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Clinico-pathological and ultrasonographic observations in canine hepatopathies

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ABSTRACT

Findings of ultrasonography were analyzed in 24 dogs with hepatopathies and in 3 dogs without hepatopathy and compared with the results of haemato-biochemical investigations. Ultrasonographic appearance of liver demonstrated diffuse hyperechoic "bright" but small liver in cirrhosis and in intrahepatic porto-systemic shunt (PSS); and diffuse fine hypoechoic hepatic changes with hepatomegaly in chronic active hepatitis. Mostly a single shunt either between intrahepatic vena cava and portal vein (most common) or intrahepatic portal and hepatic vasculature was detected sonographically, confirming the diagnosis of intrahepatic PSS. Predominance of target cells, poikilocytes, microcytes, crystals of ammonium biurate in urine was constant finding in PSS. Serum protein, serum albumin, BUN, blood glucose, SAP, ALT were on lower side in PSS. However, SAP, ALT and arginase values were high in chronic active hepatitis.

Key words: Canine, Hepatopathies,Hyperechoic, Hypoechoic, Target cells

Hepatopathies are quite common in dogs. During recent past, porto-vascular anomalies with associated hepato-encephalopathy have been increasingly recognized in dogs (Ewing *et al.* 1974, Campbell *et al.* 1980). Clinical syndromes of portal vascular anomalies, bypassing liver, and other hepatic disorders, viz. chronic active hepatitis and cirrhosis, are extremely vague and variable defying their clinical differentiation. Haemato-biochemical findings and survey abdominal radiography are of a little assistance in the diagnosis of porto-systemic shunts (Koblick *et al.* 1983). During recent years, hepatic ultrasonography seems to be of much diagnostic assistance in differentiation of canine hepatopathies (Nyland 1984) as it is in humans. Concurrent ultrasonographic and clinicopathological observations in canine hepatopathies are lacking in India.The present investigation was, therefore, undertaken to study clinico-pathological and ultrasonographic features of different hepatopathies of dogs.

MATERIALS AND METHODS

Clinical cases (24) of suspected hepatopathies in dogs (non-descript 8, spitz/pomeranian 11, German Shepherd 2, Doberman 1, Labrador 1 and Dachshund 1), referred at the Institute's Referral Veterinary Polyclinic during October 1999 to October 2000, formed the material for the present study. Three clinically healthy dogs having no symptom referable to hepatopathy were also included in the study for comparison. Haematological, coprological and urine examinations were performed employing routine standard procedures. Total serum protein, serum albumin, ALT, SAP, BUN and blood glucose were estimated colorimetrically using reagent kits. Serum arginase was also estimated colorimetrically.

Hepatic ultrasonography was performed with Scanner-200 Vet using a 5.0 MHz AAS transducer. Food withholding prior to sonographic examination was not practised in anorectic sick animals. No chemical restraint was used. Abdominal area was shaved and acoustic gel was liberally applied before sonography. Imaging was performed in dorsal right and lateral intercostal approach, using saggital, transverse and dorsal planes. Liver size was evaluated keeping age, breed and conformation of the dogs in view. Systematically porta-hepatis, liver size, and interportal vasculature were evaluated keeping caudal vena cava as a landmark. When a vessel connecting intrahepatic portal and hepatic venous vasculature was detected, diagnosis of intrahepatic porto systemic shunt was given (Holt *et al.* 1995).The images were recorded on thermal printing paper using a video copy processor.

RESULTS AND DISCUSSION

History and clinical signs suggesting hepatopathies in 24 cases included nausea/vomition (15), jaundice (3), mild anaemia (10), evidence of peritoneal fluid accumulation (10), abdominal distension (5), constipation (6), diarrhoea (2), head pressing (3), convulsion (3), hypersalivation (10), muscular tremor (3), apparent blindness (1), pyrexia (2), melena (7),

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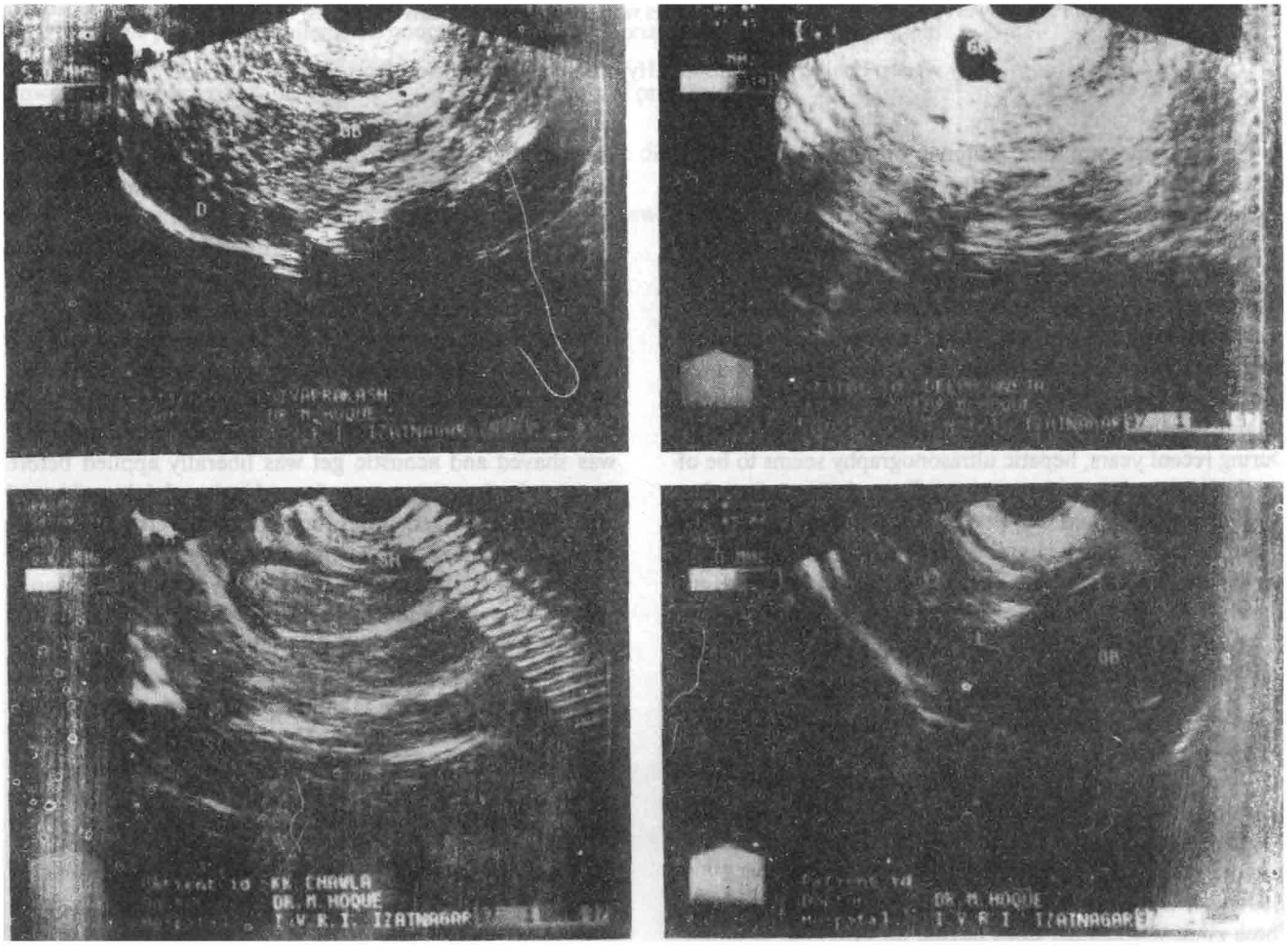
hypotension (7) and coma (2). Chronic anorexia, weakness, emaciation and depression were nonspecific signs occurring in most of the dogs. These clinical signs were extremely variable and vague related to neurologic and gastrointestinal symptoms (Holt *et al.* 1995) commonly seen in hepatopathies such as intrahepatic porto-systemic shunts, cirrhosis and chronic active hepatitis (Johnson *et al.* 1987). However, jaundice and melena were observed in chronic active hepatitis (Center 1995); bilateral hind limb oedema was evident in 6 cases of PSS. In 1 dog minor shunt jaundice was also seen. In advanced cases of cirrhosis and PSS signs referable to nervous system involvement and urinary incontinence became evident suggesting development of hepatic encephalopathy and crystalluria (Vulgamott 1985,Holt *et al.* 1995). The observation of hypotension (systemic arterial pressure 100 mm of Hg) in cases of porto-systemic shunt and cirrhosis finds support from the observation of Oster (1982). In 4 cases of PSS, history revealed craze for newspaper/polythene chewing. Haemogram revealed mild anaemia. However, preponderance of target cells (20.54%), microcytes (MCV 59.44 fl) and poikilocytes in blood smears was a consistent finding in cases of intrahepatic PSS. Hardy (1989) observed an increased number of target cells in blood smears of dogs with PSS. Nelson and Couto (1992) also reported that microcytes (MCV <60 fl) and target cells were common haematological findings in congenital PSS in dogs.

Urological examination revealed the preponderance of golden brown thorny apple shaped crystals of variable sizes, resembling with crystals of ammonium biurate (Marretta *et al.* 1981) in 15 cases of intrahepatic PSS (13) and cirrhosis (2) (Center 1995). The presence of these crystals in urine of dogs other than Dalmatian appears to be a good indicator of hepatic vascular anomalies/ cirrhosis (Breznock and Whiting 1985,Cornelius and Bjorling 1992).

The values for total serum proteins, serum albumin, blood urea nitrogen and blood glucose were considerably low in cases of PSS and cirrhosis as compared to that of healthy dogs (Table 1) and is in agreement with the findings of Willard



Figs 1-4. **1**. Sonogram of normal canine liver with a uniformly slightly coarse echotexture. **2**. Sonogram showing diffuse hyperechoic "bright" but small liver with distended gall bladder indicating cirrhosis. **3**. Sonogram showing shunt between intrahepatic vena cava and portal vein. **4** Sonogram showing diffuse fine hypoechoic liver echotexture with hepatomegaly indicating chronic active hepatitis.

(1991) in hepatic insufficiency. Low serum proteins could be ascribed to inappetence or decrease hepatic production. Hypoalbuminaemia in PSS and cirrhosis leading to chronic liver insufficiency appears due to reduced albumin synthesis and is an important feature in the development of ascites. Serum alkaline phosphatase (SAP), alanine amino transferase (ALT) values in PSS were on lower side of normal range and slightly higher in cirrhosis, though magnitude of increase was small. This is in agreement with the observations of Twedt (1985). Lower values of SAP and ALT in PSS are probably due to minimal inflammatory changes and reduced liver size with fibrosis in these conditions (Allen 1991). Serum arginase value was slightly higher than the normal in PSS. In chronic active hepatitis (CAH) a considerable increase in ALT, arginase and SAP values were evident. In 2 cases of CAH, total bilirubin was also high.

The suspected cases of hepatopathies were subjected to hepatic ultrasonography as suggested by Nyland and Park (1983) and Lamb (1990) to evaluate status of the liver. The ultrasonography has been reported to be a useful tool in diagnosis of liver diseases of dogs as it is in human beings. Ultrasonographic examination of liver of dogs, having no clinical signs referable to hepatopathy, revealed a uniformly slightly coarse ecotexture (Fig.1) with visible larger blood vessels and gall bladder. The liver was less echogenic than spleen but hyperechoic or isoechoic as compared to right kidney, using a 5 MHz transducer as also reported by Hartzband *et al.* (1989). The gall bladder was anechoic with smooth wall and acoustic enhancement was produced in the tissues deep to the gall bladder. Portal veins were recognized by well defined echogenic wall while hepatic vein lacked this characteristic. The bile duct, lobar border, hepatic arteries and small peripheral hepatic veins were not visualized in the healthy dogs with no hepatic disorder. These ultrasonographic findings are in conformity with those described by Lamb (1990) for normal canine liver. Diffuse hyperechoic "bright" but small liver with distended gall bladder (Fig.2) was evident in both the cases of liver cirrhosis as also reported earlier in

Table 1. Haemato-biochemical and ultrasonographic observations in canine hepatopathies (mean values)

Parameters	Hepatopathies			
	Intrahepatic porto-systemic shunt (13)	Cirrhosis (2)	Chronic active hepatitis (6)	Healthy dogs (3)
Haematological				
Haemoglobin (g/dl)	12.0	11.50	12.32	12.33
Haematocrit (%)	30.28	31.00	36.00	36.00
Total erythrocytes (x10⁶/µl)	5.14	5.5	5.02	5.66
Mean corpuscular volume (fl)	59.44	56.66	72.20	70.99
Target cells (%)	20.54	1.0	-ve	Nil
Poikilocytes	+ve	+ve	-ve	-
Biochemical				
ALT (µg/l)	20.65	29.50	92.20	25.33
SAP (µg/l)	22.71	30.62	126.64	38.00
Arginase (µg/l)	26.18	20.58	79.20	5.60
Total serum protein (g/dl)	3.85	4.26	4.18	6.26
Serum albumin (g/dl)	1.10	1.54	2.12	3.30
Blood urea nitrogen (mg/dl)	14.21	15.00	10.68	23.66
Blood glucose (mg/dl)	56.22	60.00	55.56	81.00
Total bilirubin (mg/dl)	-	-	9.08	-
Urological				
Crystals	Ammonium biurate	Ammonium biurate	Nil	Nil
Ultrasonographic				
Liver echos	Diffuse/patchy hyperechoic "bright"	Diffuse/hyper-echoic "bright"	Diffuse fine hypo-echoic	Uniformly coarse
Liver scanning area/ average liver size	Small / 4.82 cm	Small/ 5.32cm	Large / 10.61 cm	Normal/ 7.43 cm
Gall bladder	Distended	Distended	Distended	Normal
Bile duct	Patent	Patent	Patent	Patent
Intrahepatic shunt	Yes	No	No	No
Free fluid in abdomen	+/-	+/-	+/-	-ve
Kidneys	Normal	Normal	Normal	Normal
Urinary bladder	Distended with urine, sediment	Normal	Normal	Normal

cirrhotic humans (Gasnik *et al.* 1978) and dogs (Cartee 1981, Lamb 1990). Liver in cases of intrahepatic PSS, showed diffuse/ patchy hyperechoic small "bright" areas with an overall reduction in scanning area as also observed in liver cirrhosis. These findings are in agreement with the sonographic observations of other authors (Wrigley *et al.* 1981 and Voros *et al.* 1991). Mostly a single shunt (Fig.3) was detected either between intrahepatic vena cava and the portal vein or portal and hepatic vasculature. Of these, shunt between intrahepatic vena cava and portal vein was more common as also reported by Martin (1993). The shunting vessel was more distended and tortuous. Detection of intrahepatic shunts on hepatic ultrasonography confirmed the clinical suspicion of PSS. Hence, ultrasonography appears to be very useful in evaluating suspected cases of congenital PSS (age of the animals varied from 3 months to 7 years). Gall bladder was distended in a few cases of PSS, cirrhosis as well as of CAH. Evaluation of the size of the gall bladder was subjective, as no reference value is available for normal dogs. Free fluid in the peritoneum of dogs with PSS, cirrhosis and CAH was in conformity with the clinical findings. Sonographically free fluid in the peritoneum of 3 cases of PSS was also detected wherein clinical examination failed to establish ascites. Diffuse fine hypoechoic hepatic changes with hepatomegaly (Fig.4) were observed in dogs with chronic active hepatitis, coinciding with human reports in hepatosis/hepatitis (Gosnik *et al.* 1979, Sakuma *et al.* 1987). Hepatomegaly was again subjective criteria for the want of a quantitative data on liver size of normal dogs (Godshalk *et al.* 1988). Gall bladder distension, bile stasis and sludge formation have been reported often in association with hepatic disorders (Voros *et al.* 1991). However, distended gall bladder with patent bile duct has also been reported in anorectic dogs without obvious functional or morphological abnormalities of the liver or gall bladder (Lamb 1990, Nelson and Couto 1992).

Urinary bladder in almost all cases of PSS and cirrhosis was distended with a lot of sediment. On the basis of clinical, haemato-biochemical and ultrasonographic observations, cases of hepatopathies were differentiated as of intrahepatic PSS, liver cirrhosis and chronic active hepatitis. Out of 24 cases of hepatopathies, 14 (58.3%) were of intrahepatic PSS, 2 (8.4%) of liver cirrhosis, and 8 (33.3%) of chronic active hepatitis with a mean age of 26.8 months (median, 18 months; range 3 to 96 months), 78 months (range 72 to 84 months) and 42.1 months (median 6 months, range 3 to 96 months) respectively. Detection of intrahepatic PSS at an early age probably indicates its congenital origin (Bostwick and Twedt 1995). About 33.0% cases of intrahepatic PSS, in the present study, were more than 2 years old and the oldest was 7 years old. Center and Magne (1990) reported that cases of intrahepatic PSS could also occur in adult dogs. Most of the dogs (11/14) with intrahepatic PSS were of large breed (Mongrel-7; German Shepherd-2; Dobermann-1 and Labrador-1) as also reported by Bostwick and Twedt (1995). Significant sex associated difference in cases of intrahepatic PSS (male 8; female 6), cirrhosis (male 1, female 1) or chronic active hepatitis (male 6, female 2) could not be observed possibly because of small number of observations. From weight ranges it is apparent that mean body weight of dogs with PSS, cirrhosis and chronic active hepatitis was 14.0, 8.0 and 9.8 kg respectively.

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Effect of GnRH on fertility in crossbred cows

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ABSTRACT

The effect of GnRH on pregnancy was studied using 85 crossbred cows. Buserelin, an analogue of GnRH was administered intramuscularly at the time of standing oestrus in a 10mg dose to 25 (treatment 1), and 20mg dose to 30 (treatment 2) cows. Further 30 cows were administered normal saline solution and used as control. The pregnancy rate to first AI was increased by treatment 2 (40.0%) relative to control (23.3%) and treatment 1 (28.0%). A further 16.7% (control), 32.0% (treatment 1) and 30.0% (treatment 2) cows were found pregnant following second AI and 6.7% (control), 8.0% (treatment 1) and 10.0% (treatment 2) got pregnant to the third AI. None of these differences were significant. The cumulative pregnancy rate for all 3 services was lower for control (46.7%) than treatment 1 (68.0%) and treatment 2 (80.0%) and difference between control and treatment 2 was significant ($P<0.05$). There was no difference in service/conception between control (1.64) and treatment 1 cows (1.70) but this improved by treatment 2 (1.50). The results showed that GnRH treatment at the time of oestrus can be used to increase pregnancy rate in cows and the effect is dose dependent.

Key words: Cows, Fertility, GnRH

A calving interval of 12 months is an ideal goal for cattle husbandry system. Aberration in either ovulatory process or embryo loss may be the factor for increase in calving interval. Gonadotrophin releasing hormone (GnRH) induces ovulation or luteinization of follicles through enhanced release of luteinizing hormone (Chauhan *et al.* 1984) and may be used to improve fertility. GnRH also sustains progesterone production when injected during dioestrus (Macmillan *et al.* 1985) and thus, reduces incidence of early embryonic mortality. The use of GnRH increased conception rate of 8 to 13% and it has been reported by many workers following the use of GnRH (Leidle *et al.* 1979, Van der Westhuysen 1980). There is, however, little information regarding the effect of GnRH on pregnancy rate under tropical conditions. The experiment described here was designed to address this problem.

