), conversion rate (2.2) and feed efficiency (45.5%). Moreover, this feed showed maximum final weight of fish (1.200 g) from the initial weight of 0.175 g whereas feed 1 resulted in lowest growth (0.860 g).

Experiment 6

Fish fed with minced goat liver recorded (Table 7) the

Table 5. Results of feeding trials in golden mahseer fry stocked @ 500/m² with different feeds for a rearing period of 30 days

Particulars of experiment	Formulated feeds						Wet Feeds		Conventional Feed
	1	2	3	4	5	6	Egg-yolk	Liver	MOC + RB (1:1)
No.of fish stocked	500	500	500	500	500	500	500	500	500
Initial length (mm)	15-20	15-20	15-20	15-20	15-20	15-20	15-20	15-20	15-20
Av.initial wt. (g)	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060
Total initial wt.(g)	30.000	30.000	30.000	30.000	30.000	30.000	30.000	30.000	30.000
No. of fish harvested	256	278	293	329	378	334	342	418	245
Rate of survival (%)	51.2	55.6	58.6	65.8	75.6	66.8	68.4	83.6	49.0
Final length (mm)	24-30	25-30	26-34	27-34	27-35	25-35	26-34	29-37	23-32
Av.final wt.(g)	0.158	0.160	0.180	0.174	0.180	0.174	0.182	0.190	0.145
Total final wt.(g)	40.450	40.480	52.740	57.246	68.000	58.117	62.240	79.420	35.525
Gain in wt.(g)	10.450	10.480	22.740	27.246	37.000	28.117	40.240	49.420	5.525
Increasing rate of body weight (g)	2.6	2.7	3.0	2.9	3.0	2.9	3.1	3.2	2.4
Av.increase in body wt./day (g)	0.09	0.09	0.10	0.10	0.10	0.10	0.10	0.11	0.8
Total feed given (g)	29.260	27.250	54.575	70.840	87.400	70.290	88.528	93.900	18.790
Conversion ratio	2.8	2.6	2.4	2.6	2.3	2.5	2.2	1.9	3.4
Feed efficiency(%)	35.7	38.5	41.7	38.5	43.5	40.0	45.5	52.6	29.4

best survival rate, conversion coefficient and feed efficiency (84.9%, 1.8 and 55.5%) against conventional diet showing poor results having respective values at 54.3%, 3.4 and 29.5%. As regards to artificial diets, feed 5 recorded maximum survival (83.6%), conversion factor (2.2) and feed efficiency (45.5%) compared to other feed formulations.

Experiment 7

The rate of survival was best (43.0%) in liver fed stock followed by fish fed with artificial feed 5 (28.5%) and was least (21.5%) with MOC+ RB ration (Table 8). The total gain in final weight of the mahseer stock was insignificant and in some cases, even the loss from the initial biomass was recorded and hence further experimentation was abandoned. This indicated that the natural stock probably could not acclimatize to the captive conditions especially in small tanks of 1 m² dimension where they did not respond much to artificial feeding resulting in heavy mortalities.

DISCUSSION

The practical diet 5 gave the overall best survival, feed efficiency, conversion ratio, growth etc. amongst all the 6 feeds compounded in young stages of golden mahseer. Any balanced formula for fish diet must include sufficient indispensable amino-acids (protein) plus the energy source in the form of lipids and carbohydrates besides essential vitamins and minerals to support life and promote growth. The golden mahseer is inherently poor in biological productivity; the role of nutritionally balanced artificial feeds acquire more importance when propagated artificially in captivity as the fish growth and feed efficiency is influenced by quality of feed, feed intake and water temperature.

Protein requirement is highest at fry stage and decrease with increasing size (Garling and Wilson 1976, NRC 1977, Wilson and Halver 1986). It is recommended that to obtain maximum growth, fish fry must consume a diet containing half of dietary ingredients as protein. The high requirement of the component could be on account of relatively rapid increase of most tissues in smaller fish as they grow with positive allometry and also their generally higher metabolic turn over (Witherlay and Gill 1987). These authors stated that protein content in practical diets often ranges from 20-60% depending upon the water temperature In the feeding trials with putitor mahseer, about 45% dietary protein level appeared to be sufficient for the young stages. Joshi *et al.*(1989) recorded better feed efficiency with a diet containing 33.5% crude protein level for young ones of the same species but their results are based merely on a couple of experiments without any replicate and for a short period.