MATERIALS AND METHODS

The study was carried out at the cattle and buffalo farm IVRI, Izatnagar, during November 1999 to April 2000. The maximum, minimum temperature and humidity ranged from 22.4 to 35.8°C, 10.2 to 18.7°C and 58 to 45% during the period of study. Adult lactating crossbred cows (85), 1/2 Holstein Friesian × 1/2 Hariana, with normal calving history and body weight of 360 ±15.0 kg and milk yield 12.0 ± 1.0 kg/day with healthy reproductive organs were selected for experiment. These were maintained in 1/4 roof covered sheds under loose housing system. All cows were fed with green berseem, straw and concentrate along with 24 hr drinking water supply. Animals were also given bath using shower twice daily beginning at 0900 and 1600 to 0800 hr and 1700 to 1900 hr.

The cows observed in oestrus were allocated randomly in 3 treatment groups which were kept in single shed. The control animals (n=30) were administered a placebo of 2.5 ml (0.9%) normal saline solution (NSS) im treatment 1 cows (n=25) received 10 mg (2.5 µl receptal) and treatment 2 cows (n=30) 20 µg (5.0 ml receptal) buserelin administered im at the time of standing oestrus. Treatments were given only at first oestrus but effect on conception was analyzed up to 3 subsequent inseminations. All cows were inseminated at observed oestrus using 0.5 ml frozen semen straws according to A.M.; P.M. rule. Semen from 1 sire was used and insemination was performed by 1 inseminator during the experiment. Pregnancy was confirmed by rectal palpation 50-60 days post insemination. Pregnancy rate to each insemination, total pregnancy rate and services/conception were calculated and compared between treatments by chi-square test.

RESULTS AND DISCUSSION

The pregnancy rates to first AI were 26.7% for control,

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Table 1. Effect of buserelin on pregnancy rate

Groups	Type of treatment	No. of animals	Pregnancy rate				Services per conception
			First AI	Second AI	Third AI	Overall	
Treatment 1	10 μg GnRH	25	7 (28.0%)	8 (32.0%)	2 (8.0%)	17 (68.0%)	1.70
Treatment 2	20 μg GnRH	30	12 (40.0%)	9 (30.0%)	3 (10.0%)	24 (80.0%)	1.50
Control	2.5 ml NSS	30	7 (26.66%)	5 (16.66%)	2 (6.66%)	14 (46.66%)	1.64

28.0% for treatment 1 and 40.0% for treatment 2 cows (Table 1). The differences were nonsignificant ($P<0.05$). The pregnancy rate to the second insemination was higher for both treatments 1 (32.0%) and 2 (30.0%) than control cows (16.7%) but the difference was nonsignificant. The same trend for treatment differences was observed also for the third AI. The cumulative pregnancy rates for all the 3 services were 46.7%, 68.0% and 80.0% in control, treatments 1 and 2 group respectively. The difference between control and treatment 2 was significant ($P< 0.05$). Services per conception were different nonsignificantly between treatments (Table 1).

GnRH treatment improves fertility in dairy cows. In the study, this improvement was observed in both treatment groups but this was more pronounced in treatment 2 cows administered with the higher (20μg) dose of buserelin. The pregnancy rate increase may be due to the prevention of ovulation failure by GnRH (Zemjanis 1980, Karg *et al.* 1980). Previous workers also reported an increase of 9 to 14% in first AI conception rate following GnRH treatment (Moller and Fielden 1981, Vermerzani *et al.* 1997). Drew and Peters (1994) also observed nonsignificant effects of buserelin on fertility over control when administered on the day of insemination. The increase in pregnancy rate to first service observed for treatment 2 (16.7%) over that of control was similar to the reports of Nakao *et al.* (1983) and Aboul Ela and EL-Keraby (1986). However, Anderson and Malmo (1981) reported little effect on pregnancy rate when 250 μg synthetic GnRH was administered at the time of insemination. The pregnancy rate to the second insemination was almost similar for both treatment groups but neither was significantly higher than from the control group.

The overall pregnancy rate was also higher for both treatment groups than control by 21.3% for treatment 1 and 33.3% for treatment 2. The higher dose of GnRH may have enhanced LH secretion within 2 to 4 hr of administration in peripheral circulation which in turn increased ovulation rate (Chenault *et al.* 1990). The higher pregnancy rate observed following GnRH treatment could also be because of either changes in the cellular composition of the corpus luteum caused by GnRH induced LH release (Alila and Hansel 1984), or to better corpus luteum development during the first cycle which results in better growth and development of ovulatory follicles in subsequent cycle by endogenous GnRH secretion (Matton *et al.* 1981).

In conclusion, GnRH treatment at first oestrus improved conception rate at each of the first to third insemination as well as the overall pregnancy rate. Administration of 20 μg GnRH was more effective than 10 μg.

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Hypophyseal and gonadal response to $PGF_2\alpha$ and GnRH at onset of puberty in Murrah buffalo heifers (*Bubalus bubalis*)

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ABSTRACT

The objective of the present investigation was to study the releasing pattern of FSH, LH, prolactin, estradiol and progesterone at onset of puberty in Murrah buffalo heifers. Twelve Murrah buffalo heifers included in experiment had not exhibited behavioural estrus but had follicles or luteal tissue, or inactive ovaries at 32 months of age. The heifers with follicular phase had significantly ($P<0.01$) higher concentration of plasma FSH (31.43 ± 3.25 ng/ml), prolactin (127.93 ± 7.36 ng/ml) and estradiol (57.04 ± 12.53 pg/ml) than the concentration of FSH (14.63 ± 1.39 ng/ml and 9.93 ± 1.54ng/ml), prolactin (74.08 ± 1.46 ng/ml and 89.70 ± 8.36 ng/ml) and estradiol (23.67 ± 0.81 pg/ml and 23.70 ± 1.67 pg/ml) in heifers having, respectively, luteal phase and inactive ovaries. The progesterone concentration was higher in heifers having luteal phase (2.37 ± 0.23 ng/ml) than the heifers of follicular phase (0.49 ± 0.10 ng/ml) and having inactive ovaries (0.33 ± 0.07 ng/ml). However, plasma LH concentration in heifers having follicles (0.56 ± 0.04 ng/ml), corpus luteum (0.72 ± 0.05 ng/ml) and inactive ovary (0.67 ± 0.19 ng/ml) were similar. $PGF_2\alpha$ caused luteolysis with subsequent follicular growth, elevation of estradiol, FSH and prolactin release. Administration of GnRH in heifers having inactive ovaries induced follicular growth, increased estradiol, FSH and prolactin concentrations with subsequent ovulation and corpus luteum formation.

Key words: Buffalo heifers, GnRH, Hormones, $PGF_2\alpha$, Reproduction

Few information available on the effect of hypothalamic hormone on hypophyseal hormone release with subsequent alteration of gonadal activities towards the onset of puberty in Murrah buffalo heifers (Singh and Madan 1998, 1998a) are not enough to ascertain the relationship of hypophyseal and gonadal system at peripubertal period. An information of such relationship is required to understand the endocrine mechanism involved during peripubertal period in Murrah buffaloes. An investigation on temporal relationship of hypothalamo-hypophyseal and gonadal system at onset of puberty in Murrah buffalo heifer is therefore, very much required keeping in view the specific role of hypophyseal and gonadal hormone at onset of puberty in farm animals (Hafez 1987).

MATERIALS AND METHODS

Murrah buffalo heifers (12) (*Bubalus bubalis*) 30 months-old were selected from institute's herd. All animals were observed for exhibition of behavioural estrus symptom between 30 and 32 months of age. Each heifer was injected intramuscularly with $PGF_2\alpha$ (lutalyse, 25 mg) on the day they attained the age of 32 months (designated as day 0). Synthetic GnRH (fertagyl, 200 µg) was injected intravenously on day 10 after $PGF_2\alpha$ administration. Both $PGF_2\alpha$ and GnRH were administered on respective days between 7.00 AM and 9.00 AM. All the animals were maintained under standard management and nutritional condition (ambient temperature was ranging from 22 to 36°C). Genitalia were examined per rectum and a potent teaser bull with trained attendants was used at 6 hr daily to detect the estrus. Blood samples from each animal were collected through indwelling jugular canulae on day - 2, 0 (just before $PGF_2\alpha$ injection), 2, 4, 5, 10, 11, 13, 15, 19, 23 and 28 of $PGF_2\alpha$ in chilled heparinized test-tubes. Plasma separated was divided in different aliquots and stored at –20°C for estimation of estradiol, progesterone, FSH, LH and prolactin. Estradiol, progesterone, FSH, LH (Singh and Madan 1998) and prolactin (Singh and Madan 1998ab) were estimated by radioimmunoassay.

The pair 'T' test between the groups and 2-way analysis of variance was done to compare the difference in hormone concentration between groups and between samples (Snedecor and Cochran 1967).

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RESULTS AND DISCUSSION

None of the heifers studied had exhibited behavioural estrus symptoms by 32 months of age. Moreover, 4 heifers (R_1, R_8, R_9 and R_{10}) were having palpable follicles and plasma estradiol, progesterone, FSH, LH and prolactin in these 4 heifers were ranging from 32.52 pg/ml to 86.15 pg/ml, 0.28 ng/ml to 0.75 ng/ml, 24.08 ng/ml to 39.81 ng/ml, 0.50 ng/ml to 0.66 ng/ml and 112.41 ng/ml to 147.31 ng/ml respectively. In another 4 heifers (R_2, R_3, R_4 and R_{11}) the plasma progesterone concentration was more than 1 ng/ml (1.84 ng/ml to 2.93 ng/ml) although corpus luteum on the ovaries could not be palpated in those heifers. The plasma estradiol, FSH, and prolactin concentration were lower ($P<0.01$) than the heifers of follicular phase. A significant ($P<0.01$) decrease in progesterone concentration by 48 hr after $PGF_2\alpha$ injection was detected. This was followed with elevation in plasma estradiol, FSH and prolactin associated with folliculogenesis and ovulation. The ovaries of remaining 4 heifers (R_5, R_6 R_7 and R_{12}) were neither having follicles not corpus luteum. GnRH administration induced follicular growth, elevation of FSH, estradiol and prolactin within 7 to 9 day with subsequent ovulation and corpus luteum formation in 2 heifers (R_5 and R_6). The pre-treatment plasma estradiol progesterone, FSH, LH and prolactin concentrations of individual heifers have been presented in Table 1.

The plasma estradiol, FSH and prolactin concentrations were significantly ($P<0.01$) higher in 4 heifers of follicular phase than the heifers of luteal phase and inactive ovaries. Similarly, the pretreatment plasma progesterone concentration was significantly ($P<0.01$) higher in the heifers having corpus luteum than the heifers having follicles and inactive ovary. The plasma LH concentration was maintained at similar level in all 3 groups of heifers throughout the experiment.

The distinct follicle with elevated levels of estradiol, FSH and prolactin in 4 heifers (R_1, R_8, R_9 and R_{10}) while more than 1 ng/ml progesterone associated with lower level of estradiol, FSH and prolactin in another 4 heifers (R_2, R_3, R_4 and R_{11}) revealed that the hypothalamo-hypophyseal and gonadal axis had been established at onset of puberty in these heifers is similar to the cycling animals (Haferz 1987, Schoenemann *et al.* 1985).

Secretion of progesterone more than 1 ng/ml in 4 heifers (R_2, R_3, R_4 and R_{11}) without having palpable corpus luteum in 2 heifers (R_3 and R_4) agreed with the report of elevated levels of progesterone without having palpable corpus luteum at onset of puberty in beef heifers (Glencross 1984). The detection of LH at similar concentration in all heifers having different ovarian status was due to the fact that collection of single blood sample daily was not sufficient to detect LH surge, as preovulatory LH surge occurs only during estrus for 6 to 10 hr (Hafez 1987, Swenson and Reece 1996).

The detection of plasma progesterone concentration more than 1 ng/ml on day 0 in 4 heifers followed with its decline to below 1 ng/ml by 48 hr after $PGF_2\alpha$ injection suggests that these heifers were having functional corpus luteum in their ovary as $PGF_2\alpha$ is a potent luteolytic agent in sexually mature cows (Lebedev *et al.* 1980) and buffaloes (Kamonpotana *et al.* 1979). The lower concentration of plasma FSH in luteal phase heifers during progesterone dominance might be due to the negative feedback effect of progesterone on GnRH and FSH release, respectively, from hypothalamus and pituitary

Table 1. Hypophyseal and gonadal hormone concentration in Murrah buffalo heifers receiving $PGF_2\alpha$ and GnRH administration during follicular, luteal and non-cycling phases

Animal No.	Estradiol (Pg/ml)	Progesterone (ng/ml)	FSH (ng/ml)	LH (ng/ml)	Prolactin (ng/ml)
			Follicular phase		
R_1	40.20	0.75	39.81	0.56	122.41
R_8	32.52	0.46	24.08	0.51	112.41
R_9	69.32	0.46	29.96	0.66	129.57
R_{10}	86.15	0.28	31.85	0.50	147.31
Mean ± SE	57.05±12.53	0.49±0.10	31.45±5.25	0.56±0.04	127.93±7.36
			Luteal phase		
R_2	23.10	2.51	12.08	0.78	73.97
R_3	21.81	1.84	18.15	0.80	77.36
R_4	25.65	2.93	12.75	0.74	74.71
R_{11}	24.11	2.21	15.53	0.56	70.28
Mean ± SE	23.67±0.81	2.37±0.23	14.63±1.39	0.72±0.05	74.08±1.46
			Non-cycling phase		
R_5	22.51	0.46	5.37	0.47	102.75
R_6	28.36	0.36	11.00	1.23	105.46
R_7	20.50	0.35	11.17	0.52	76.92
R_{12}	23.44	0.13	12.19	0.46	73.67
Mean ± SE	23.70±1.67	0.33±0.07	9.93±1.54	0.67±0.19	89.70±8.36

gland similar to the suppression of GnRH and FSH release in sexually matured farm animals (Hafez 1987). The negative effect of plasma progesterone might have been removed following decrease in progesterone concentration after $PGF_2\alpha$ injection and resulted thereafter, in spontaneous FSH secretion similar to the established feedback mechanism in farm animals (Hafez 1987, Mc Donald 1989). The detection of sustained elevated levels of estradiol, FSH and prolactin with presence of growing follicles at onset of puberty suggests that the estradiol at this stage might have not reached at threshold concentration for stimulation of LH surge. That caused prolongation of follicular phase without exhibition of behavioural estrus at onset of puberty in Murrah buffaloes.

The alteration in plasma estradiol followed with the similar coincident increase or decrease in prolactin concentration in the heifers of present experiment suggests that elevated level of estradiol might have stimulated prolactin release in these heifers similar to the stimulation of prolactin secretion by estradiol in normally cycling animals (Swenson and Reece 1996, Hafez 1987, Me Donald 1989). Hypophyseal and gonadal hormone recorded during pre- and post-$PGF_2\alpha$ treatment in the heifers having ovarian follicles and / or corpus luteum was identical to the relationship of these hormones in sexually matured cattle, sheep and goat (Hafez 1987), pig (Brinkley 1981), swamp buffaloes (Kanai and Simizu 1984), and Murrah buffaloes (Razdam *et al.* 1982).

The elevation of FSH and estradiol on day 7 and day 10 after GnRH injection with subsequent ovulation, corpus luteum formation and progesterone secretion in 2 heifers (R_5 and R_6) having inactive ovaries was similar to the response of GnRH reported in 24-month- old Murrah buffalo heifers (Singh and Madan 1998a).

The observations suggested that hypothalamo-hypophyseal and gonadal system of 32-month- old Murrah buffalo heifers are fully matured with developed receptors at hypothalamo-hypophyseal level for feedback effect of gonadal hormones. Estradiol during follicular phase stimulates prolactin release in Murrah buffalo heifers. The Murrah buffalo heifers at this age achieve puberty and have potential for folliculogenesis, ovulation and functional corpus luteum formation for full cycle length.

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Seroprevalence of chlamydiosis among cows and buffaloes in Himachal Pradesh

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Key words: Buffaloes, *Chlamydia psittaci*, Cows, Seroprevalence

The insidious disease chlamydiosis has been reported globally from several livestock species as well as wild fauna and is caused by *Chlamydia psittaci*. It induces variety of disease syndromes depending upon the susceptibility of host species and virulence of the chlamydial strain. From this region, the seroprevalence of chlamydiosis has earlier been reported mostly from sheep and goats (Hazarika and Dhingra 1985, Mahapatra *et al.* 1987, Krishna *et al.* 1988, Batta *et al.* 1996). However, reports on seroprevalence among cows and buffaloes are scare (Sharma and Baxi 1983, Dhingra *et al.*1986, Joshi *et al.* 1997). This study was conceived to screen cows and buffaloes of the area by employing agar gel precipitation (AGPT), complement fixation (CFT) and elementary body agglutination tests (EBAT).

In this investigation altogether 216 serum samples emanating from apparently healthy 176 cows and 40 buffaloes accrued from both organized farms (120 cows and 12 buffaloes) and unorganized sector (cows 56 and buffaloes 28) were seroscreened for the presence of antibodies against *C. psittaci* employing a battery of serological tools, viz. agar gel precipitation test (AGPT), complement fixation test (CFT) and elementary body agglutination test (EBAT). The collected serum samples were stored at —20°C until processed. The method of Page (1975) with minor modifications in centrifugation steps, was followed for the preparation of precipitating and complement fixing antigen by propagating a locally isolated abortion strain of *C. psittaci*, The 6-8-day-old embryonated chicken eggs were inoculated via intrayolk sac route. The AGPT was performed according to Ouchterlony (1968). Complement fixation test in microtitre plates was carried out as per Kolmer (Alton *et al.* 1975), while elementary body agglutination test (EBAT) was performed following the method of Grimes *et al.* (1994).

The species-wise seroprevalence discerned by different serodiagnostic tests is depicted in the Table 1. The perusal of the Table 1 revealed that buffaloes and cows of this region are seropositive for chlamydiosis reflecting the overall percentage positivity up to the tune of 17.50 and 14.20 by AGPT, 18.34 and 21.62 by CFT and 12.50 and 10.23 by EBAT, respectively. Therefore, the disease was more prevalent among buffaloes than cows.

In fact, the isolation of *C. psittaci*, is a foolproof method of diagnosing chlamydiosis, but it is quite cumbersome, expensive and time consuming due to highly fastidious nature of the organism. The investigations of Page *et al.* (1974), Meyer-Ropke *et al.* (1977) and Grimes (1985) have also reported CFT, AGPT and EBAT as inexpensive simple, sensitive and specific serological tests. In all these tests group specific lipopolysaccharide antigen of chlamydiae is used. In present investigation, CFT was adjudged more sensitive

Table 1. Seroprevalence of *C.psittaci* among different species by employing AGPT, CFT and EBAT

Species	Sera collected	Sera tested in CFT	Percentage sero positive by		
			AGPT	CFT	EBAT
Cows	176	169	26 (14.77)	31 (18.34)	18 (10.23)
Buffaloes	40	37	7 (17.50)	8 (21.62)	5 (12.50)
Total	216	206	33 (15.27)	39 (18.93)	23 (10.64)

All the serum samples (176 cows and 40 buffaloes) were tested by AGPT and EBAT; *titre of 1:16 and above was taken as positive; **haemolytic samples were discarded.

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followed by AGPT and EBAT. Such observations were also documented by Anonymous (1995) while comparing fidelity of these tests in sheep and goats.

SUMMARY

The chlamydiosis, caused by *Chlamydia psittaci* has global distribution and manifested as variety of disease syndromes among many livestock species as well as from wild fauna. In this investigation, altogether 216 serum samples emanating from 176 cows and 40 buffaloes accrued from both organized farms and unorganized sector were seroscreened for the presence of antibodies against *C. psittaci* employing serological tests, viz. agar gel precipitation test (AGPT), complement fixation test (CFT) and elementary body agglutination test (EBAT). This study reflected overall percentage positivity of 14.77 and 17.50 by AGPT, 18.34 and 21.62 by CFT and 10.23 and 12.50 by EBAT, among cows and buffaloes respectively.