Fats being the ready source of energy have the distinct advantage of almost completely digestible need to be supplied in diets for quick growth and maintenance of healthy fish stocks. Fat requirements of Indian carps are 4-6% (Singh 1991) whereas Jafri *et al.*(1992) stated that fish feeds normally incorporate 4-9% fat. NRC (1981) reported that lipid not less than 10% and not more than 20% can be added to coldwater fish diets with excellent results. Takeuchi *et al.* (1978) observed that the optimum ratios in the rainbow trout is 35% protein to 15-20% lipid content. Weatherly and Gill (1987) stated that when dietary lipid is generally increased to a certain limit, the feed conversion efficiency of the fish may increase proportionately and further opined that

Table 6. Results of feeding trials in golden mahseer advance fry stocked @ 300/m² with different feeds for a rearing period of 30 days

Particulars of experiment	Formulated feeds						Feeds egg-yolk	Conventional Feed MOC + RB (1:1)
	1	2	3	4	5	6		
No.of fish stocked	300	300	300	300	300	300	300	300
Initial length (mm)	30-35	30-35	30-35	30-35	30-35	30-35	30-35	30-35
Av.initial wt.(g)	0.175	0.175	0.175	0.175	0.175	0.175	0.175	0.175
Total initial wt.(g)	52.500	52.500	52.500	52.500	52.500	52.500	52.500	52.500
No.of fish harvested	166	183	205	232	251	220	267	163
Rate of survival (%)	53.3	61.0	68.3	77.3	83.6	73.3	89.0	54.0
Final length (mm)	42-48	41-50	44-51	45-68	50-60	47-58	53-64	38-45
Av.final wt.(g)	0.860	0.875	0.915	1.100	1.200	0.900	1.310	0.695
Total final wt.(g)	142.760	160.125	187.575	255.200	301.200	198.000	349.770	113.285
Gain in wt.(g)	90.260	107.625	135.075	202.700	248.700	145.500	297.270	60.785
Increasing rate of body weight (g)	4.9	5.0	5.2	6.3	6.9	5.1	7.5	4.0
Av.increase in body wt./day (g)	0.08	0.08	0.09	0.10	0.12	0.08	0.13	0.06
Total feed given (g)	261.700	290.590	324.180	466.210	547.140	378.300	535.100	200.590
Conversion ratio	2.9	2.7	2.4	2.3	2.2	2.6	1.8	3.3
Feed efficiency(%)	34.5	37.0	41.6	43.5	45.5	38.5	55.6	30.3

Table 7. Results of feeding trials in golden mahseer advance fry stocked @ 400/m² with different feeds for a rearing period of 30 days

Particulars of experiment	Formulated feeds						Feeds egg-yolk	Conventional Feed MOC + RB (1:1)
	1	2	3	4	5	6		
No.of fish stocked	400	400	400	400	400	400	400	400
Initial length (mm)	30-35	30-35	30-35	30-35	30-35	3-35	30-35	30-35
Av.initial wt.(g)	0.175	0.175	0.175	0.175	0.175	0.175	0.175	0.175
Total initial wt.(g)	70.000	70.000	70.000	70.000	70.000	70.000	70.000	70.000
No.of fish harvested	207	241	265	296	325	293	340	217
Rate of survival (%)	51.7	60.3	66.2	74.1	81.2	73.3	84.9	54.3
Final length (mm)	41-46	41-48	45-52	44-57	49-60	46-54	51-62	38-44
Av.final wt.(g)	0.845	0.870	0.895	1.010	1.120	0.910	1.230	0.690
Total final wt.(g)	174.915	209.670	237.175	298.960	364.000	255.630	418.200	149.730
Gain in wt. (g)	104.915	139.670	167.1715	228.960	294.000	196.630	348.200	79.730
Increasing rate of body weight (g)	*4.8*	*5.0*	*5.1*	*5.8*	6.4	5.2	*7.0*	4.0
Av.increase in body wt./day (g)	0.08	0.08	0.08	0.10	0.10	0.09	0.12	0.06
Total feed given(g)	293.760	391.100	401.220	572.400	676.200	491.575	626.760	271.100
Conversion ratio	2.8	2.8	2.4	2.5	2.3	2.5	1.8	3.4
Feed efficiency (%)	35.7	35.7	41.7	40.0	43.5	40.0	55.5	29.4

generally in fish diets, the fat level may range from 5-25% without adverse effects on growth and the acceptable lipid level should be determined in fish tissues to be able to adjust dietary lipid level accordingly. Based on the analysis of lipid content (approximately 17%) in the golden mahseer fry tissues, the level and diets containing 13-14% crude fat gave the best performance. Much higher values did not prove effective causing high rate of mortality and low rate of feed efficiency in the test fishes.