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Efficacy of dot- ELISA and Biken test for the detection of heat-labile enterotoxin (LT) of *Escherichia coli**

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Enterotoxigenic *Escherichia coli* (ETEC) is the most common etiological agent associated with neonatal diarrhoea. ETEC produces one or both of the two types of enterotoxin, i.e. heat - labile (LT) and heat- stable (ST) (Holmgren 1985). LT enterotoxin of *E. coli* induces diarrhoea by altering intestinal transport mechanism without causing any significant alteration of mucosal histology. Enterotoxic activity of *E. coli* has been demonstrated by using a variety of biological as well as immunological test systems such as rabbit-ligated illeal loops (RLIL), rabbit skin permeability test, Chinese hamster ovary (CHO) cell assay, staphylococcal coagglutination (CoA) test, Biken test, ELISA, GM1- ELISA (De and Chatterjee 1953, Vadivelue *et al.* 1987, Goswami *et al.* 1995). In the present study, dot - ELISA and Biken test were used and compared for detection of heat -labile (LT) enterotoxin of *E.coli* isolated diarrhoeic piglets.

Bacterial strains

Strains (89) of *E. coli* isolated from cases of piglet diarrhoea were included in the study (Table 1). A reference enterotoxigenic *E. coli* (LT) strain was used for production of LT enterotoxin and a known non-enterotoxigenic *E.coli* strain (C-600) was used as negative control. The strains were maintained on nutrient agar slants before use.

Enterotoxin preparation

Cell- free culture supernatant (CFCS) was prepared from each *E. coli* strain (Singh *et al.* 1983). The organism was grown in brain heart infusion (BHI) broth on a rotary shaker (160 rpm) for 18 hr at 37°C. The broth culture was centrifuged (10 000 g at 4°C for 30 min), supernatant was collected and membrane filtered (0.22 mm). The CFCS was stored at 4°C for further use.

Partial purification of enterotoxin and antiserum preparation

The CFCS prepared from the reference strain was partially purified by successive ammonium sulphate precipitation (60% saturation level) and dialysis (Rahman *et al.* 1994). Protein concentration of the partially purified toxin was determined (Lowry *et al.* 1951) and was tested for enterotoxic activity in rabbit ligated illeal loops (De and Chatterjee 1953). The antiserum to partially purified enterotoxin was raised in rabbits

Table 1. Enterotoxigenicity (LT toxin) of different serogroups of *E. coli* as detected by immunological tests

Serotype	Number of isolates tested	No of isolates positive with	
		Biken test	Dot-ELISA
O20	12	5	7
O8	9	5	9
O9	9	4	9
O137	6	3	4
O45	5	2	5
O95	5	-	5
O39	4	1	4
O4	4	2	3
O21	4	1	3
O22	4	2	3
O154	4	2	3
O90	4	-	-
O25	2	2	2
O107	2	2	2
O24	1	1	1
O50	1	1	1
O55	1	1	1
O55	1	1	1
O76	1	1	1
O81	1	-	1
O84	1	1	1
O86	1	1	1
O99	1	-	1
O61	1	1	1
Rough	2	-	1
Untypable	4	1	3
Total	89	39	72

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according to procedure described by Rahman *et al.* (1994). Serum was absorbed on to the whole cell of reference strain of *E.coli* (LT +) to remove the nonspecific antibodies and specificity of the antiserum was determined by serum neutralization test (Jiwa 1981).

Dot- ELISA

Dot- ELISA was standardized for detection of enterotoxin (LT) of *E. coli* as described by Rahman (1999) with minor modification. Two ml CFCS of each isolate of *E. coli* was dotted on nitrocellulose (NC) membrane strips and dried at 37°C for1 hr. The unsaturated sites were blocked by immersing the strips in 1% solution of skim milk powder in PBS (0.01 M, pH 7.2) for 1hr at 37°C. The strips were washed 3 times in PBS-T (0.01M PBS, pH 7.2 with 0.5% tween 20) for 5 min. The strips were dipped in the antiserum raised against LT enterotoxin in rabbits, diluted 1:500 (predetermined) in Tris buffer (0.05M of Tris buffer, pH 7.2) and incubated at 37°C for 1hr. After incubation, the strips were washed thrice in PBS-T and again incubated with anti-rabbit IgG horse radish peroxidase (HRPO) conjugate at a dilution of 1: 1 000 for I hr at 37°C. Finally the strips were washed thrice in PBS-T and immersed in freshly prepared substrate solution (1 chloro-4 napthol) containing 1 ml / ml of 30% H_2O_2. Washing the strips in running tap water stopped the enzymatic reaction and a positive reaction was indicated by the appearance of deep purple dot against a white background within 10 min.

The Biken test was performed as described by Honda *et al.* (1981). Two test cultures and two control strains, one positive and one negative, were spot inoculated in to Biken agar plate. After incubation at 37°C for 48 hr a disc containing 500 IU polymyxin -B, was placed on each colony and a well was punched in the center at equal distance from the colonies. The plates were further incubated at 37°C for 6 hr and 100 ml of antiserum was added to the well. The plates were incubated at 37°C and read for line of precipitation occurring between colony and well after 24 hr.

The CFCS of the reference *E. coli* strain induced marked fluid accumulation in the RLIL test with a dilatation index of 0.74. The partially purified LT enterotoxin was highly immunogenic in rabbits. The antiserum raised in rabbits produces marked precipitation line when tested by AGPT test and the antiserum completely neutralized the LT toxin of *E. coli* in RLIL. The antiserum thus prepared was used for standardization and screening of heat- labile enterotoxin of *E.coli* by dot- ELISA and Biken test (Table 1). Of the 89 isolates tested 72 (80.89%) were positive for LT toxin production by dot- ELISA. These isolates belonged to 22 different O groups, 3 untypable and 1 rough strain. Organism giving faint to a marked precipitation line in Biken agar was considered positive for LT production. Of the 89 isolate tested, 39 isolates (42.82%) belonging to 19 different serogroups and 1 untypable isolate were positive for LT toxin. In comparison to the Biken test, dot- ELISA was more sensitive for detection of LT enterotoxin of *E.coli*. All the strains (39) that were positive in the Biken test were also found positive for LT production by dot- ELISA. However, 33 strains were positive in dot-ELISA but were negative in Biken test. Various bioassay models like RLIL, rabbit skin permeability factor, mouse paw edema and suckling mouse intragastric test have been described for detection of *E. coli* enterotoxin, however, in terms of sensitivity and specificity, these tests are not comparable with the newer immunological tests like staphylococcal co- agglutination test, Biken test and dot-ELISA (Rahman *et al.* 1991, Goswami *et al.* 1994). In this study, 2 immunological tests, Biken test and dot-ELISA were performed for detection of heat - labile (LT) enterotoxin production by *E.coli* strains isolated from diarrhoeic and dead piglets. Biken test is a modified form of immunodiffusion test, which is easy to perform and does not require any special equipment. Similarly dot- ELISA, is a highly sensitive and simple test. In this present study, dot-ELISA was more sensitive than the Biken test.

Although Biken test has extensively been used to detect enterotoxigenicity of *E. coli* strains (Honda *et al.* 1981), however, further studies on comparative efficacy of different *in vitro* and *in vivo* tests for detection of LT toxin of *E. coli* is necessary for drawing a final conclusion. Biken test is very simple and convenient to carry out in ordinary microbiological laboratory, however, it requires a large amount of antiserum and takes 3 - 4 days to obtain the results, which may limit its application as a routine test. Although information on the comparative efficacy of these 2 tests for detection of LT toxin of *E coli*, is very meager, some researchers have found ELISA and dot - ELISA to be highly sensitive for detection of both *E. coli* and *Salmonella* enterotoxin (Osek 1996, Saxena and Sharma 1987, Rahman *et al.* 1991, Rahman 1999). The colour developed in dot - ELISA could be clearly read by visual examination and did not fade. The NC paper strips could be stored as a permanent record for future reference.

In this study, dot- ELISA was more sensitive than the Biken test for detection of LT enterotoxin production by *E. coli*. The test could detect LT production by as high as 80.89% strains of *E. coli*. Therefore, dot-ELISA may be preferred over Biken test for assaying *E.coli* LT enterotoxin, as the test is sensitive, rapid and simple to perform.

SUMMARY

Dot-ELISA and Biken test were standardized and performed for detection of heat- labile (LT) enterotoxin production by 89 strains of *Escherichia coli* isolated from 85 cases of piglet diarrhoea. Biken test revealed 39 strains (43.83%) as positive for LT enterotoxin whereas dot- ELISA could detect LT production by 72 (80.89%) strains.

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Economics of mastitis

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Key words: Mastitis, Treatment, Economics, Dairy cattle

Mastitis alone causes economic losses to Indian dairy industry to the extent of Rs 1 940 crores annually (Manickam 1998). The economic losses sustained by dairymen because of mastitis has been identified as reduced milk production, discard of milk, decreased market value of animal, cost of veterinary services and medicines, and increased labour to care infected animal. Dhanda and Sethi (1962), Nielson (1976) and Sharma *et al.* (1987) reported enormous economic losses due to mastitis in India and abroad. Many studies have been undertaken on clinical, preventive and microbiological aspects of mastitis. However, the studies on economics involved in treatment of this diseases in India are scanty. Hence, this study was undertaken to ascertain the cost of treatment and the economic losses due to mastitis.

The procedure followed by Sharma *et al.* (1987) was adopted with little modifications. Daily milk yield records of 30 affected crossbred cows treated with mastitis in an organized herd in Hyderabad were studied. Depending upon the type of infection, number of quarters affected and availability of drugs, they were treated using antibiotics alone or by combination of drugs. To keep the variation in milk yield at a minimum level because of various factors affecting lactation curve, 15 days milk yield before and after the treatment were obtained from the records. The total expenditure incurred on medicines and chemical equipment during treatment period of all the 30 cows was calculated. The services rendered by veterinary staff were calculated in terms of money based on the average time spent by them on each cow. On an average a veterinary doctor spends 5 min for examining and suggesting the treatment, a compounder takes about 5 min to administer the medicine and an attendant needs 10 min for restraining the animal during examination and treatment. The loss of milk during treatment period was calculated by the difference between the average milk potential of each cow before and after treatment and it was multiplied by Rs 12 (price of cow milk/kg) to arrive at the value of milk loss due to mastitis.

Number of days taken on an average to treat the animal was 6.25 (Table 1), which is lower than that observed by Sharma *et al.* (1987). Average milk yield dropped from 12.56 to 9.82 kg/day during treatment period amounting to a drop of 2.74 kg/day. This is slightly more than the observations reported by Kuzmina (1978) and Sharma *et al.* (1987). On recovery, the affected cows milk yield increased from 8.90 to 11.02 kg/day amounting to 12.26% lesser than the milk yield before treatment. This finding is in agreement with those observed by Steen (1978) who recorded 12.85% less milk yield and slightly more than those observed by Sharma *et al.* (1987) who reported 10% less milk yield. During treatment period, the total loss of milk amounted to 8.90 kg , which is

Table 1. Economics of mastitis treatment

Parameters	Range	Mean
Treatment period (days)	2-21	6.25
Average milk yield of cows/day before treatment (kg)	2.80-28.90	12.56
Average milk yield of cows/day before after treatment (kg)	1.67-19.05	11.02
Average milk yield of cows/day during treatment (kg)	1.90-14.80	9.82
Total loss of milk during treatment period (kg)	0-27.5	8.90
Total loss of milk in terms of money during treatment period (Rs)	12.00-330.00	106.8
Expenditure on medicines/cow (Rs)	10.97-282.85	90.85

Economics of veterinary measures

Item	Cost/cow (Rs)	Total cost for 30 cows (Rs)	% cost
Medicines	90.85	2725.50	41.50
Equipment and chemicals	5.90	177.00	2.71
Sub total	96.75	2902.50	44.21
Salaries of veterinary staff	122.09	3662.72	55.71
Total	218.84	6565.22	100.00

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slightly lower than that reported by Sharma *et al.* (1987). The expenditure on medicines for treating mastitis ranged from a minimum of Rs 10.97 to a maximum of Rs 282.85 with an average of Rs 90.85. On an average the total cost of treating mastitis per cow is Rs 218.84, which included the cost of medicines (41.50%), chemicals (2.71%) and salaries of veterinary staff (55.79%). Sharma *et al.* (1987) reported that cost of medicines, equipment and chemicals used and salaries were 35.34%, 2.18% and 62.48% respectively. The loss of milk in terms of rupees was Rs 106.8, thereby, total monetary loss for each affected cow on an average was Rs 325.64 which includes the treatment and loss of milk cost. Noorlander *et al.* (1965) observed that loss of production due to mastitis per cow was about 15 dollars, but net treating amount to 60 to 100 dollars per year because of loss in production in addition to lowering productive life of the cow. Sharma *et al.* (1987) reported that loss of production was Rs 170.40/cow.

SUMMARY

In a mastitis affected cow, on an average it took about 6 days for treatment with the cost of treating per cow as Rs 218.84 which includes the cost of medicines (41.50%), chemicals (2.71%) and salaries of veterinary staff (55.79%). The total loss of milk yield on an average accounted for 8.90 kg/day during treatment period, and 12.26% lesser milk yield after treatment as compared to before treatment was observed. The loss of milk in terms of money was Rs 106.80 thereby total monetary loss for each affected cow on an average was Rs 325.64 which includes the cost of treatment and loss of milk.

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Use of fresh and preserved bovine amnion grafts in urinary bladder reconstruction of goats (*Capra hircus*)

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In the present study an attempt was made to clinically evaluate the success of reconstruction of urinary bladder in goats using fresh and preserved amnion for its suitability. The present study was conducted in 18 nondescript healthy female goats of 8 to 15 months old and weighing between 15 to 20 kg. The animals were randomly divided into 3 groups of 6 animals each and in all the animals subtotal cystectomy was performed. Urinary bladder reconstruction was then done using fresh amnion (FA) graft in group 1, frozen and boiled in 70% alcohol (FrA) amnion graft in group 2, and 1% glutaraldehyde PBS treated and heat dried (GTA) graft in group 3.

Preparation of graft

Bovine amnion collected from a healthy and normal parturated cow was stored at 4°C in sterile normal saline solution and used within 72 hr of collection in group 1. The amnion graft for group 2 was frozen at - 4° C for 30 days and then preserved in 94% glycerol at room temperature. Ten to fifteen min before grafting the amnion was washed thoroughly with normal saline and boiled in 70% alcohol for 3 min. The amnion graft for group 3 was treated with 1% glutaraldehyde phosphate buffer saline (pH 7.4) for 24 hr at 4°C and then kept in 70% isopropyl alcohol at 4°C. Finally 3 layers of amnion were taken on the stent to mold them as dome. It was dry heated at 70°C for 15 min and stored as dry state in sterile packs or in 70% alcohol at room temperature.

The animals were kept off feed and water 24 and 12 hr respectively. They were first tranquilized with triflupromazine hydrochloride at 0.15 mg/kg IM and then under local infiltration analgesia the urinary bladder was approached through a 10 cm midline incision given caudal to umbilicus. One-third of the dome of the bladder was resected out after securing it with Doyen's intestinal clamp. The resected bladder was then reconstructed using respective amnion grafts by double layer of silk no 3/0 using Connell's sutures in first and Cushing's for the second layer. The skin incision was closed in routine manner. Postoperatively 1 g of streptopenicillin was given for 5 days and daily dressing was done until skin sutures were removed on day 10 postoperatively.

The animals were observed clinically for general condition, frequency of urination, efforts made while urination and for change in temperature, pulse and respiration rates at day, 1, 2, 3, 5, 7, 10, 15, 30 and 60 postoperatively. The urine was collected at day, 3, 7, 15, 30 and 60 postoperatively and examined for colour, blood, albumin, glucose, cellular and non-cellular casts. The data were analyzed with ANOVA using paired 't' test (Snedecor and Cochran 1973).

The amnion from 1 cow was sufficient to prepare 15 fresh amnion grafts and 6 to 8 preserved amnion grafts. FrA preservation caused shrinkage of amnion whereas GTA-preserved grafts did not cause shrinkage, and colour changed to yellowish brown. The double layer suturing was found essential for reconstruction of bladder wall. The FA grafts were fragile and needed careful suturing whereas preserved implants were strong enough to hold the sutures. Similar was the experience of Fishman *et al.* (1987). The strength and hardness of both types of grafts may be attributed to change of amino protein to aminoplastin (Chao *et al.* 1940) and denaturation of proteins causing fixation of amnion.

Clinical behaviour of animals of different groups was nearly same. Dullness and depression were seen for first few days as a result of surgical stress (Shiva Prakash 1990). However, animals started taking feed and water from the next day of operation. Rectal temperature, pulse and respiration rate recorded at different intervals are presented in Figs 1,2 and 3 respectively. Rectal temperature, pulse and respiration rate showed significant increase ($P<0.01$) up to day 15 in all the groups. The values gradually decreased then return to preoperative levels by day 30. The maximum increase was recorded at day 7 in all the 3 groups. However, significant difference ($P<0.01$) was not recorded between the 3 groups at different intervals.

Increased frequency of urination in all the groups was suggestive of reduced urinary bladder capacity and irritation caused by the presence of foreign graft, suture material and

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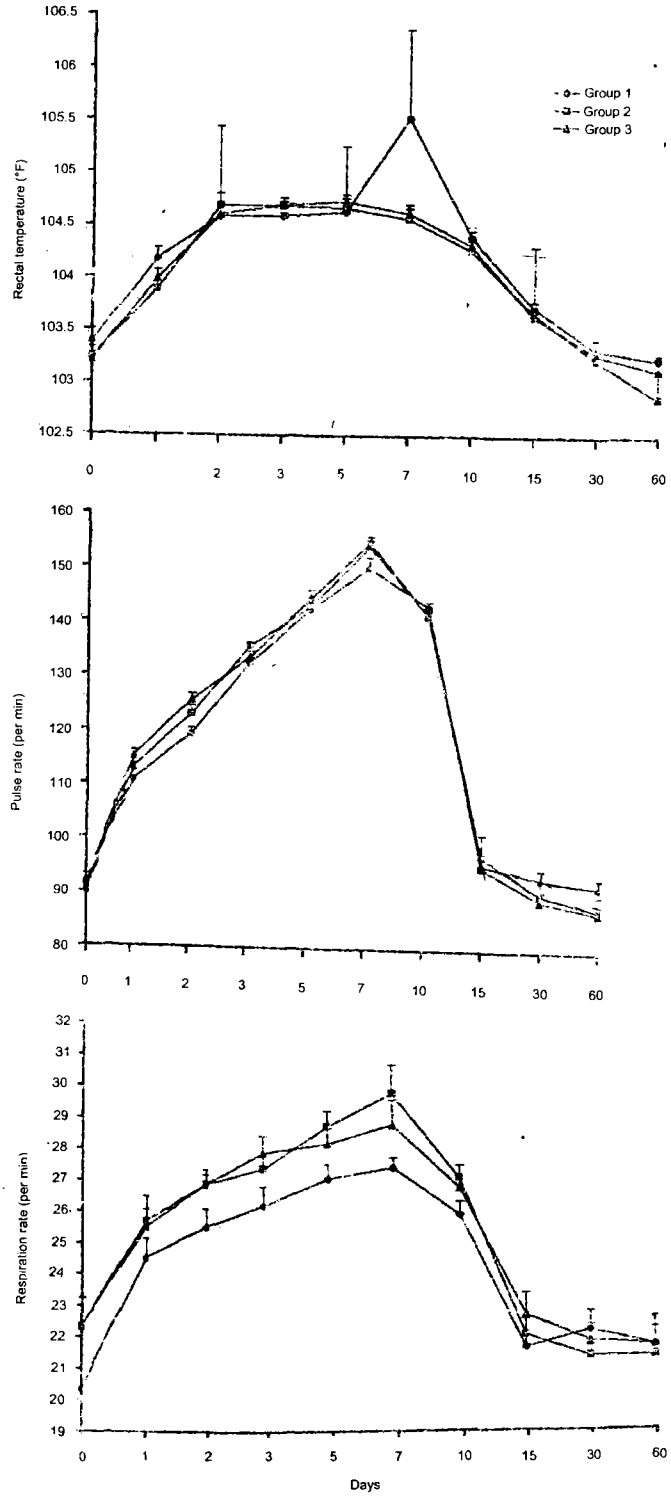

surgical manipulation. Urinary incontinuence was observed in 2 animals of group 1 only and needed catheterization for the first few days.