Carbohydrates to a certain limit can replace the dietary fats to meet the metabolic energy needs of fish. Since carbohydrates are least expensive compared to protein and fat so it could be given in feed formulations besides they also get as binders/fillers in feed preparation. In formulated diets, carbohydrates can vary from 10-50% and the efficiency of utilization as energy may range from 40-90% (Hastings 1979). Bergot (1979) indicated that digestible carbohydrate level can be increased to 15-30% without any adverse effects

Table 8. Results of feeding trials with advance fry of mahseer collected from natural systems on laboratory formulated and conventional feeds for a period of 60 days

Particulars of experiment	Formulated feeds			Conventional Feed	
	4	5	6	Liver	MOC + RB (1:1)
No.of fish stocked	200	200	200	200	200
Initial length (mm)	30-40	30-40	30-40	30-40	30-40
Av.initial wt.(g)	0.190	0.190	0.190	0.190	0.190
Total initial wt.(g)	38.000	38.000	38.000	38.000	38.000
No.of fish harvested	47	57	49	86	43
Rate of survival (%)	23.5	28.5	24.5	43.0	21.5
Final length (mm)	46-51	47-52	45-52	47-54	44-50
Av.final wt.(g)	0.800	0.825	0.785	0.920	0.580
Total final wt.(g)	37.600	47.025	38.465	79.120	24.940
Gain in wt.(g)	-	9.025	0.465	41.120	-

Table 9. Comparison of survival rate, feed efficiency, increasing rate of body weight,growth rate/day and conversion ratio of various feeds in different stages of golden mahseer

Particulars of experiment		Formulated feeds						Feeds		Conventional Feed
		1	2	3	4	5	6	Egg-yolk	Liver	MOC + RB (1:1)
Survival(%)	a.Swim-up fry	40.7	46.8	52.5	54.9	65.1	64.2	55.4	72.6	31.8
	b.Fry	48.3	52.1	57.9	63.4	74.8	69.1	65.2	82.1	45.5
	c.Advance fry/ fingerling	52.6	60.5	64.4	75.2	82.6	73.3	-	86.7	53.9
Feed efficiency(%) (Gain in wt. Feed given × 100)	a.Swim-up fry	31.4	31.5	36.9	37.9	41.7	34.9	42.9	46.8	26.7
	b.Fry	35.7	37.6	40.8	39.2	43.5	40.9	47.1	52.6	28.8
	c.Advance fry fingerling	34.8	36.2	41.2	42.1	44.6	39.7	-	55.9	29.5
Increasing rate of body wt.(g) (Final wt./ Initial wt.)	a.Swim-up fry 7.1	7.3	8.4	8.2	8.8	7.3	8.8	9.3	7.1	
	b.Fry	2.5	2.7	3.0	2.9	3.0	2.9	3.1	3.2	2.4
	c.Advance fry/ fingerling	4.7	4.9	5.1	6.3	6.6	5.1	-	7.3	4.0
Av.Increase in body wt./day(g) (Feed efficiency/No.of feeding days)	a.Swim-up fry	0.23	0.24	0.26	0.27	0.29	0.25	0.29	0.31	0.31
	b.Fry	0.08	0.09	0.10	0.10	0.10	0.10	0.11	0.11	0.08
	c.Advance fry/ fingerling	0.08	0.08	0.09	0.10	0.11	0.08	-	0.12	0.06
Conversion ratio (Feed given/ weight gained)	a.Swim-up fry	3.4	3.2	2.7	2.7	2.4	2.8	2.3	2.1	3.7
	b.Fry	2.8	2.7	2.4	2.5	2.3	2.5	2.2	1.9	3.4
	c.Advance fry/ fingerling	2.8	2.7	2.4	2.4	2.2	2.5	-	1.8	3.3

on the growth and survival of rainbow trout. Sen *et al.* (1978) and Singh (1991) observed that the gross protein and carbohydrate requirements for carp fry and fingerlings varied from 36-47% and 22-28%, respectively, at 28.0-32.0°C. The present studies show that the feed containing 20.0-22.0% carbohydrate suited best for the survival and growth of mahseer fry/advance fry.

Compared to the artificial feeds barring minced liver diet, egg yolk emulsion gave better performance in young stages of putitor mahseer and conventional diet comprising rice bran and mustard oil-cake mixture proved to be least efficient which is in agreement with the findings of Joshi *et al.*(1989). Further during the feeding trials, it was undoubtedly observed that higher food quotient, survival and feed efficiency was observed when mahseer young ones were fed with wet diet of minced sheep/goat's liver over the dry compounded diets. However, this may not be economical and practical as the cost of wet diets have escalated tremendously which may

upset the whole aquaculture programme when taken up in a big way besides many a times, the regular supply of such products may not be ensured and also there is the risk of introducing pathogens to the recipient fish stock, if regularly used.

In early rearing stages of mahseer, varying densities (300-500/sq.m.) did not appear to have significant deleterious effect when fed with various diets on fish growth, feed efficiency conversion factor or survival rate which suggests that if mahseer fry at higher density up to a optimum level are properly fed, there may not be any negative impact provided the water quality parameters are conducive and sufficient water supply is regulated at required level. However, the overall performance of various diets (both dry and wet) reduced when the fish density of advance fry (30-35 mm) was enhanced from 300 to 400/sq.m. The overall performance in terms of survival rate, feed efficiency, increasing rate of growth, growth/day and conversion ratio was recorded best in fish fed with minced liver followed by egg-yolk emulsion and poor in conventional feed (Table 9).

On the basis of various feeding experiments carried out with different diets so far, it can be said that it is just a start to be followed by more detailed studies especially the field trials in a big way. Such an attempt is aimed towards standardization of feeding schedule/techniques with final goal of golden mahseer farming on a commercial scale. Mahseer culture is a very young field so many more studies need to be undertaken to make sure that the formulated feeds prepared from locally available raw ingredients are balanced and nutritive for better growth, higher efficiency and fish health besides being cost effective. All possible efforts are to be made on feed formulations for Himalayan mahseer laying more emphasis on analytical knowledge than on empirical practices to avoid any wastage of costly nutrients besides studies on computerized feed formulations could be taken up. However in this study, among the 6 formulations, these parameters were highest with formula 5 at various growing phases of mahseer compared to other artificial diets.

ACKNOWLEDGEMENTS

Financial grant from ICAR, Krishi Bhavan, New Delhi under ICAR Cess Fund Scheme is gratefully acknowledged. Thanks are also due to Mrs Susheela Tewari for computer assistance in preparing the manuscript.

REFERENCES

AOAC. 1984. *Official Methods of analysis*. 14th edn. Association of Official Analytical Chemists, Arlington, Verginia.

Bergot F. 1979. Carbohydrates in rainbow trout diets : Effect of level and source of carbohydrates and number of meals on growth and body composition. *Aquaculture* **18**:157-67.

Garling D L and Wilson R P. 1976. Optimum dietary protein to energy ratio for channel cat fish fingerlings. *Ictalurus punctatus. Journal of Nutrition* **106** : 1368-75.

Hastings W H. 1979. Fish nutrition and fish feed manufacturer. *Advances in Aquaculture*. (Eds.) Pillay T V R and Dill W A.Fishing News Book, Faranham.

Jafri A K, Jafri D K, Khan M A, Anwar M P, Hassan A and Erfanullah. 1992. On the use of locally available feed stuffs in formulated fish feeds : Proximate composition and gross energy content. *Aquaculture Research Needs for 2000 AD*.pp. 143 - 51.IBH Publishing Co..New Delhi .

Joshi C B, Sehgal K L and Malkani K C. 1989. Experimental trials on feeding of *Tor putitora* with formulated diets at Bhimtal in Kumaon Himalayas. *Indian Journal of Animal Sciences* **59**(1) : 206-209.

NRC. 1977. *Nutritional Requirements of Warm Water Fishes*. Washington D.C. National Academy of Sciences Press.

NRC. 1981. *Nutritional Requirements of Coldwater Fishes* No.16.63 P. Nutritional requirements of domestic animals. Washington D.C. National Academy of Sciences Press.

Raina H S, Shyam Sunder and Naulia U. 1993. Nutritional requirements for the juveniles of golden mahseer *Tor putitora* (Ham.).*The Third Indian Fisheries Forum Proceedings* (11-14 Oct.,1993). Pantnagar.5-8.

Sen P R, Rao N G S, Ghosh S R and Rout M. 1978. Observation on protein and carbohydrate requirements of carp. *Aquaculture* **13** : 121-29.

Singh B N. 1991. Nutrition and feed development strategies for aquaculture in India. *Journal of Inland Fisheries Society of India* **23**(2) : 99-112.