The animals of all the 3 groups passed blood tinged urine for 1-2 post-operative days, which gradually changed to yellowish by day 7. Blood tinged urine for first 2 post-operative days could be attributed to surgical trauma, resection and reconstruction of the urinary bladder.

Traces of glucose found in urine of all the animals up to day 3 postoperatively could be due to false positive reaction of streptomycin in the urine (Coles 1974) as discontinuation of streptopenicillin administration gave negative glucose. None of them had detectable albumin in their urine. Bladder epithelial cells and triple phosphate crystals were seen in the urine till day 60 postoperatively was a normal finding in ruminants as their urine is alkaline in nature (Coles 1974). Animals of group 3 at day 60 showed amnion strands in urine.

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Figs 1-3. **1.** Rectal temperature (°F) at different time intervals in groups 1, 2 and 3. **2.** Pulse rate (per min) at different intervals in groups 1, 2 and 3. **3.** Respiration rate (per min) at different intervals in groups 1, 2 and 3.

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Effect of season and different FSH-P regimen on superovulation in crossbred cows

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The success of embryo transfer technique depends largely on the reliable method for the induction of superovulation to harvest maximum number of normal fertile ova or embryos. Although various advances have been achieved in superovulation technique, it still remains a weak link in the chain of events influencing the final outcome of embryo transfer in cattle. The number of ovulation occurring after a specific superovulatary treatment may vary because of individual variation, the hormones, the batch of hormones, the breed, season, nutrition and the method of determining ovulation rate (Walton and Stubbings 1986).

Thus the study of superovulatory response under Vidharbha agroclimatic conditions in winter and summer with FSH-P (folltropin-v) treatment in crossbred cows will be useful to bring about more specific understanding of this new front line biotechnology.

Crossbred cows (8) showing regular cyclic activity for 2 consecutive cycles and those with active corpus luteum were selected and divided into 4 groups. Group-A winter season (n=4) and group-B summer season (n=4), group-A again divided into 2 subgroups. Group1 (n=2) treated with folltropin-v with total dose of 400 mg at constant daily doses and group 2 (n=2) treated with folltropin-v with a total dose of 400 mg in tapering daily doses. Group-B summer season (n=4) also divided into 2 sub groups, group 3 (n=2) received superovulatory treatment with a total dose of 400 mg at constant daily doses, and group 4 (n=4) received superovulatory treatment with folltropin-v with a total dose of 400 mg in tapering daily doses. Synchronization of oestrus was done in all the groups by injecting prosolvin (PGF2α luprostiol 7.5 mg / ml).

Superovulatory treatment was started from ninth day of induced oestrus for 4 days at constant or tapering dose regimens. In the evening of third day, along with sixth dose of folltropin-v (FSH-P 20 mg/ml), injection lutalyse (PGF2α), 25 mg was administered intramuscularly for luteolysis. Artificial insemination with frozen semen was carried out on day of oestrus from 0 hr for 3 times at an interval of 12 hr using 2 doses each time. Flushing of embryos by Dulbecco's phosphate buffered saline (DPBS) was carried on day 7 after last insemination, and evaluation and gradation of embryos was done after collection of embryos.

Time interval from $PGF_2\alpha$ to onset of oestrus

Time required for onset of oestrus in winter for group 1 cows was 55.0 ± 3.00hr and group 2 cows it was 48.0 ± 12.03 hr. In summer the time required from PGF2α to onset of oestrus for group 3 cows was 38.0 ± 0.0 hr and for group 4 cows it was 30.0 ± 6.01 hr.

The time required for onset of oestrus in winter (51.5 ± 5.43 hr) was longer as compared to that in summer (34.0 ± 3.36 hr) with an overall aggregate average of 42.75 ± 4.45 hr for both the seasons. Present findings are similar to Pandit (1992), Pawshe *et al.* (1992), Kathiresan *et al.* (1997) and Khanna *et al.* (1997), while Looney *et al.* (1981) and Calder and Rajmahendran (1992) reported higher values.

The length of oestrus with superovulatory treatment in crossbred cows for group 1 cows was 28.0 ± 4.01 hr, and for group 2 cows, it was 27.5 ± 1.50 hr. The length of oestrus for group 3 cows was 33.1 ± 0.0 hr and for group 4 cows, it was 36.0 ± 2.00 hr. The length of oestrus in the winter was shorter (27.75 ± 1.75 hr) as compared to that in summer (34.5 ± 1.19 hr) with an overall aggregate average of 31.12 ± 1.61 hr for both the seasons. Present findings are similar to those of Pawshe *et al.* (1992) but Khanna *et al.* (1997) recorded higher values.

Superovulatory response

The number of corpora lutea and unovulatory follicles in winter for group 1 cows was 4.5 ± 2.50 and 1.0 ± 1.00, respectively, and for group 2 cows, it was 7.0 ± 2.0 and 1.0 ± 1.0, respectively. The number of corpora lutea and unovulatory follicles in the summer for the group 3 cows was 4.5 ± 2.50 and 1.0 ± 1.0, and for group 4 cows it was 8.0 ± 1.00 and 0.5 ± 0.5, respectively.

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In winter the number of corpora lutea (5.75 ± 1.49) was lower and unovulatory follicles (1.00 ± 0.57) were more than in summer (6.25 ± 1.59 and 0.75 ± 0.47, respectively), with an overall aggregate average of 6.00 ± 0.98 and 0.87 ± 0.35 corpora lutea and unovulatory follicles, respectively. Present findings are similar to those of Totey *et al.* (1991), Singla and Madan (1990), Chauvan *et al.* (1994b) and not in agreement with Chauvan *et al.* (1994a) and Agrawal *et al.* (1993) who recorded higher corpora lutea / donor.

Total number of embryos and transferable embryos

Total number of embryos and transferable embryos in winter for the group 1 cows, were 2.0 ± 2.0 and 1.5 ± 1.5, respectively, and for group 2 cows these were 3.5 ± 2.5 and 2.0 ± 2.0, respectively. Total number of embryos and transferable embryos in summer for the group 3 cows were 1.5 ± 1.5 and 1.0 ± 1.0, respectively, and for group 4 cows these were 4.5 ± 4.5 and 3.5 ± 3.5, respectively.

Total number of embryos and transferable embryos were lower in the winter (2.75 ± 1.37 and 1.75 ± 1.03, respectively), than that in summer (3.00 ± 2.12 and 2.25 ± 1.05, respectively), with an overall aggregate average of 2.87 ± 1.17 and 2.0 ± 0.90 total embryos and transferable embryos per donor, respectively.

The present findings are similar to those of Totey *et al.* (1991) and Umashankar *et al.* (1998), and not in agreement with Singla and Madan (1990), Agrawal *et al.* (1993) and Chauvan *et al.* (1994a).

SUMMARY

In superovulatory treatment of crossbred cows lower oestrus response and longer duration for the onset of oestrus was observed in winter. Length of oestrus due to superovulatory treatment was shorter in winter as compared to that in summer. Higher number of corpora lutea and lower numbers of unovulatory follicles were observed in summer. The embryos recovery rate and transferable embryos was more in summer. Superovulatory response and yield of embryos with FSH-P in tapering doses for 4 days was superior than that with constant doses in both the seasons.

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Superovulatory response in Syrian golden hamsters (*Mesocricetus auratus*) for recovery of eggs

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Key words: Hamster, Superovulation, Gonadotrophin

Hamsters are unique laboratory animal species, the eggs (ova) of which lack a complete species-specificity, and thus permit the penetration of spermatozoa from many other mammalian species. Yanagimachi (1972) described the uniqueness of their vitelline surface and this ultimately led to the development of a heterologous *in vitro* fertilization (IVF) protocol by Yanagimachi *et al.* (1976). The test evolved was called "zona free hamster oocytes (ZFHO) penetration assay" and since then it has been used extensively for assessment of fertilizing capacity of spermatozoa from human as well as other livestock species (Yanagimachi 1981). Application of this test, however, requires regular supply of large number of hamster eggs for generating data on the status of male fertility. A suitable protocol for augmenting the superovulatory response of this rodent species will be thus useful for its routine use in fertility assessment studies. The present investigation was carried out to determine the efficiency of a superovulation treatment regimen for Syrian golden hamsters for production of large number of hamster eggs.

Female Syrian golden hamsters (*Mesocricetus auratus*) were obtained from the CCSHAU, Hisar, and maintained at the Small Animal House of NDRI, Karnal. Animals were provided with high-energy feed and water *ad lib.* and kept under natural diurnal light cycle. For breeding, adult females (> 6 weeks age) were kept with a male in the ratio of 3:1 for 7 days and suspected pregnant animals were managed separately around the time of kidding. Pups born were kept with mother for 15 days before separating them. Pups were sexed around 25 days of age. Adult animals were injected intraperitonially with pregnant mares serum gonadotrophin (PMSG), followed by injection of human chorionic gonadotrophin (hCG) 98 hr later. PMSG and hCG were injected in 3 dose combinations. Group 1 received PMSG and hCG @ 50 IU each, animals in group 2 were injected with 50 IU PMSG and 60 IU hCG and those in group 3 received 60 IU PMSG and 75 IU hCG. PMSG treatment was started towards the end of natural day light cycle in the evening. Oocytes were collected from animals 17.5 hr after hCG treatment. After performing laparotomy oviducts were dissected out and collected in a culture dish containing mBWW medium supplemented with 0.3% bovine serum albumin (BSA). The cumulus mass containing oocytes was released by puncturing the oviducts at ampullar region under a stereo-zoom microscope, which were desegregated subsequently by a brief treatment with 0.1% hyaluronidase. The released oocytes were picked up in another dish and washed twice with mBWW with 0.3% BSA.

After the dose of gonadotrophin for superovulation was standardized, through the first phase of this experiment, the most effective dose i.e. 60 IU PMSG and 75 IU hCG was attempted on animals of 2 different age groups i.e., 2 to 4 weeks (group 1, 10 animals) and 4 to 8 weeks (group 2, 23 animals), to study the effect of age of the animals on oocyte recovery.

Data with respect to oocyte recovery for different treatment groups are presented in Table 1. Only 3 out of 13 animals treated with 50 IU each of PMSG and hCG responded to the superovulation treatment with an average yield of 6±3.4 oocytes. Many stimulated but unovulated follicles were observed on the surface of ovaries of these animals. However, animal treated with 50 IU of PMSG and 60 IU of hCG yielded on an average 19±7.5 oocytes and the recovery was further

Table 1. Effect of dose of gonadotrophins on oocytes recovery

Dose (IU) of gonadotrophin	No. of animals treated	No. of animal responded	Recovery rate/ animal
50 IU PMSG 50 IU hCG	13	3[a]	6±3.4[a]
50 IU PMSG 60 IU hCG	18	13[b]	19±7.5[b]
60 IU PMSG 75 IU hCG	27	27[c]	35±5.0[c]

Figures with different superscripts indicate significant difference. $P < 0.05$.

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improved with increase in the dose of gonadotrophin i.e. 60 IU of PMSG and 75 IU of hCG where average recovery was 35±5.0 oocytes/ animal.

Literature suggests that under the influence of gonadotrophins, few oocytes in resting stage enter growth phase that would result either in ovulation or atresia. Growth phase lasts for about 16 days and includes development of small follicles, larger pre-antral and the antral follicles (Jones 1978). In hamster, some follicles begin to grow each day during the 4 day estrus cycle. Only 10 to 12 of the largest follicles present on the first day of the cycle ovulate 4 days later. Follicles (5-6) in each ovary become vesicular, hyperemic and large during third and fourth day of estrus cycle. An equal number of large follicles become atretic. According to Greenwald (1978), use of exogenous hormones causes 3 different groups of oocytes to ovulate. In addition to those that would ovulate under normal conditions, those that would have undergone atresia and those not quite ready to ovulate until 4 days later also take part in ovulation process and thus, exogenous injection of gonadotrophins results in a large number of recoverable oocytes.

In this study, it was observed that the dose of gonadotrophins had a significant effect on the number of oocytes recovered by superovulation. It was in agreement with the findings of Church and Shea (1976), who established that ovulation rate increased with PMSG dose in cattle. Rudak (1981), however, opined that the quality of hormone is crucial and the failure of animal to ovulate is most frequently the result of using old and poor quality gonadotrophins. Similar effect was encountered during this experiment also. Differences of ovulation rate with different batches of gonadotrophin used might be explained through the well-documented variability in the FSH: LH ratio particularly in PMSG preparations (Stewart *et al.* 1996, Allen and Stewart 1978).

Based on the interpretation of the experiment on the effect of dose of gonadotrophins on superovulation response, the most effective dose i.e. 60/75 IU of PMSG/hCG was attempted on animals of 2 different age groups. In the first group, total 10 animals, 2 - 4-week-old, were treated and 80% of these animals responded, recovery rate per animal was 15±5.0 oocytes. In second group, however, 23 animals, 4 - 8 week old, were treated and all of them responded with an average recovery of 35±5 oocytes/ animal. Response in 4 to 8 weeks age group was significantly higher than the young stock. The sexual maturity of female is a major factor affecting the recovery of eggs following superovulation. In this investigation the hamsters of 2-4 weeks age group failed to respond optimally to superovulatory treatment. This was, however, not in agreement with Pryor (1986) who obtained 40 eggs/ animal from 25 - 30-day-old immature hamsters. Most of the investigators (Imai *et al.* 1979, Martin *et al.* 1988, Takahashi *et al.* 1989) have used only the adult female hamsters for superovulation. Probably by this stage of development, a wave of follicle growth is normally initiated that increases the number of follicles capable of responding to PMSG.

SUMMARY

The present study concluded that a combination of 60 IU PMSG/ 75 IU hCG was optimal for superovulation of hamsters, and animals of 4 - 8 weeks of age responded better to the superovulatory treatment than the younger animals.

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Effect of sub-acute oral administration of deltamethrin on certain haematological parameters in rats

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Key words: Deltamethrin, Haematology, Rats

Deltamethrin, a synthetic dibromo-pyrethroid insecticide is highly effective against a wide range of insects, and is extensively used on crops, in public health programmes for vector control and as an ectoparasiticide on animals (Kok *et al.* 1996, Moretti *et al.* 1997). Pyrethroids are easily biodegradable and relatively safer to mammals (Leahay 1985). However, they are not totally free from toxicity to non-target mammalian species. In the present study it was proposed to assess the toxicity of deltamethrin to haemopoietic organs by studying certain haematological parameters in rats.

Healthy young adult male Wistar rats, 5 to 6 weeks old, weighing from 100 to 140 g used in the study. They were identified uniquely and were kept in standard polypropylene rat cages for at least 10 days prior to the start of the study to allow for acclimatization to the laboratory conditions. They were maintained under standard laboratory conditions (NRC 1996). Feed and water were provided *ad lib.* Necessary approval of the Institutional Ethics Committee was obtained before starting the study. The animals were randomly divided into 4 groups consisting of 6 male rats in each group. The doses used were as follows: Group 1 (control group, administered vehicle), group 2 (low dose group, deltamethrin 3mg/kg), group 3 (mid dose group, deltamethrin 6mg/kg) and group 4 (high dose group, deltamethrin 12mg/kg). The dose levels were selected as per Gaitonde committee guidelines (1979) based on the LD_{50} value obtained for the insecticide in our laboratory in an earlier study (Shivakumar *et al.* 2000).

The animals in deltamethrin treatment groups were dosed once daily with deltamethrin for 28 days by giving a single dose using a stomach tube. General clinical observations were made at least once a day for any morbidity and mortality.

Haematological parameters were estimated using blood samples collected at the end of the feeding period. Disodium EDTA was used as the anticoagulant. The haematological parameters, namely, total erythrocyte count (TEC), haematocrit (Hct), haemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total leukocyte count (TLC) and DLC, were determined following standard methods (Jain 1986).

The data were expressed as mean ± SE. The test of significance was performed using Student's 't' test (Snedecor and Cochran 1967).

Clinical signs of hyperexcitability and salivation were noted in the high dose group. These were indicative of CNS involvement and form part of choreoathetosis salivation syndrome described for alpha-cyano pyrethroid compounds (Gammon *et al.* 1982).

The data on haematological parameters are presented in Table 1. None of the parameters related to RBC or WBC in the treated groups differed significantly ($P>0.05$) from those of control group. The observed nonsignificant effect on WBC, Hb and Hct in this study are in concurrence with the findings of Malone and Chester (1970), who reported that bioresmethrin at 1 000mg/kg for 14 days did not alter any of these haematological parameters. Hend and Butterworth (1976) reported no significant changes in RBC values in rats upon administration of cypermethrin at 1600mg/kg for 3 months. However, in the studies on bioresmethrin and cypermethrin the technical grade compounds were used, whereas, in this study an emulsifiable concentrate was used. Emulsifiable concentrates more toxic than the pure compounds (Shiva Kumar *et al.* 2000).

From the study it was concluded that deltamethrin emulsifiable formulation did not cause any adverse changes on the haematological parameters at the doses and duration used in the study.

SUMMARY

Deltamethrin was administered @ 3, 6, 12 mg/kg body weight daily orally for 28 days to male rats to study the effect of sub-acute oral administration of deltamethrin on certain haematological parameters. The blood samples were analyzed

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Table 1. Effect of deltamethrin on haematological parameters in rats in the 28 days oral toxicity study

Haematological parameter	Dose of deltamethrin			
	Group 1 (vehicle control)	Group 2 (3 mg/kg)	Group 3 (6 mg/kg)	Group 4 (12 mg/kg)
RBC (10^6/mm^3)	8.8±0.16	8.55±0.19	8.68±0.10	8.66±10.83
Hb (g/dl)	13.8±0.17	13.83±0.09	13.80±0.13	13.86±0.13
Hct (%)	43.16±1.00	41.33±0.07	43.41±0.40	43.10±0.54
MCV (μ^3)	48.64±1.10	48.50±1.44	49.39±0.60	49.79±1.13
MCH (%)	15.55±0.19	16.19±0.34	15.89±0.17	16.00±0.16
MCHC (pg)	32.04±0.81	33.45±0.51	31.79±0.34	32.20±0.53
WBC (10^3/mm^3)	9.58±0.28	9.23±0.26	9.48±0.29	9.43±0.23
Neutrophils (%)	18.00±0.73	17.83±0.65	18.00±1.03	17.50±0.71
Lymphocytes (%)	80.16±1.04	80.66±0.55	80.50±1.40	80.66±0.95
Monocytes (%)	1.66±0.61	1.16±0.47	1.50±0.61	11.83±0.65
Eosinophils (%)	0.16±0.16	0	0	0

Values are mean±SE; P>0.05; n=6.

at the end of the test period for estimating different haematological parameters (TEC, Hb, Hct, MCV, MCH, MCHC, TLC and DLC). None of the parameters differed significantly (P>0.05) from the control group indicating lack of adverse effect on the haemopoietic organs at the doses.