Sunder Shyam and Raina H S.1995. Development of artificial diets for *Tor putitora* (Ham.) juveniles - their relevance to aquaculture. *Nature Workshop on Aquaculture Nutrition. CIFE (29-30 December 1995), Bombay* : 14.

Takeuchi T, Watanabe T and Gino C O. 1978. Optimum ratio of protein to lipid in diets of rainbow trouts. *Bulletin of Japanese Society of Science Fisheries* **44**(6) : 683-88.

Wilson R P and Halver J E. 1986. Protein and amino-acid requirements of fishes. *Annual Review of Nutrition* **6** : 225-44.

Witherley A H and Gill H S. 1987. *The Biology of Fish Growth*. pp.443. Academic Press Inc.(London) Ltd.

CALL FOR ABSTRACTS

1999 ANNUAL MEETING OF THE INTERNATIONAL EMBRYO TRANSFER SOCIETY

10-12 January, Quebee City Convention Center, Province of Quebee, Canada

Free communications will be presented as posters, abstracts of which will be published in the January, 1999 issue of *THERIOGENOLOGY.* Abstracts must be in English, and prepared according to *THERIOGENOLOGY's* guidelines for direct offset reproduction; **abstracts must adhere rigidly to the guidelines on format, paper quality, and font size.** The abstract must report **unpublished, original data,** and contain objectives, experimental methods, results, and conclusions. Due to space **restrictions**, no one may be first author of more than one abstract or co-author of more than four abstracts. Each abstract will be subjected to **rigorous scientific review** by at least two referees. A **signed commitment** to **present the data in the poster session** at the annual conference is required. Students may enter their abstract in a competition sponsored by the IETS Foundation, and also are eligible to compete for the US$500 travel awards. The Foundation also offers to young international scientist a grant to be awarded competitively on specific merit and need, consisting of US$ 1500 to be applied toward travel and lodging; registration fees will be waived. Request details of eligibility, judging procedures, and prizes from the IETS office before preparing abstracts.

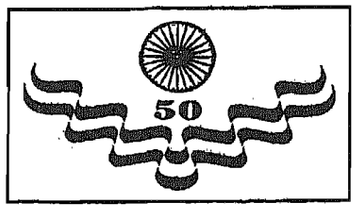
The **firm deadline** for **receipt** of the abstract (original and three photostatic copies) is **15 August 1998.** Abstracts received after this date will be returned. Do not fold you abstract and please provide some stiff backing to protect the abstract during transit. In addition to the hard copies, submission on disk (3.5 inch) is encouraged to facilitate processing. Acceptable word-processing formats are WordPerfect 5.1 or higher and Word 5.1 or higher, Fax copies and electronic submissions of abstracts will not be accepted. (However, we ask authors to provide their E-mail address if possible, to allow electronic correspondence). The abstracts should be mailed (postmark deadline by 1 August (outside North America) or 10 August (within North America) to :

Dr Marc Andre Sirard
1999 IETS Program Chairman
Departement des sciences animales
Universite Laval
Ste-Foy, Quebec G1K 7P4
CANADA
Tel. : 418-656-7359
Fax : 418-656-3766
E-mail : Marc-Andre-Sirard@CRBR.ulaval.ca

Instructions *of preparation of abstracts as well as information about the student competition and travel awards may be obtained from;*

Ms Fran Gardner, Executive Secretary,
International Embryo Transfer Society
1111 N. Dunlap Ave., Savoy, IL 61874 U.S.A.
Telephone : 217-356-3182; Fax : 217-398-4119
E-mail : frang@assochq.org

Topics of the invited papers include practical considerations for in vitro embryo production, fertilization, maintaining embryo viability in vitro, and methods and applications of animal genetic modifications.



To Celebrate

50 YEARS OF INDEPENDENCE

ICAR Presents Gold-mine of Agricultural Information

	Publications	Price (Rs.)	Postage (Rs.)
1.	50 Years of Crop Science Research in India	900.00	20.00
2.	ICAR Now & Ahead...	200.00	20.00
3.	Acid Soils of India	100.00	15.00
4.	Agricultural Bio-technology in the ICAR Research Institutes	150.00	15.00
5	Rice Research in India	60.00	15.00
6.	Handbook of Agriculture	150.00	15.00
7.	Handbook of Animal Husbandry	125.00	15.00
8.	The Rose in India	175.00	20.00
9.	Techniques in Diagnostic Virology	30.00	10.00
10.	A History of Agriculture in India (Vol.I-IV)	260.00	(Inclusive of postage)
11.	Indian National Agricultural Bibliography (Vol.I-IV)	1200.00	(Inclusive of postage)
12.	Proceedings of Second World Buffalo Congress (Vol.I-IV)	1000.00	(Inclusive of postage)

	Journals	Annual Subscription (Rs.)
1.	Indian Farming (English Monthly)	84.00
2.	Indian Horticulture (English Quarterly)	28.00
3.	The Indian Journal of Agricultural Sciences (English Monthly)	900.00
4	The Indian Journal of Animal Sciences (English Monthly)	900.00
5.	Kheti (Hindi Monthly)	84.00
6.	Phal Phool (Hindi Quarterly)	28.00
7.	Krishi Chaynika (Hindi Quarterly)	32.00

* Rush your order alongwith advance payment by **Demand Draft** or **Indian Postal Order** in favour of Business Manager, ICAR, New Delhi.

* Complete price list is available on request.

For further information please write to:

Business Manager
Indian Council of Agricultural Research
Krishi Anusandhan Bhavan, Pusa
New Delhi 110 012, Phone : 011-5713657
Fax : 011-5731282, E.mail : bmicar @ kab.delhi.nic.in

THE INDIAN JOURNAL OF ANIMAL SCIENCES

GUIDELINES TO AUTHORS

1. *The Indian Journal of Animal Sciences* is published every month. The following types of material will be considered for publication in the Journal.

2.1 PAPERS ON ORIGINAL RESEARCH COMPLETED, not exceeding 3 500 words (approximately 10-12 typed pages), should be exclusive for the journal. They should present a connected picture of the investigation and should not be split up into parts.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

6. A recent issue of the journal should be consulted for the methods of citation of References in the text as well as at the end of the article. The biliography should have the names of the authors, initials, year of publication, full title of the paper, name of the journal (spelt out in full), volume, preferably the issue within parentheses and complete page-range (not merely the first page). Authors should ensure that all references in the text appear at the end of the paper and vice versa, and that names and dates at the two places correspond.

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

8 Papers should be TYPE-WRITTEN, and double-spaced throughout (including references and tables) on white, durable bond paper of size 22 cm × 28 cm, with a 5-cm margin at the top, bottom and left-hand side. Articles (including illustrations) should be sent in duplicate, after a careful check-up of typographical errors.

9. Though no rigid rules are recommended for writing, authors are requested to consult *The Style Manual for Biological Journals*, 4th edn, American Institute of Biological Science, Washington DC.

10. PROOF-CORRECTION should be in ink, in the margin. All queries marked in the article should be answered. Proofs are supplied for a check-up of the correctness of type-setting and facts. Excessive alterations may by charged to the authors. The proofs should be returned within 10 days.

11. Contributors will receive 50 copies of OFFPRINT Free (to be shared with the co-authors).

R. N. 14357/57 Delhi Postal Regd. No. DL-18027/97

Highlights of the Forthcoming Issue (May 1998)

●ELISA and co-agglutination test for detection of K88ac fimbrae in *Escherichia coli* strains ●Review of animal health services in India ● True acrosome reaction in buffalo (*Bubalus bubalis*) spermatozoa ●Effect of estradiol-17 β priming on superovulatory response, embryo recovery and progesterone profile in goats ●Individual variation among buffalo bulls in fertilization and subsequent embryonic development *in vitro* ●Sero-incidence of bovine brucellosis in Peshawar district of Pakistan ●Normal haematology of yak (*Poephagus grunniens* L.) ●Evaluation of berseem based complete feed blocks in growing buffalo calves ●Comparative evaluation of gas production and dry matter digestibility of straw substrates *in vitro* ● Nutrients utilization pattern in weaner goats under berseem (*Trifolium alexandrinum*) silage based feeding

THE INDIAN JOURNAL OF ANIMAL SCIENCES

is indexed in the AGRINDEX, published by the FAO; the *Science Citation Index* and the *Current Contents* (Agriculture, Biology and Environmental Sciences), published by the Institute for Scientific Information, Philadelphia. It is also indexed in the abstracting journals of the Centre for Agriculture and Biosciences International as well as all the major abstracting services of the world.

Published by Dr R D Sharma, Director, (Directorate of Information and Publications on Agriculture), Indian Council of Agricultural Research Krishi Anusandhan Bhavan, Pusa, New Delhi 110 012. Lasertypeset at Rashtriya Press, 12-B Annexe, Rajendra Park, New Delhi 110 060 and printed in India at the Nutan Printers, F-89/12, Okhla Industrial Area, Phase-I, New Delhi 110 020.