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Blood urea concentration in crossbred calves*

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The blood urea concentration in crossbred female calves was studied to investigate the base line concentration of blood urea in newly evolved crossbred neonatal calves from birth onwards in tropical climate. Crossbred (Friesian 87.5% × Hariana 12.5%) female calves (6) each at age group from day 0 (at birth, normally delivered), 5 months old and 10 months old, were selected from Animal Production Research Institute, Pusa, Bihar, during February to April (1999) when ambient temperature was ranging from 10°C to 38°C. Single blood samples were collected from each neonatal female at birth (before colostrum feeding), day 7, day14, day 21 and day 28. Single blood samples were also collected by jugular venipuncture in chilled heparin treated test - tubes between 7.00 and 9.00 hr. Blood urea concentration was estimated from the freshly collected blood using diagnostic reagent based on diacetyl monoxime method (Natelson 1957). Analysis of variance was used to test significance of differences in blood urea concentration between different age groups (Snedecor and Cochran 1967).

The concentration (mean ± SE) of blood urea in crossbred calves on day 0, day 7, day 14, day 21 and day 28 was respectively, 32.33 ± 1.20 mg/dl, 28.83 ± 2.34 mg/dl, 31.33 ± 3.04 mg/dl, 36.00 ± 2.32 mg/dl and 18.17 ± 1.22 mg/dl. The value at 5 months and 10 months were 28.83 ± 1.54 mg/dl and 32.17 ± 1.40 mg/dl. The values of blood urea on day 0, 7, 14, 21, 5 months and 10 months did not differ, and remained almost similar and these values were significantly higher ($P< 0.01$) than the values recorded on day 28.

The significantly higher values of blood urea from birth to day 21 might be because of the fact that the calves were maintained on milk from birth to day 21, and the milk protein might have been absorbed from the GI tract through the enzymatic digestion in similar fashion as it happened in monogastric animals. The amino acids thus absorbed were made available to the liver for its assimilation and conversion to urea at similar intensity (Swenson and Reece 1996).The possible reason for lower concentration of blood urea in the calves on day 28 after birth might be because of the fact that the development of rumen in calves is initiated after birth but the availability of ruminal microflora associated with microbial and ruminal enzymes are established with its digestive function by about 20 to 30 days after birth (Swenson and Reece 1996). The ruminal microflora of different classes are multiplied and propagated fastly during fourth week after birth and the available nitrogenous constituents in the microbial protein synthesis. Under the situation it may be presumed that the available nitrogenous constituents in developing rumen by day 28 might have been utilized for multiplication and propagation of microflora in developing rumen as established earlier (Swenson and Reece 1996).

The higher concentration of blood urea in calves of 5 and 10 months of age than calves on day 28 might be the cause of the fact that the rumen development in cattle is completed by 5 to 6 months of age (Swenson and Reece 1995). Thereafter, they consumed sufficient quantities of feeds and fodder as per need and utilized the different nutrients of feeds for their growth and other physiological processes in normal fashion and maintained the optimum blood urea concentration as reported in normal adult ruminants (Sharma *et al.* 1995). Further, the blood urea concentrations in crossbred calves were higher than that in crossbred lactation cows (Shrikhande and Sarode 1999) and buffaloes (Nayyar *et al.* 1996) may be due to the higher metabolic rate of younger and growing animals than the adult (Shaffer *et al.* 1981).

SUMMARY

The blood urea concentration in crossbred calves from birth to day 21 and at 5 to 10 months of age was higher ($P<0.05$) than the blood urea concentration on day 28 after birth. The initiation of development of rumen function with synthesis of microbial population between day 21 to 30 after birth might be responsible to the lower availability of nitrogenous constituent to the liver for synthesis of urea.

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Chemical castration in pigs

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Pork is highly accepted meat in India and abroad. In order to meet the increasing demand of animal protein by ever-increasing population, emphasis has been given for the production of pig due to its appreciable faster growth. Pork of castrated pig is also preferred to uncastrated one due to the meat quality and aestheticity. The traditional surgical method of castration in pig is highly technical, costly and difficult to adopt by common pig farmer. Thus experiment was planned to evolve a suitable technology of chemical castration in pigs which may be easy to practice with low cost and better growth performance.

Experiment was conducted in crossbred (Hampshire × Khasi local) 'F_1' strain of pigs at the ICAR Research Complex for NEH Region, Umiam, Meghalaya. Piglets (20) of 3 months of age were randomly divided into 2 groups of 10 in each. In group 1 (T_1) the conventional method of surgical castration (Dollar 1998) was done with 5 days antibiotic coverage, whereas in group 2 (T_2), chemical method of castration was conducted by injecting 2 ml of prepared chemicals (0.25 g potassium permanganate + 17 ml glacial acetic acid + 83 ml distilled water) in each testis as per technique of Lipatnikov (1980). Administration of chemicals was done by sharp needle and syringe pushed through epididymis into the body of testis to discharge the fluid from one end to the other in a withdrawing fashion. Animals of both groups were maintained on uniform feed and managemental condition up to 180th day of castration. Birth weight, body weight at castration and final body weight of pigs were recorded.

Histopathological study of the atrophied testis recovered from chemically castrated group (T_2) was conducted to find out the changes in the testis and epididymis. The cost-benefit effect among the 2 methods of castration in pigs was calculated. Finally statistical analysis was done (Snehdechor and Cochran 1968).

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General behaviour

It was observed that after 12 hr of chemical castration, there was inflammation of testis which persisted up to third day and by seventh day it was absolutely vanished. It was probably due to the corrosiveness of the chemicals used. However, during the inflammatory period, feed intake and body temperature were normal as the inflammation was localised one. No behavioural change of the animal was marked except swollen testis with mild pain. After 1 week of castration, it was found that in T_2 there was gradual decrease in size of testis and finally by sixth month of castration, they were atrophied and rudimentary in structure.

Growth performance

Birth weight, age of castration, weight at castration, final body weight on 180th day of castration and average daily weight gain of both treatment groups has been depicted in Table 1. It was observed that birth weight, age at castration and body weight at castration were not significantly different between groups. This is in agreement with the findings of Saeow and Parkasa (1986) who found no significant effect on growth rate, feed efficiency or carcass quality of pigs castrated at different days of age. However, final body weight at 180th day of castration in T_2 (33.79 ± 0.71 kg) was significantly ($P<0.01$) higher than T1 (29.68±0.61 kg). So the average daily weight gain (g/day) in chemically castrated animal (149.60±4.23) was significantly ($P<0.01$) higher than the surgically castrated animal (Table 1). In chemical castration, the germinal cells are mostly affected, but the interstitial cells are partially immune to it which was envisaged from the histo-architecture of testicular biopsy. As such the production of testosterone is not completely ceased and the increase in body weight might be due to the effect of this anabolic hormone which is supported by the findings of Mann and Mann (1981). However, in surgical method, testes are completely removed and the same effect is not felt.

Histopathology

The rudimentary testis recovered from all the pigs of T_2 through minor surgery were subjected for the histo-pathological study. It revealed different degrees of

Table 1. Growth performance of pigs to different methods of castration

Particulars	T_1	T_2
Birth wt. (kg)	0.729±0.03	0.761±0.03
Age at castration (days)	84.10±2.07	82.80±2.26
Weight at castration (kg)	7.41±0.39	7.37±0.36
Final body weight (kg)**	29.68^{b}±0.61	33.79^{a}±0.71
Average daily wt.gain (g/day)**	123.72^{b}±1.47	149.60^{a}±4.23

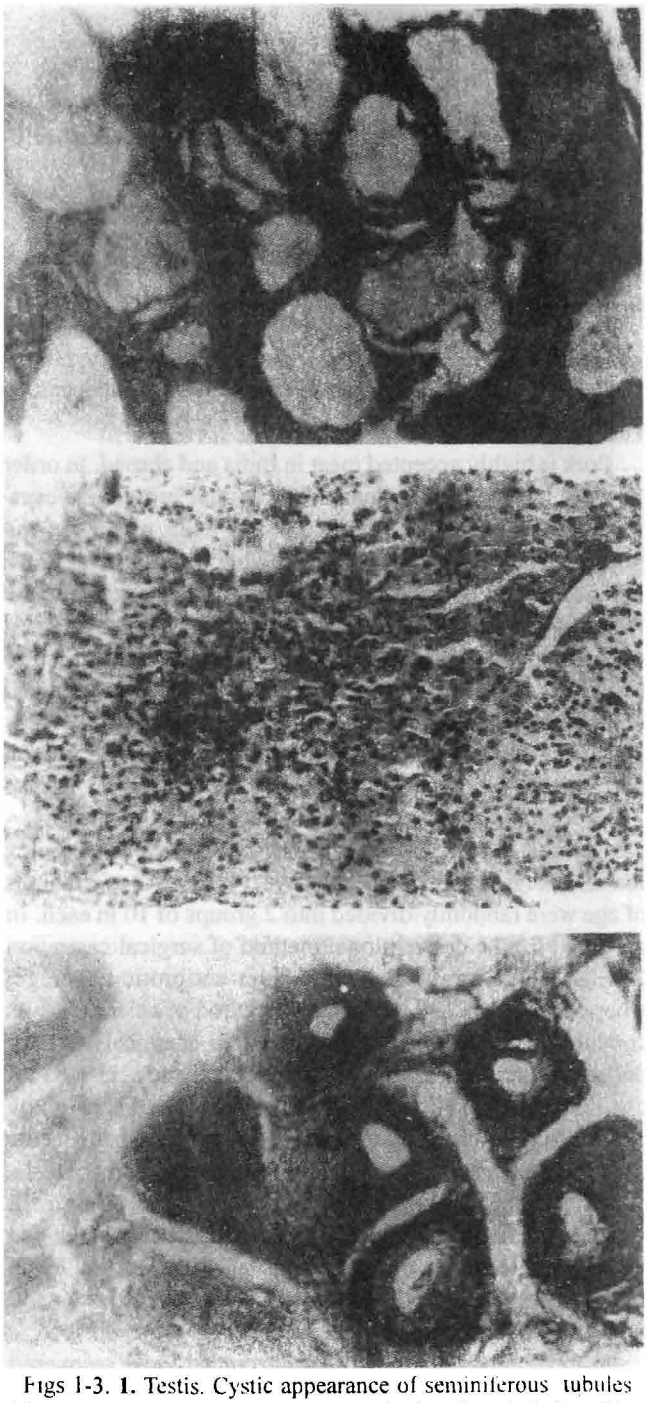
Values in same row having different superscripts differ significantly: ** (P<0.01).

degenerative changes in the spermatogonial cells characterised by pyknosis, karyorrhexis and karyolysis of the nuclei. There was increased eosinophilia of the cytoplasm with varying degrees of rarefaction of cells including cystic appearance of the tubules due to complete disappearance of germinal cells (Fig. 1). Most of the seminiferous tubules were devoid of spermatozoa. The interstitial cells showed hypertrophy giving an adenoid appearance (Fig.2) with necrobiotic changes in a few cells. There was mild congestion of interstitial tissue and fibrosis in tunica vaginalis. It might be concluded that due to irritation caused by chemicals, the seminiferous tubules were devoid of spermatozoa and there was disapperance of germinal cells due to its delicate structure and high sensitivity to irritants. However, the interstitial cells being less sensitive to such irritants got better nourished in post inflammatory period and become hypertrophied with adenomatous appearance, which might be due to continued secretion of androgen.

Similarly in epididymis, the tubules were empty, dilated and devoid of spermatozoa. The lumen of some of the tubules contained proteinacious material. Most of the tubules were fibrosed, obliterated and loss of cilia of lining columnar cells. The inner epithelial cells showed hypertrophy and hyperplasia with polypoid projections. However, there was marked intertubular fibrosis with fibrosed tunica albugenia (Fig.3).

Economics

The economics was calculated on the basis of cost of surgical materials (anaesthesia + suture + antibiotic) and the chemicals used in castration. It was observed that an extra expenditure of Rs 33 was incurred which excluded the charges to be paid to technical person employed to perform surgery. Again due to increased weight gain found in chemically castrated group of animals, an additional amount of Rs 160 of profit/animal was made than that of surgically castrated group (pork @ Rs 40 of on live weight basis fixed by the concerned committee in ICAR research Complex NEH Region, Umiam). Therefore an overall profit of Rs 193/animal was obtained by adopting the method of chemical castration in pigs.



Figs 1-3. **1.** Testis. Cystic appearance of seminiferous tubules with complete disappearance of germinal cells. H & E × 30 **2.** Testis. Interstitial cells showing hypertrophy having adenomatous appearance. H &E × 75. **3.** Epididymis: Fibrosed obliterated tubules with loss of cilia in the lining of columnar cells. H & E × 30.

SUMMARY

Chemical method of castration in pigs done by injecting 2 ml of prepared chemical (0.25 g potassium permanganate + 17 ml glacial acetic acid + 83 ml sterile distilled water) to each testis and its subsequent effect in terms of body weight

gain and histopathology of testis was compared with the conventional surgical method of castration in the same species. It was found that chemical castration in pigs was simple, economical and easier to adopt with significantly higher ($P<0.01$) weight gain incurring more profit to the farmer than that of surgical method of castration.

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Algebraic models of the lactation curve in Sahiwal, Frieswal and other grades of Friesian cattle*

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ABSTRACT

Comparative efficiency of inverse polynomial and gamma type algebraic models of the lactation curve were studied to estimate milk production trends during a lactation on 487 records of Sahiwal, Frieswal and other grades of Friesian cattle extending over 14 years (1980-1993). Principles of least squares analysis were used to solve the equations. The slope of ascending phase was steeper than the descending phase indicating high rate of increase in milk yield in ascending phase in all the genetic groups. The inverse polynomial and gamma type models estimated an initial ascending phase followed by a peak and then a descending phase with the advancement of the lactation. The inverse polynomial was marginally superior to gamma type and considered as model of choice for individual lactation curve (R^2>98%) in all the genetic groups. The gamma type function estimated the lactation curves most efficiently in Sahiwal and crossbred.

Key words: Algebraic models, Cattle, Frieswal, Lactation, Inverse polynomial, Gamma type function

The knowledge of milk production pattern across various stages of lactation would enable the breeder to have an early preliminary sire and cow evaluation, and early culling of poor producers. To realize this trend various linear and non-linear algebraic models have been tried in different breeds of dairy cattle. A comparison between two algebraic models, viz. inverse polynomial and gamma type, in 5 genetic groups of cattle, viz. Sahiwal, Frieswal, 5/8 FS, ¾ FS and 3/8 FS have been made and their fitness and suitability have been discussed.

MATERIALS AND METHODS

Data, extending over 14 years (1980-93), on weekly milk yield records up to 40 weeks of lactation in Sahiwal, ½ Friesian+½ Sahiwal, 5/8 Friesian+3/8Sahiwal (5/8 FS), ¾ Friesian+ ¼ Sahiwal (¾ FS) and 3/8 Friesian + 5/8 Sahiwal (3/8 FS) cows maintained at Military Farm, Meerut, were analyzed for the trend of milk production across different stages of lactation. The breeding policy was to maintain 3/8 to 5/8 Friesian inheritance and for that alternative breeding i.e. forward crossing with exotic bulls and back crossing with Sahiwal bulls was followed. Abnormal performance records like those resulting from abortions, premature births, still births and incomplete lactation due to death and culling were excluded. The 2 models employed to study the trend of lactation were:

Inverse polynomial model

Nelder (1966) described this model algebraically in the form of following equation

$$Y_t = t (b_0 + b_1 t + b_2 t^2)^{-1}$$

where, Y_t is the milk yield in tth week of lactation, t is the time in terms of weeks on the lactation, b_0 is the intercept for theoretical value of Y at the time of parturition, b_1 is average slope of decline, and b_2 is the co-efficient for quadratic term.

Gamma type model

Wood (1967) described this model in the form of following equation

$$Y_t = at^b e^{-ct}$$

where, Y_t is the test day weekly yield in tth week of lactation, a is the initial yield, t is the time (number of weeks), e is the base of natural logarithm and b and c are constants for rising and falling trend of milk yield, respectively.

Both of the intrinsically non-linear models, viz. inverse polynomial and gamma types, were linearized after log-linear transformation. The method of least-squares analysis was applied to solve different equations. The efficiency of different

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Table 1. Parameters of lactation function (inverse polynomial) for milk production in different genetic groups

Inverse polynomial function	Sahiwal	Frieswal	5/8 FS	3/4FS	3/8FS
B_0	0.02	0.01	0.01	0.00	0.04
b_1	0.385**	0.473**	0.526**	0.620**	0.195**
SE (b_1)	0.002	0.001	0.001	0.004	0.0211
b_2	0.622**	0.534**	0.481**	0.386**	0.803**
SE (b_2)	0.003	0.001	0.001	0.001	0.002
R^2	99.96	99.89	99.86	99.85	98.57

** Indicates significance at P<0.01

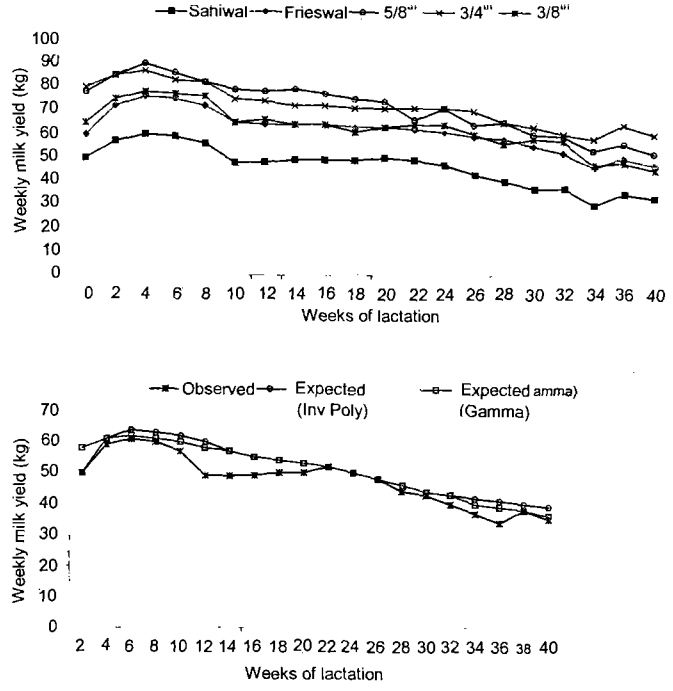
models was compared by R^2 values and the deviations of curves from the observed curve.

RESULTS AND DISCUSSIONS

The observed lactation curve based on test day yields of the 5 genetic groups from 1st to 40th week of lactation (Fig. 1) revealed that the milk production during lactation had 3 phases, viz. an ascending phase, peak phase and a descending phase. The slope of ascending phase was steeper than the descending phase indicating high rate of increase in ascending phase. The peak phase was observed to be very short-lived and the initial milk yield was higher than the yield at drying off. These observations corroborated with the Abubkar and Buvanendran (1981), Yadav and Sharma (1985) and Yadav and Rathi (1991) in different breeds of dairy cattle.

On comparing the lactation curves of different genetic groups in the same figure it was observed that the curves of different genetic groups were found to have almost similar milk production pattern across all the stages of lactation. All the 5 genetic groups had an ascending phase of 2-6 weeks, peak phase at sixth week and a descending phase in the remaining period (seventh week onward). At the initial stages, genetic groups' differences for milk yield were less pronounced as compared to those at the peak yield and subsequently. The curves of ¾ FS and 5/8 FS genetic groups were similar and sharing overlapping position at some places and similar was the case with Frieswal and 3/8 FS.

The estimates of constants and regression coefficients with their standard errors and coefficients of determination for both models are given for inverse polynomial (Table 1) and for gamma type function (Table 2).



Figs 1-2. **1.** Genetic group-wise plot of observed weekly milk yield. **2.** Observed and expected weekly milk yield in Sahiwal.

Inverse polynomial function

This function is superior to ordinary polynomial as it is non-negative and bounded with in-built symmetry (Nelder 1966). The constant intercept 'b_0' had lower values for all the genetic groups, indicating that initial milk yield was higher in all the genetic groups (Table 1).

The constant 'b_1' and 'b_2' had significant contribution

Table 2. Parameters of lactation function (gamma type) for milk production of different genetic groups

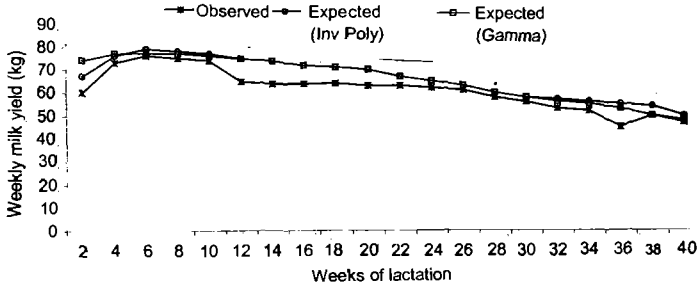
Gamma function	Sahiwal	Frieswal	5/8 FS	3/4FS	3/8FS
$\log_e^a$	3.97	4.17	4.35	4.42	4.08
b	-1.618**	-1.738**	-1.581**	-1.299**	1.831**
SE (b)	0.001	0.001	0.001	0.001	0.002
c	0.706**	0.867**	0.672**	0.357**	0.998**
SE (c)	0.018	0.022	0.018	0.017	0.030
R^2	97.17	94.51	95.68	94.18	91.69

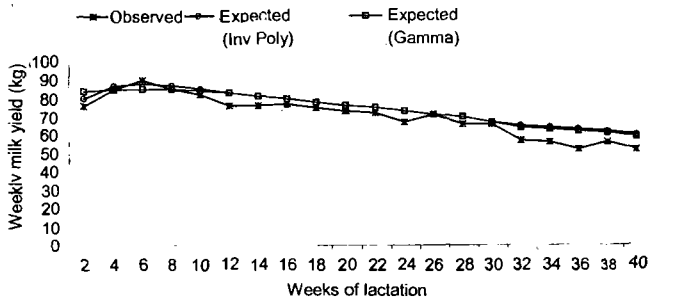
**Indicates significance at P<0.01.

towards reduction of variation in all the genetic groups. Further, the R^2 values were 99.96, 99.89, 99.86, 99.85 and 98.57 % in Sahiwal, Frieswal, 5/8 FS, ¾ FS and 3/8 FS groups, respectively, indicating that the function/model had better fit than gamma function, for all the genetic groups. The observed and expected values of weekly milk yield in Sahiwal, Frieswal, 5/8 FS, ¾ FS and 3/8 FS, are presented in Figs 2, 3, 4, 5, 6, respectively. The shape of the lactation curves estimated by this model in all genetic groups was very close to the observed values. However, the initial milk yield was over- estimated in all genetic groups (except 3/8 FS) and this model under-estimated the peak yield. The descending phase as predicted by this model in all the genetic groups were almost similar to the observed values except showing slight deviation from 8 to 20 week of lactation. Hence, it is evident from the Table 1 and Figs 2-6 that the inverse polynomial is a model of choice for individual lactation curves in all the 5 genetic groups of cattle. Sudarwati *et al.* (1995) found the inverse polynomial function to be more accurate in predicting the 305-day milk yield. The inverse polynomial function is the best fit model for the lactation curve (R^2 = 99%) in Sahiwal and Jersey × Sahiwal cross (Gore *et al.* 1996). This function was recently found to perform well in Holstein cattle (Tozer and Huffaker 1999).

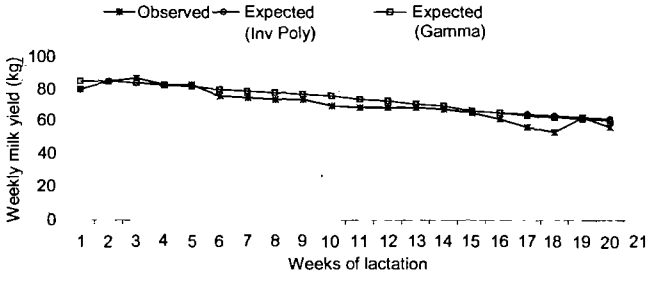
Gamma type model

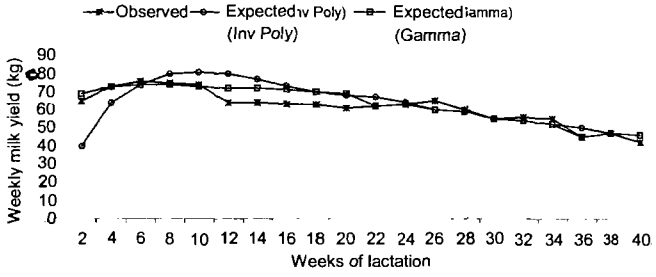
The gamma type model, postulated by Wood (1967), revealed that the value of pure constant intercept $\log_e$, a measure for the initial milk yield (multiplier) which changes the height of the curve all times, and having no impact on the shape of the curve, was lowest in Sahiwal and highest in ¾ FS followed by 5/8 FS, Frieswal and 3/8 FS grades of Friesian cattle.





Figs 3-4. **3.** Observed and expected weekly milk yield in Frieswal. **4.** Observed and expected weekly milk yield in 5/8 FS.





Figs 5-6. **5.** Observed and expected weekly milk yield in 3/4 FS. **6.** Observed and expected weekly milk yield.

The constant 'b', a measure of ascending trend was, observed negative in all the genetic groups except for 3/8 FS and the constant 'c', a measure of descending trend, was positive for all the genetic groups. However, both the constants 'b' and 'c', had significant contribution towards reduction of error variance in the model. The value of 'c' indicated that constant for descending phase was minimum (0.357) in ¾ FS and maximum (0.998) in 3/8 FS. In this function, the constant 'b/c' determined the duration of ascending phase, which had greater value for 3/8 FS genetic group. The per cent R^2 values were 97.17, 94.51, 95.68, 94.18 and 91.69 in Sahiwal, Frieswal, 5/8 FS , ¾ FS and 3/8 FS, respectively. Shape of the lactation curves estimated by this model (Figs 2-6) in all the genetic groups were close to the observed ones. However, this model was found to overestimate the initial milk yield in all the genetic groups, underestimate the peak yield in 5/8 FS, 3/8 FS and ¾ FS and overlap in the later part of the descending phase in almost all the genetic groups. The gamma function has also been reported to give best fit for the first lactation (Sherchand *et al.* 1995, Singh *et al.* 1996, Singh *et al.* 1997).

From this study, it can be concluded that both inverse polynomial and gamma type models could be used for estimating the shape of lactation curve. The gamma type model would be a model of choice for estimating the average shape of lactation curves in a given genetic group, whereas the inverse polynomial model could estimate the shape of individual lactation curves. However, this may not be an indication for estimating the actual trend as explained by Nelder (1966). Therefore, before arriving at some conclusion the results of inverse polynomial need more detailed examination for the behaviour of its constants on individual

records and a set of records.

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Precision in estimation of heritability

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ABSTRACT

The various effects such as year and period of calving, season of calving, parity and lactation length were included in the model. The effects of year of calving, season of calving and parity dam were significant ($P<0.001$) for the various traits. The estimates of heritability using 16 linear models showed a wide range of variation. The values ranged from 0.062 to 0.439 for total milk yield, 0.075 to 0.482 for 305 days' milk yield, 0.085 to 0.589 for peak yield and 0.06 to 0.432 for daily milk yield. The inclusion of lactation length in the model as a co-variable reduces the error mean squares, thereby increasing the efficiency (R^2 value) of the model. It also increased the heritability estimates, however, it reduced the mean square due to sires. The R^2 value was maximum for the model, which included year, season, parity, sire within year and regression of lactation length (linear and quadratic) for all traits. The results indicated that environmental factors adjustment affected the estimates of heritability and the magnitude of such variation usually depended upon the number of adjustments made.

Key words: Cattle, Environmental factors, Heritability, Linear models, Production traits, Tharparkar

An owner of a dairy enterprise would always try to maximise his profit by providing improved management and feeding practices. However, for a continuing enterprise he should require bulls of high genetic merit, selected with finest accuracy. For planning an effective selection and breeding programme knowledge of genetic architecture of his herd is necessary, and accuracy in the estimation of genetic parameters should be considered paramount important. For estimation of genetic parameters, a variety of methodologies are available. The least squares methodology is the one of them, in which adjustment of data is tried for significant environmental effects, before obtaining estimates of genetic differences. Hence, when different assumptions are made, estimate of genetic parameters tend to vary. This is especially so in tropical and sub-tropical climates, where year to year differences tends to mask relatively feeble sire to sire differences. It is, therefore, very essential that due consideration is made to the accuracy of procedures used for estimating genetic parameters for the data generated under such environmental situations. This has been one of the objectives of the present study. This work is expected to provide clues to similar procedural problems for ultimately increasing the accuracy of genetic parameters, which may directly and favourably, influence genetic progress and improvement.

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MATERIALS AND METHODS

The data for this analysis consisted of 2380 normal lactation records from 1968 to 1996 of Tharparkar cows maintained at the Central Cattle Breeding Farm, Suratgarh. These animals were the progeny of 66 sires. The habitability of total lactation yield, 305 day's yield, peak yield and daily milk yield were estimated using 16 linear models. The total milk yield referred to the amount of milk produced from sixth day after calving till cow went dry, while 305 days' yield referred to the yield from sixth day to 305 days or the day of drying whichever was earlier. The peak yield referred to the maximum amount of milk produced on any one day within 3-4 months of calving. The daily milk yield referred to the milk yield per day of lactation length for individual cow. The analysis of variance ((LSMLW, Harvey, 1990) to obtain precise estimates of genetic parameters for production traits, was undertaken, which investigate the change in the estimates of heritability when data are adjusted for different environmental factors. The environmental factors were year/ period and season of calving, parity of calving, lactation length and age at first calving. The linear and quadratic regression effect of lactation length and age at first calving were studied. In order to improve the accuracy of heritability estimate by

increasing the number of records per sire and per year, season, the multiple lactation records (first to eighth lactation) were included.

At the first instance all 5 environmental factors were included in the model along with sire and genetic parameters along with standard error were estimated. Then the changes in genetic parameters were studied by estimating them from the models that did not include environmental effects. Lastly genetic parameters were also estimated from model (model I), which include the sire effect only. In models XII to XIV, the years were grouped into periods based on period of calving. In case of models XI and XVI, a nested analysis was done with years/period and sires nested in periods. The mean squares due to sires and error, and the sire component of variance in model I to XVI were expressed as percent of the same obtained in model I, which include only sire effect for comparison purposes. The following models were used in this analysis.

I –Yjm = μ + Sj + ejm
II –Yijm = μ + Vi + Sj + eijm
III –Yijkm = μ + Vi + Sj + Mk + eijkm
IV –Yijklm = μ + Vi + Sj + Mk + Ll + eijklm
V –Yijklm = μ + Vi + Sj + Mk + Ll + bL (Aijklm-AA) + eijklm
VI –Yjklm = μ + Vi + Sj + Mk + Ll + bL (Aijklm-AA) + bQ (Aijklm-AA) + eiklm
VII –Yjklm = μ + Sj + Mk + Ll + ejklm
VIII –Yjllm = μ + Sj + Mk + Ll + bL (Aijklm-AA) + bQ (Aijklm-AA) + ejkllm
IX –Yijlm = μ + Vi + Sj + Ll + bL (Aijklm-AA) + bQ (Aijklm-AA) + eijlm
X –Yijkm = μ + Vi + Sj + Mk + bL (Aijklm-AA) + bQ (Aijklm-AA) + eijkm
XI– Yijklm = μ + Vi + Sij + Mk + Ll + bL (Aijklm-AA) + bQ (Aijklm-AA) + eijklm
XII– Yijkm = μ + Pi + Sj + Mk + bL (Aijklm-AA) + bQ (Aijklm-AA) + eijkm
XIII –Yijkm = μ + Pi + Sj + Mk + eijkm
XIV– Yijklm = μ + Pi + Sj + Mk + Ll + eijklm
XV –Yijklm = μ + Pi + Sj + Mk + Ll + bL (Aijklm AA) + bQ (Aijklm-AA) + eijklm
XVI –Yijklnm = μ + Pi + Sij + Vin + Mk + Ll + bL (Aijklm-AA) + bQ (Aijklm-AA) + eijklnm

where,

Yijklm is the observation on mth cow sired by jth sire in ith year/period, kth season and lth parity; μ is the overall mean; Sj is the random effect attributed to jth sire; Vi / Pi is the fixed effect of year/period of calving; Mk is the fixed effect of season of calving; Ll is the fixed effect of parity; bL, bQ is the linear and quadric regression of lactation length; Aijklm is the lactation length corresponding to Yijklm; AA is the average lactation length; Yin is the nested effect of nth year with in ith period of calving; eijklm is residual error under standard assumption, which makes analysis valid i.e. NID (0,2)

RESULTS AND DISCUSSION

The various effects such as year and period of calving, season of calving, parity and lactation length included in the model I to XVI showed significant ($P<0.001$) effect on all the production traits.

The heritability values (Table 1) showed wide variation when different models were used for their estimation. The heritability values ranged from 0.062 to 0.439 for total lactation yield, 0.075 to 0.482 for 305 days yield, 0.085 to 0.589 for peak yield and 0.060 to 0.432 for daily milk yield. The values were higher for total milk yield, 305 days milk yield, peak yield and daily milk yield when estimated from the model XII. The heritability values were lowest for total lactation

Table 1. Heritabilities and their standard error of production traits estimated from different models

Models	Total yield	305 days yield	Peak yield	Daily milk yield
XII	0.439 ± 0.084	0.482 ± 0.089	0.587 ± 0.102	0.432 ± 0.083
X	0.369 ± 0.075	0.419 ± 0.081	0.521 ± 0.094	0.368 ± 0.075
VIII	0.169 ± 0.047	0.175 ± 0.048	0.190 ± 0.050	0.156 ± 0.045
XVI	0.164 ± 0.047	0.174 ± 0.048	0.198 ± 0.050	0.137 ± 0.045
I	0.146 ± 0.043	0.232 ± 0.056	0.479 ± 0.098	0.358 ± 0.074
VII	0.129 ± 0.041	0.140 ± 0.043	0.181 ± 0.049	0.159 ± 0.046
XIII	0.119 ± 0.039	0.224 ± 0.055	0.582 ± 0.102	0.424 ± 0.082
VI	0.114 ± 0.039	0.131 ± 0.042	0.195 ± 0.051	0.098 ± 0.037
IX	0.114 ± 0.039	0.129 ± 0.041	0.197 ± 0.051	0.098 ± 0.036
V	0.113 ± 0.039	0.115 ± 0.039	0.195 ± 0.051	0.096 ± 0.036
III	0.111 ± 0.038	0.191 ± 0.050	0.516 ± 0.093	0.353 ± 0.073
XV	0.108 ± 0.038	0.123 ± 0.040	0.168 ± 0.047	0.097 ± 0.036
XI	0.107 ± 0.060	0.097 ± 0.060	0.085 ± 0.059	0.060 ± 0.058
II	0.102 ± 0.037	0.182 ± 0.049	0.518 ± 0.093	0.349 ± 0.072
IV	0.073 ± 0.033	0.085 ± 0.035	0.180 ± 0.049	0.095 ± 0.036
XIV	0.062 ± 0.031	0.075 ± 0.033	0.154 ± 0.044	0.095 ± 0.036

yield, 305 days yield, peak yield and daily milk yield when estimated from the model IV and XIV. The higher estimate of heritabilities was due to the effect of period, season and lactation length in the model. The parity effect was possibly confounded with the sire effect, which resulted in higher estimate of heritabilities. The lower estimate of heritabilities was obtained from the model, which included the effect of year/period, season and parity. This might be probably due to the fact that in the present set of data the effect of lactation length was could not be separated out from sire effect and it went to error component, which resulted in to lower estimate of heritabilities.

These results showed that the estimates of heritability values change markedly with different models that include effects, which are not independent of each other. The effect of parity was clearly evident when heritability value was estimated from the model VI which included period, season, parity and regression on lactation length (linear and quadric) as compared to the model X which did not include parity effect.

The results of this study varied from those obtained by Rao and Dommerholt (1981) and Sachdeva and Gurnani (1995). Rao and Dommerholt (1981) used several procedures for estimating heritabilities of production traits of Tharparkar cows in India, and the heritability estimates for 305 days yield, total milk yield and average daily yield ranged from 0.03 to 1.48, 0.01 to 1.19 and 0.11 to 1.58, respectively, depending upon the model used. They reported highest estimates of heritability for production traits (total milk yield, 305 days milk yield and daily milk yield), when estimated from the model that included sire, season and linear regression of lactation length and age at first calving. The lowest estimates were obtained when estimated from the model that included periods, years and sire within periods. Sachdeva and Gurnani (1995) estimated the heritability of first lactation milk records using 8 linear models for adjustment of environmental factors. They found that heritability (0.07) was highest when data were adjusted for farm only and heritability (0.025) was lowest when data were adjusted for all significant effects like genetic groups, age groups, farms and periods. The heritability of un-adjusted data was 0.51.

An attempt was made to investigate the reasons for such variability in the heritability estimates by looking at the mean square and components of variance due to sire and error. The R^2 values were lower in the model, which did not include the regression of lactation length in comparison to other models that included regression of lactation length.

A perusal of mean squares due to sires and errors, and sire components of variance for different models for these traits, indicated that the inclusion of parity along with the year/period of calving in the model resulted in a decrease in both the mean sum of squares due to sires and sire components of variance. The decrease in sire components of variance and sire mean squares was much more in comparison to decrease in error mean squares for production traits. The mean sum of squares due to sire and sire variance decreased 38 and 60% for total milk yield, 52 and 69% for 305 days yield, 66% and 74% for peak yield, 67% and 80% for average daily milk yield, respectively, whereas error component of variance reduced only 9 % for total milk yield, 10 % for 305 days milk yield, 22% for peak yield, and 16 % for average daily milk yield. This clearly indicated that the effect of sires and effect due to parity and year could not be separated even when they are estimated separately and created doubt about the real contribution of sires to the variation in the traits analysed.

The inclusion of lactation length in the model contributed to significant reduction in the error mean sum of squares. The error mean squares reduced by 54% for total milk yield, 38% for 305 days yield, 16% for dry period, 50% for service period and 51 % for calving interval when lactation length was included in the model (models IV v/s VI and XIV v/s V). Therefore, increased the R^2 value of the model for these traits. This indicated that inclusion of lactation length in the model as a regression variable would reduce the error mean squares and increase the reliability of heritability estimates. However, inclusion of lactation length also resulted in a decrease in mean sum of squares due to sires. The reduction of error mean squares was very small for peak yield and daily milk yield (2-3%) indicating that inclusion of lactation length in these traits did not change mean squares or sire components of variance. The grouping of years in periods and the analysis of data using nested model with years and sire nested in years/periods (model XI and XVI) also did not change the error mean squares markedly. On the other hand there was reduction of the mean squares due to sires and sire component of variance, thereby resulting in lower heritability estimates.

The effect of inclusion of lactation length in the model, grouping of years in to periods and nested effect of sires within years/period obtained by Rao and Dommerholt (1981) in Tharparkar cows were similar to the findings of the present investigation. Basu (1983) also reported that inclusion of additional variable i.e. lactation length in the models affected the estimates of heritability (0.0 to 0.09). Schaeffer (1975) suggested that grouping of years in to period may improve the estimation of effects and thereby to increase the precision of parameter estimated. The present investigation showed that such grouping would not help in estimating reliable genetic parameters in the present set of data.

The results indicated that environmental factors adjustment affected the estimates of heritability and the magnitude of such variation usually depended upon the number of adjustments made. There was, perhaps, a serious confounding of the effects of sires with those of environmental factors and also with time scale. It is concluded that it would be difficult to estimate the contribution of sires to the variability in a trait independent of the other effects in case of a spread of data for long time. Too many years in a set of data therefore may not be desirable. This will further complicate the situation as the

small sets of data per year, that are inherent in animal groups of tropical areas, may make any such effort entirely wasteful.

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Estimates of genetic trends in a closed flock of Bharat Merino sheep

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ABSTRACT

The genetic and phenotypic trends over 15 years (1982-96) of multiple trait selection in a closed flock of Bharat Merino sheep were estimated. The estimates of genetic trend in body weights at birth, 3, 6 and 12 months of age, pre- and post-weaning growth rates, first, second and adult six-monthly greasy fleece weights, staple length, fibre diameter and medullation percentage were -0.064±0.057, -0.327±0.264, -0.335±0.343, and -0.180±0.477 kg, -2.842±2.722, and 0.191±1.428 g, 0.059±0.021, 0.014±0.026 and 0.040±0.006 kg, -0.029±0.123 cm, -0.160±0.279 microns and 0.970±0.837, respectively. These estimates revealed significant genetic improvement only in the first and adult six-monthly greasy fleece weights but little or no improvement in rest of the traits. The phenotypic trends only in 6-mo body weight (0.603±0.183 kg) and fibre diameter (-0.315±0.146 microns) were desirable and significant. There is, therefore, a need for reviewing the ongoing breeding plans for faster genetic improvement in this flock of Bharat Merino sheep.

Key words: Fleece, Genetic trends, Growth, Sheep

Knowledge of the change in the performance of farm livestock with time due to changes either in the genetic structure of the population or improvement in the management and environment is of fundamental interest to the animal breeder. Such an information would help in assessing the effectiveness of breeding programme undertaken for improvement of productivity and adopting remedial measures if the progress has been short of the expectations. The flock of Bharat Merino sheep has undergone 7 generations of multiple trait selection so far. A study of genetic trends in important economic traits is, therefore, very necessary for knowing its performance status over the years for developing breeding strategies for further genetic improvement of productivity of this flock of sheep. Hence, the present investigation was undertaken to obtain estimates of genetic trends in directly selected and in correlated traits.

MATERIALS AND METHODS

Development of Bharat Merino breed of sheep

The Bharat Merino, a fine wool synthetic breed of sheep was developed at the Central Sheep and Wool Research Institute, Avikanagar (Rajasthan, India), which is situated in the semi-arid region of North-Western India. In 1982, 3/4th crossbreds of Rambouillet and Russian Merino with Chokla, Jaisalmeri, Malpura and Nali breeds were assembled into a foundation sheep breeding population. Since then, the flock was closed to the introduction of germplasm from any other source, and multipletrait selection of ram lambs and *inter se* breeding was practiced for the genetic improvement of the productivity of this flock.

Management of the flock

The flock was housed in asbestos-roofed sheds during summer. The rams, ewes and lambs were grazed separately under the semi-free range grazing management system. The grazing area consisted of forest land with natural fodder trees/ bushes and surface vegetation including the improved pastures of *Cenchrus ciliaris*. Grazing resources are scarce from March to June so sheep were supplemented with hay of *Cenchrus*, cowpea and dolichos; pala leaves *(Zizyphus)* and fodder tree loppings, and fed 300 g of concentrate mixture daily throughout the year.

The breeding of ewes was restricted to spring (March-April) and autumn (September-October) only. The ewes in heat were mated with rams alloted as per the breeding programme, which took care of inbreeding in the flock.

The ewe's weight at lambing and the birth weight of lamb were recorded within 24 hr of lambing. The lambs were subsequently weighed at 3 (weaning), 6 and 12 months of age. The shearing of the animals was carried out twice a year

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in the spring and the autumn seasons. The animals were washed 1 or 2 days before shearing. The fleece of each animal was weighed separately and recorded. The fibre diameter and medullation percentage was measured on the basis of wool samples taken from the lateral mid-side region at about 90 days of age and staple length was measured at about 180 days of age. Standard laboratory techniques were used for evaluating the wool quality.

The genetic and environmental trends in the Bharat Merino flock of sheep were measured (Smith 1962). These methods are based on the comparison of the changes in the mean performance of successive progeny groups of individual sires (paternal halfsibs) as compared with the changes in the population at the phenotypic level. The only requirement of this method is the continuity of genotypes over different years. In the sheep farm data, the progeny of rams born in different years provide such continuity. These methods measure the genetic change due to changes in the array of sires used for breeding over the years and assume the same rate of change in both the sexes. Since the ewes are daughters of the rams of the previous generations, the rate of change in the 2 sexes over a period of time would be expected to be almost the same.

The estimates of genetic trend were obtained by 2 methods. The estimate of genetic trend in the first method is obtained as the difference between the regression of population performance on time and the pooled intra-sire regression of individual sire progeny performance on time and this estimate measures only one-half of the genetic improvement in a population per unit of time. In the second method, the estimate of genetic trend is obtained as the pooled intra-sire regression of the progeny performance on time, each record taken as deviation from their contemporaries. This requires the assumption that the mates of the sires are a random sample of those available. The second method provides a better estimate of the genetic change than the first method because it takes care of the year-year fluctuations in the environment. The phenotypic change in the performance of this flock was estimated as the linear regression of population performance on time measured in years.

Table 1. Distribution of lamb records and their sires, and trait means for Bharat Merino breed of sheep

Trait	Lamb records	Sires	Mean
Body weight (kg) at			
Birth	1508	62	3.60
3 -mo	1346	60	17.12
6-mo	1240	61	23.74
12-mo	1005	60	30.95
Daily gain (g)			
Pre-weaning	1346	60	150.012
Post-weaning	1005	60	51.006
Six-monthly clip (kg)			
First	1366	76	0.931
Second	895	54	1.022
Adult	4758	110	1.173
Staple length (cm)	619	35	3.96
Fibre diameter (microns)	736	41	18.80
Medullation %	736	41	3.92

RESULTS AND DISCUSSION

The distribution of lamb records and their sires, and means for growth and fleece traits of Bharat Merino sheep examined are presented in Table 1. The estimates of phenotypic, genetic and environmental trends in the traits studied are presented in Table 2. Only estimates of genetic trends obtained

Table 2. Estimates of annual phenotypic (ΔP), genetic (ΔG) and environmental (ΔE) trends in growth and fleece traits of a Bharat Merino flock

Trait	ΔP	ΔG		ΔE
		1st method	2nd method	
Body weight (kg) at				
Birth	0.018±0.028	-0.055±0.057	-0.064±0.57	0.045±0.058
3 -mo	0.137±0.133	0.130±0.271	-0.327±0.264	0.072±0.272
6-mo	0.603**±0.183	-0.442±0.371	-0.335±0.343	0.824*±0.373
12-mo	-0.249±0.251	1.142*±0.511	-0.180±0.477	-0.820±0.513
Daily gain (g)				
Pre-weaning	1.201±1.378	2.274±2.799	-2.842±2.722	0.064±2.810
Post-weaning	-1.251±0.735	3.165*±1.494	0.191±1.428	-2.833±1.500
Six-monthly clip (kg)				
First	0.004±0.011	0.016±0.23	0.059**±0.021	-0.004±0.023
Second	-0.036*±0.014	0.052±0.027	0.014±0.026	-0.063*±0.027
Adult	-0.038**±0.003	0.031**±0.007	0.040**±0.006	-0.053**±0.007
Staple length (cm)	-0.008±0.062	0.086±0.129	-0.029±0.123	-0.050±0.130
Fibre diameter (microns)	-0.315*±0.146	0.573±0.300	-0.160±0.279	-0.601*±0.302
Medullation %	-0.464±0.431	-0.399±0.884	0.970±0.837	-0.264±0.889

* $P < 0.05$; ** $P < 0.01$.

by second method are discussed in detail below whereas those obtained by first method are presented for comparison.

Phenotypic trends

The estimates of phenotypic trend in 6-month body weight and fibre diameter revealed significant improvement in these traits over the years. The corresponding annual improvements as per cent of population mean were 2.54 and -1.68, respectively. The wool fibres became finer over the years. The phenotypic trends in greasy fleece weight at second and adult 6-monthly clips were negative ($P<0.05$). Correspondingly, the annual decreases as per cent of population mean were -3.52 and -3.24, respectively. Contrary to these results, Singh and Dhillon (1990, 1991) reported undesirable and significant phenotypic trends in 6-month body weight and fibre diameter in Avikalin and Avivastra lambs under similar climatic conditions. The estimates of phenotypic trend in rest of the traits studied were statistically non-significant.

Genetic trends

The significant annual genetic advance was observed in the greasy fleece weight at first and adult 6-monthly clips. The observed annual genetic trends in the first and adult 6-monthly clips as per cent of population mean were 6.2 and 3.4, respectively. The estimates of genetic trend in rest of the traits were not significant. The desirable genetic trends in greasy fleece weight and/ or fibre diameter were reported by Shelton (1984) in Rambouillet, Klerk *et al.* (1990) in Dohne Merino, Singh and Dhillon (1990) in Avikalin, Wyk *et al.* (1994) in South African Merino and Morris *et al.* (1996) in Romney sheep. Undesirable genetic changes in staple length and/ or medullation percentage were also reported by Singh and Dhillon (1990, 1991) in Avikalin and Avivastra lambs. Contrary to these results, undesirable genetic trends in greasy fleece weight (Mansour *et al.* 1977, Singh and Dhillon 1990, 1991), fibre diameter (Singh and Dhillon 1991), desirable genetic trends in staple length (Shelton 1984), medullation percentage (Singh and Dhillon 1990, 1991) and early growth traits (Shrestha *et al.* 1996) were also reported.

Environmental trends

The environmental trends in 6-month body weight and fibre diameter were significant and in the favourable direction. The environment appears to play an important role in improving 6-month body weight and reducing the fibre diameter. The environment trends in rest of the traits were nonsignificant.

A perusal of the estimates of genetic trend in the growth and fleece characteristics revealed genetic improvement in the greasy fleece weight at first and adult 6-monthly clips. The genetic improvement in wool production potential of Bharat Merino sheep, however, could not be realised at phenotypic level because of possibly poor environmental and management conditions. These results revealed that the ram lamb selection based on the selection index incorporating 6-month body weight and first 6-monthly greasy fleece weight, was effective in improving the wool production potential and ineffective in improving the wool quality and growth performance of Bharat Merino sheep. This warranted a critical review of the ongoing breeding programme in this flock. The overall means for adult annual greasy fleece weight (2.346 kg) and 6-month weight (23.74 kg) emphasized the need for rigorous efforts for genetic improvement in the fleece and growth characteristics of Bharat Merino flock to achieve the target of an average annual greasy fleece weight of 3.0 kg and 32.0 kg body weight at 6 months of age by 2020 as laid down in the objectives of the project. Short staple length (<5 cm) and higher medullation percentage (>1%) also warranted a review of the breeding strategies so that Bharat Merino may be evolved as a fine wool breed of sheep under Indian climatic conditions. Furthermore, improvement in the environmental conditions and management practices would also be required for full realization of the genetic potential for growth and fleece characteristics of this breed of sheep.

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Time trends of genetic parameters in a White Leghorn population subjected to long term single trait selection

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ABSTRACT

A total of 2 263 pullets of 127 sires and 1 046 dams of 7 consecutive generations were studied. Females on the basis of their first 100 days egg production (EP_{100}) and cocks, on the basis of their sire family averages for EP_{100} were selected every generation. The average correlated phenotypic response of age at sexual maturity (ASM) was negatively significant and of egg mass (EM), was positively significant. The average direct (of EP_{100}) and correlated (other than EP_{100}) phenotypic responses of the remaining traits were not significant. The pooled heritability estimates of ASM, WAM (weight at sexual maturity), EP_{100} and EM were low to moderate while, for CS (clutch size), it was very low. The time trends of heritability estimates of WAM, EP_{100}, CS and EM were all negative except for ASM, which was positive. This study indicated that additive genetic variance of primary trait (EP_{100}) was reduced due to selection. The pooled estimates of genetic, environmental and phenotypic correlation of ASM with EP_{100}, CS and EM were all negative but between ASM and WAM, the estimate was positive. The estimates of correlation of WAM with other traits were small in magnitude (though significant) and inconsistent in direction. The pooled estimates of genetic, environmental and phenotypic correlation of EP_{100} with CS and EM and between CS and EM were all highly ($P<0.01$) positive. The pooled estimates of genetic correlation between traits were mostly significant. The time trends of the estimates of genetic, environmental and phenotypic correlation were mostly negative and not significant. However, the time trends of the estimates of environmental correlation between ASM and WAM and CS and EM were significant.

Key words: Phenotypic response, Genetic parameters, Poultry, Time trends, White Leghorn

Evidence of changes in the genetic constitution of populations under selection is manifested by changes in heritability estimates in different generations (Falconer and Mackay 1996, Chatterjee *et al.* 2000). It has been postulated that heritability decreases during the course of selection in a closed flock due to utilization of genetic variation, and also because of increasing homozygosity from inbreeding, which is inevitable because of small population size. However, Lerner (1958) presented theoretical models which allow for a change from a positive to a negative genetic correlation between 2 traits on which selection was applied.

The main objectives of the present study were to observe average phenotypic performances of the traits, changes over a period of time in the heritabilities and in the genetic, environmental and phenotypic correlations between certain traits of economic importance subjected to long-term single trait selection.

MATERIALS AND METHODS

The data of 2 263 pullets of 127 sires and 1 046 dams pertaining to 7 consecutive generations of a White Leghorn population were utilized. Nearly 150 females on the basis of their first 100 days egg production were selected every generation and 20 males every generation were selected on the basis of sire family averages for first 100 days egg production. Fullsib and halfsib mating were avoided. The selected males were transferred to single sire pens with 7-10 females. The traits measured were ASM, WAM, EP_{100}, CS and EM. Average clutch size (CS) was measured as the total number of eggs laid by a bird up to the 280 days of age divided by the number of cycles of egg laying to that age. Egg mass (EM) for each bird was obtained by multiplying the total number of eggs laid up to 280 days of age with mean EW.

The data were corrected for significant hatch effects in

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each generation separately using least square constants (Harvey 1966). Heritability estimates by using Harvey's (1987) mixed model least squares and maximum likelihood (LSMLMW) programme and standard errors (SE) as per Dickerson (1960). The pooled heritability estimates were obtained by weighting the individual estimates inversely to their variances (Enfield *et al.* 1966). The correlations, estimated initially for each generation separately, have been pooled over generations (Enfield *et al.* 1966). The time trends of the estimates of heritability and genetic, environmental and phenotypic correlation were calculated from regression of the estimates on generation numbers (Snedecor and Cochran 1989).

RESULTS AND DISCUSSION

There was significant decline of ASM (P<0.05) and significant improvement of EM (Table 1) as a correlated phenotypic response in those traits. The negative trend (average phenotypic response) of ASM might be due to negative genetic correlation of the primary trait (EP_{100}) with ASM (Table 3). Similar findings were obtained by Chatterjee *et al.* (2000) in White Leghorn. The positive phenotypic trend of EM might be due to high positive genetic correlation of primary trait with EM (Table 4) as reported by Chatterjee and Misra (2000). The average phenotypic response of WAM was negative and nonsignificant, while, that of EP_{100} and CS was positive and nonsignificant (Table 1). The reasons for small positive response in the primary trait (EP_{100}), probably were small population size, as suggested by Nordskog *et al.* (1967). In CS, the positive correlated phenotypic response was perhaps due to a high positive genetic correlation between EP_{100} and CS (Table 4). The small negative correlated phenotypic response in WAM might be due to negative genetic correlation between EP_{100} and WAM in some of the generations (Table 3). Similar observations of EP_{100} and CS were recorded by Chatterjee *et al.* (2000). The average positive phenotypic response of EP_{100}, CS and EM and negative phenotypic response in ASM were desirable, because all these will improve egg production.

Table 1. Average phenotypic response in the performance of the traits

Traits	Average or pooled phenotypic response (b± SE)
ASM	-3.28± 1.06*
WAM	-7.40± 8.59
EP100	1.18±1.02
CS	0.07±0.05
EM	134.94±52.29*

The pooled heritability estimates of all the traits were highly significant (Table 2) but were variable in magnitude. These values for WAM, EP_{100} and CS corresponds well with the reports of previous workers (Bais 1996, Chatterjee *et al.* 2000).

The time trends of heritability estimates for ASM was positive, but negative and significant for EP_{100} while, for rest of the traits the trends were negative. This indicated the selection was effective in primary trait (EP_{100}) to some extent but was not effective to change the secondary traits. The heritability estimates of the traits were variable over generations (Table 2) and probably were due to small population size and/or non-normal distribution of data (Ibe and Hill 1988).

The pooled estimates of genetic correlation of ASM with WAM was positive and highly significant (P<0.01), while, with EP_{100}, CS and EM were all highly negative (Table 3). These values were in agreement with Shrivastava *et al.* (1993). The pooled estimates of genetic, environmental and phenotypic correlation between WAM and EP_{100} were small positive (Table 3). The pooled estimates of genetic correlation of WAM with CS and EM were negative and significant (Table 4), but phenotypic correlations were either small positive or small negative. The pooled estimates of genetic,

Table 2. Time trends of heritability estimates of the traits

Generation	ASM	WAM	EP100	CS	EM
	h^2_s ± SE	h^2_s ± SE	h^2_s ±SE	h^2_s ± SE	h^2_s ±SE
S_0	0.26±0.13*	0.31± 0.11*	0.21±0.11	0.22±0.11*	0.31±0.12*
S_1	-	-	0.17±0.08*	0.16±0.17	0.21±0.07**
S_2	-	0.27±0.13*	0.18±0.12	-	0.07±0.12
S_3	0.14±0.15	0.07±0.12	0.24±0.08**	0.02±0.18	0.16±0.15
S_4	0.07±0.12	-	0.14±0.11	0.24±0.18	0.05±0.11
S_5	0.11±0.58	0.20±0.57	0.16±0.52	-	0.16±0.59
S_6	0.24±0.19	0.14±0.12	0.16±0.13	0.01±0.07	0.26±0.16
Pooled	0.16±0.005**	0.20±0.004**	0.19±0.002**	0.09±0.003**	0.18±0.002**
TT	0.0124±0.021	-0.0254±0.0234	-0.014± 0.007*	-0.025±0.0264	-0.0096±0.0196

TT - time trend of h^2 estimates, *significant at P<0.05, **significant at P<0.01.

Table 3. Estimates of genetic, environmental and phenotypic correlations between different traits and their time trends

Gen	ASM × WAM			ASM × EP_{100}			ASM × CS			ASM × EM			WAM × EP_{100}		
	rG(s)	rE(s)	rP	rG(s)	rE(s)	rP	rG(s)	rE(s)	rP	rG(s)	rE(s)	rP	rG(s)	rE(s)	rp
S_0	0.38	-	0.58	-0.20	0.06	0.05	-0.21	0.06	-0.10	-0.89	-0.48	-0.65	0.75	0.06	0.08
S_1	-	-	0.07	-	-0.14	-0.44	-	-	-0.48	-	-	-0.78	-	0.10	0.21
S_2	-	-	0.14	-	0.34	-0.03	-	0.08	0.15	-	-0.48	-0.63	0.37	0.21	0.22
S_3	0.64*	-0.13	-0.04	-0.31	-0.24	-0.18	-0.41	0.12	-0.14	-0.85	-0.52	-0.57	-0.03	-0.12	-0.10
S_4	-	0.03	0.23	-0.58	-	-0.62	-0.61	-0.40	-0.42	-0.28	-0.82	-0.84	-	-	-0.18
S_5	0.52	0.48	0.48	-0.64*	-0.14	-0.29	-	-0.46	-0.22	< -1	-0.66	-0.80	0.23	0.02	-0.02
S_6	0.55	0.07	0.18	-0.70*	0.25	-0.27	-0.74**	-0.13	-0.21	-0.83	-0.49	-0.65	-0.27	-0.20	0.09
Pooled	0.54**	0.11	0.23	-0.47**	0.02	-0.25	-0.48**	-0.12	-0.20	-0.49**	-0.58	-0.70	0.14**	0.01	0.04
TT	0.024	-0.247*	-0.01	-0.09	0.0094	-0.045	-0.091	-0.0742	-0.046	-0.029	-0.023	-0.009	-0.139	-0.022	-0.03

TT - Time trend of correlation estimates,*significant at P<0.05, **significant at P<0.01.

Table 4. Estimates of genetic, environmental and phenotypic correlations between different traits and their time trends

Gen	WAM × CS			WAM × EM			EP_{100} × CS			EP_{100} × EM			CS × EM		
	rG(s)	rE(s)	rP	rG(s)	rE(s)	rP	rG(s)	rE(s)	rP	rG(s)	rE(s)	rP	rG(s)	rE(s)	rp
S_0	0.36	-0.01	-0.005	0.13	0.01	-0.35	0.37**	0.69	0.71	0.68	0.66	0.67	>1	0.58	0.60
S_1	0.56	-	-0.04	-	0.12	-0.02	-	0.68	0.72	0.94*	0.81	0.83	0.68	0.74	0.71
S_2	-	0.02	0.04	-	-0.23	0.09	> 1	-	0.61	> 1	0.76	0.76	0.001	0.35	0.38
S_3	-0.12	0.09	0.05	-0.81	0.40	0.17	0.46	0.48	0.48	0.67*	0.82	0.83	0.58	0.48	0.43
S_4	0.36	0.32	-0.06	-0.68	0.48	-0.19	0.58	0.12	0.67	0.35	0.87	0.88	0.11	0.59	0.62
S_5	-	-0.02	-0.12	0.30	-0.49	-0.39	0.59	0.32	0.69	0.97	0.82	0.77	0.89 **	0.68	0.58
S_6	-0.32**	0.006	-0.006	-0.40	-0.02	-0.09	> 1	0.61	0.61	0.92 **	0.86	0.85	0.82	0.56	0.57
Pooled	-0.32 **	0.07	-0.02	-0.13 *	0.04	-0.11	0.50 **	0.48	0.64	0.90 **	0.80	0.80	0.82 **	0.57	0.56
TT	-0.115	0.007	-0.0094	-0.075	0.021	-0.009	0.05	-0.049	-0.011	0.014	0.026	0.019	0.083	0.0021± 0.0007**	-0.004 ±0.024

TT - Time trend of correlation estimates, *significant at P<0.05, **significant at P<0.01.

environmental and phenotypic correlation of EP_{100} with CS and EM were all highly positive (P<0.01) and significant (Table 4). These findings between different combinations of traits are in agreement with Renganathan *et al.* (1983). The time trend of estimates of environmental correlation between ASM and WAM (Table 3) was negatively significant while between CS and EM (Table 4) was positively significant. The time trends of estimates of genetic, environmental and phenotypic correlation between different combinations of traits except above combinations of traits were all nonsignificant, either negative or positive. Similar type of observations were recorded by Chatterjee *et al.* (2000). Thus, the nonsignificant time trends in estimates of genetic correlation did not provide a strong argument against the general tendency for negative trends in the genetic correlation observed by Frairs *et al.* (1962).

The realized phenotypic response of ASM and EM were significant in desirable directions and for other traits the response were not significant. The additive genetic variance of the traits in the population was slightly reduced as indicated by significant negative time trend (though small in magnitude) of heritability estimate of the primary trait and nonsignificant negative time trends of heritability estimate of the secondary traits except for ASM. The pooled estimates of correlation between traits were in good agreement with other workers. Mostly, there was no time trend of correlation estimates. The average (or realized) phenotypic response, estimates and time trends of heritability and correlations have given indications for the traits to be included in selection index or else, the selection criterion is to be changed for the studied population, or the population size ought to be increased within generation.

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Scope for utilization of sunflower heads as animal feed in Karnataka state

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ABSTRACT

A survey was carried out to assess the utilization pattern of sunflower heads (SFH) in the districts of Bellary, Raichur and Chitradurga, which are the major sunflower growing areas of the Karnataka state. The survey revealed that currently sunflower heads are not being fed to any class of livestock, and are mainly used for composting or burnt or dumped on way side. Even though sunflower heads are not being fed, the survey revealed that animals consumed SFH in a limited way. Small ruminants have a greater liking for SFH than the large ruminants. The major reason ascribed for not feeding SFH is lack of tradition and availability of other feeds in sufficient quantities. The chemical composition and *in vitro* studies revealed that SFH is nutritionally better than many of the commonly used roughages. Proper extension and demonstration of utilization of SFH is required for popularizing the use of SFH as livestock feed.

Key words: Feed, Nutrition, Nutritive value, Proximate composition, Sunflower heads

In view of the shortage of feed resources there is a continuous need to identify newer feed resources which could be used to augment the existing feed resources. Nearly one million tonnes of deseeded sunflower heads (SFH), a by-product of sunflower crop, is estimated to be available in India (CMIE 2000). Sunflower heads has been successfully incorporated at different levels ranging from 15 to 50 % in complete diets of cattle and sheep (Reddy *et al.* 1986, Goud *et al.* 1987, Reddy and Reddy 1998, Rao *et al.* 1999). In view of its availability in abundance and its nutritive value it has a good potential to be used as a livestock feed. Hence the present survey was undertaken in the major sunflower growing districts of Karnataka state to have a first hand information on the utilization pattern of SFH and to know the farmers' perception about its nutritive value/ suitability as livestock feed.

MATERIALS AND METHODS

Raichur, Bellary and Chitradurga districts, the major producers of sunflower in Karnataka state, were surveyed. Two tehsils in each district and two villages in each tehsil were selected for the survey. At least 15 sunflower growing farmers from each village were surveyed as per the format developed to assess the utilization pattern of SFH and to know their perception about its value as animal feed. Samples of popularly grown sunflower varieties were collected from the farmers' field and All India Coordinated Research project on Sunflower from the University of Agricultural Sciences, Bangalore. The samples collected were analysed for the proximate composition (AOAC 1984) and *in vitro* organic matter digestibility (Goering and Van Soest 1970).

RESULTS AND DISCUSSION

Utilization pattern of sunflower heads

Survey results on 130 farmers surveyed are presented in Table 1. Currently SFH is not being fed to any class of livestock by the farmers and the main disposal methods followed by the farmers surveyed was by composting (37%), dumped on the way side (32%) or burnt (31%). The mode of disposal varied across the districts, SFH was disposed chiefly by burning in Raichur while composting was common in Bellary and Chitradurga districts. Even though SFH is not being fed, majority of the farmers (59%) observed that SFH is consumed by animals and their numbers were maximum in Chitradurga district (85%) while majority (62%) of the farmers in Raichur were of the opinion that SFH is not consumed by the animals. Within the animals the acceptability of SFH varied and it was reported that small ruminants had a greater liking than the large ruminants. Sheep had the maximum liking for SFH followed by goats, buffaloes and least by the cattle. However 40% of the respondents in the Bellary district felt that buffaloes had the maximum liking for SFH. With regard to the consumption pattern of SFH majority of respondents (77%) reported that SFH is not consumed continuously by the animals and the animals generally eat little and then move away from it and return to

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Table 1. Utilization pattern of sunflower heads (SFH) by the farmers in the districts of Karnataka

Particulars		Name of district			
		Raichur	Bellary	Chitra dudga	Average
Number of farmers surveyed		49	44	37	130
		(in percentage)			
Utilization of sunflower head	Used as manure	24.24	40.00	57.89	37.41
	Burnt	43.94	25.71	13.16	30.94
	Fed to animals	00.00	00.00	00.00	00.00
	Dumped on way side	31.82	34.29	28.95	31.65
Whether animals eat sunflower	Yes	38.88	43.90	85.29	58.06
	No	61.11	56.10	14.71	41.94
Which species of animals like it more	Cattle	00.00	00.00	13.64	07.40
	Buffaloes	00.00	40.00	09.09	22.22
	Sheep	28.57	34.29	45.45	41.98
	Goats	00.00	25.71	31.82	28.40
Eating pattern of animals	Eat continuously	17.24	00.00	40.00	23.26
	Eat little and leave the rest	75.86	100.0	60.00	76.74
Why SFH is not being stored when animals eat?	Tradition is not there	35.71	32.05	37.93	34.90
	Feeding value not known	00.00	16.67	06.90	08.85
	Apprehension that it may cause some ill effect	10.71	05.13	22.41	11.98
	Availability of sufficient quantity of other feeds and fodders	19.64	33.33	24.14	26.56
	Any other factor	33.93	12.82	08.62	17.71
If the feeding value of SFH is demonstrated, will you	Use SFH as animal feed	70.83	63.27	78.38	74.07
	Continue with the traditional feeding	00.00	00.00	00.00	00.00
	Others	13.58	13.64	13.51	13.58
If some agency procures SFH on payment basis	Store SFH for selling	85.30	77.27	93.33	73.11
	Will throw off	13.56	20.45	06.67	13.10
	Any other	00.00	02.27	00.00	01.19

Table 2. Proximate composition, *in vitro*-organic matter (IVOMD) and neutral detergent fibre (IVNDF), digestibility of different popular hybrids of sunflower heads

Sl.No	Hybrids	DM %	OM %	CP %	EE %	CF %	Ash %	NFE %	NDF %	IVOMD %	IVNDFD %
1	Mahyco-1	90.22	93.44	8.22	4.29	29.86	06.56	51.07	38.23	76.49	43.75
2	Mahyco-17	89.49	86.75	9.02	4.41	21.26	13.25	52.06	35.06	74.97	39.36
3	NSP-92	91.04	87.27	9.42	3.64	20.11	12.73	54.10	36.79	72.89	34.97
4	DSH-1	92.27	87.84	7.76	7.37	17.69	12.16	44.98	33.13	78.43	42.81
5	Modern	92.11	86.56	7.20	2.72	18.19	13.44	58.45	43.39	66.56	33.29
6	Ganga Kaveri	89.85	84.46	9.64	3.87	24.63	15.54	46.32	45.82	71.96	48.37
7	KBSH-1	90.44	87.79	11.43	3.66	24.16	12.21	48.54	45.84	74.78	51.68
8	MSFH-17	92.35	84.73	11.66	2.95	24.69	15.27	45.43	41.88	71.84	43.02
9	PAC-1091	86.98	86.18	10.14	2.39	25.97	13.82	47.68	45.15	72.90	48.38
10	Sungene-85	89.64	86.11	11.59	2.65	26.70	13.89	45.17	52.09	69.31	49.28
Average		90.44±	87.11±	9.61±	3.80±	23.33±	12.89±	49.38±	41.74±	73.01±	43.49±
		0.52	0.79	0.51	0.46	1.24	0.79	1.41	1.86	1.09	1.95
Composition of some commonly used roughages in the region											
Para grass		85.00	87.60	5.30	2.0	45.70	12.40	34.60	65.61	75.01	67.47
Ragi straw		90.00	89.30	3.70	1.1	57.20	10.70	33.00	69.66	45.37	28.45
Paddy straw		90.00	90.90	3.10	2.8	46.10	9.10	28.90	-	-	-
Sorghum stover		90.00	91.70	3.80	1.3	51.00	8.30	35.60	70.00	64.00	52.00

it after a while indicating an intermittent pattern of feeding.

Reasons for not feeding sunflower heads

Analyzing various reasons for not feeding SFH by the farmers revealed that majority of the farmers (35%) attributed the lack of tradition as the major reason for not feeding SFH to livestock. The sunflower is a new crop and introduced only two decades back and as such the tradition of feeding SFH does not exist. Traditions have an important place in the rural lifestyles where they profoundly influence the livestock rearing practices. Of the farmers surveyed, 27% were of the view that SFH is not utilized for feeding of animals because of adequate availability of other feeds and fodder. Some of the respondents (18%) attributed poor palatability to be the reason for not feeding it to livestock. Some of the farmers (12%) were not feeding SFH because of apprehensions that feeding SFH may cause digestive disorders, respiratory problems and dullness in the livestock. However these apprehensions were not supported by any clinical reports in the villages surveyed. Few respondents (8%) attributed lack of awareness as the reason for not feeding SFH.

The farmers were of the opinion that if the feeding value of SFH was demonstrated, they would like to use the SFH as animal feed. However, very few felt that the use of SFH depends on the convenience of usage (as sole roughage/mixed with other feeds/as a part of complete feed) and the method of feeding. The farmers were ready to store SFH, if some agency procures SFH on payment basis as it would give them an additional income.

Chemical composition of sunflower heads

The proximate composition, *in vitro*-organic matter digestibility (IVOMD) and neutral detergent fibre digestibility (IVNDFD) of different popular hybrids and the chemical composition of the commonly used dry forages and roughages are given in Table 2. Among the different hybrids the crude protein content varied from 7.20% (Modern) to 11.66% (MFSH-11). The crude fibre and neutral detergent fibre ranged from 17.69 to 26.70 and 33.13 to 52.09% in DSH-1 and Sungene-85 respectively. The ether extract ranged from 2.39% (PAC-1091) to 7.39% (DSH-1) with an average value of 3.80%. The differences observed could be because of differences in the variety, agro-climatic conditions and management practices. The proximate composition of SFH in terms of protein, crude fibre, fat and neutral detergent fibre was superior to many of the commonly used roughages like ragi straw, paddy straw, sorghum stover, maize stover etc. The average crude protein content (9.6%) of SFH was twice or thrice the protein content of the commonly used roughages. Similarly the crude fibre and neutral detergent fibre levels were lower than the values present in the commonly used roughages sources indicating that the fibre levels cannot be the limiting factor in utilization of SFH. Further the *in vitro* organic matter and neutral detergent fibre digestibility of SFH was higher than the values of the other commonly used roughages. The chemical composition and the *in vitro* digestibility values of SFH indicated that it has the potential to be used as livestock feed resource. Earlier SFH has been successfully incorporated in complete feeds at different levels i.e., 20% in lactating cows (Rao *et al.* 1999), 29% in growing bulls (Reddy and Reddy 1998) and 50% in sheep (Reddy *et al.* 1986) without affecting their production performance. Mohan *et al.* (1997) have reported that SFH can be used as a sole roughage source in rations to meet the maintenance requirement of crossbred bulls.

The survey work undertaken and the chemical composition of the different varieties of SFH has clearly indicated that the by-product has a potential to be used as animal feed especially in the sunflower growing districts of Karnataka state. Tapping of this new feed resource for animal feeding would add to the total availability of the feed resources. However, for propagating the use of SFH as animal feed the points to be considered for implementation are- (i) frontline demonstration, (ii) creating awareness through appropriate extension methods, and (iii) use in preparation of complete feeds thereby creating a commercial value for the by-product.

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Growth performance, nutrient utilization, rumen fermentation and enzyme activities in calves fed on *Saccharomyces cerevisiae* supplemented diet

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ABSTRACT

To study the effect of dietary supplementation of yeast cells (YC) on body weight gain, nutrient digestibilities, production of ruminal metabolites and enzymes activities in the rumen, 16 crossbred cattle calves of 150.3±14.3 kg live weight were used. The animals were distributed into 2 groups and fed 65% concentrate mixture and 35% wheat straw diet. Calves of the experimental group were given a daily dose (10 ml) of yeast cell suspension (YC) containing 5×10^9 cells/ml of *Saccharomyces cerevisiae* ITCCF 2094 for 159 days. There was no difference in the body weight gain, feed intake, feed conversion efficiency, digestibilities of nutrients and the nutritive value of diet between the groups. The activities of rumen enzymes, viz. carboxymethyl cellulase, amylase, xylanase, β-glucosidase, urease, aspartic and alanine transaminases were unaffected but protease was lower ($P<0.05$) in yeast fed animals. Production of TVFA remained unaffected but NH_3-N and lactic acid decreased ($P<0.05$) and pH increased ($P<0.05$) in the rumen liquor of YC group. There was no effect of YC feeding on the number as well as types of ciliate protozoa in the rumen liquor of calves.

Key words: Calves,Enzyme activity, Growth, Nutrient utilization, Rumen fermentation, *Saccharomyces cerevisiae*

Nature has provided unique gift to ruminants in the form of rumen for the utilization of fibrous feeds through the process of rumen fermentation. There is scope for altering or manipulating rumen fermentation to increase the nutrient utilization efficiency of the animals. During the last 30 years various rumen manipulating agents have been used (Dawson 1993). The increasing concern over the use of antibiotics and growth stimulants has increased the interest towards the use of live microbial cultures in livestock production (Nisbet and Martin 1990). *Saccharomyces cerevisiae* is one of the most widely used probiotic in the livestock feeding. Though the supplementation of microbial cultures is well accepted in livestock production but their effects are highly variable (Fuller 1989, Douraeu and Jouany 1998). There was an increase in milk production in cows fed on yeast supplemented diet (Putnam *et al.* 1997, Wohlt *et al.* 1998). No improvement in live weight gain in bulls and grazing steers by yeast supplementation was observed (Cabrera *et al.* 2000). There are many factors which govern the probiotic expression of yeast inside the body such as, environment, nutritional status, type of microbe, mode of feeding etc. Therefore, the present experiment was conducted to explore the efficiency of selected yeast strain ITCCF 2094 as a probiotic in the crossbred calves under set conditions.

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MATERIALS AND METHODS

Animals

Sixteen crossbred (inter se, *Bos* indicus × *Bos taurus*) calves (7 months, average body weight 150.3 kg) were distributed in 2 equal groups on the basis of body weight. The treatments were (1) yeast cells (YC) and (2) control. The calves of group 1 received a daily dose of 10ml yeast cell suspension containing 5×10^9 cells/ml. *Saccharomyces cerevisiae* ITCCF 2094 was cultured on agar surface in culture flasks on YEPD agar, containing peptone 1%, dextrose 2%, yeast extract 0.3% and agar 2%. These flasks were incubated at 39°C for 48hr. The yeast cells grown on the agar surface were collected by washing with sterile normal saline. The desired concentration of yeast cells (5×10^9 cells/ml) was obtained by diluting the suspension with normal saline and adjusting optical density at 540nm. The calves were housed under hygienic conditions in experimental shed with individual feeding facility for each animal. The calves were offered the calculated amount of concentrate mixture and wheat straw *ad lib.* to support 500g daily gain (NRC 1989). The concentrate mixture contained 60% crushed maize, 27% groundnut-cake, 10% wheat bran, 2% mineral mixture and 1% common salt on fresh basis. Chemical composition of

Table 1. Chemical composition of concentrate mixture and wheat straw (g/100gDM)

Item	Concentrate mixture	Wheat straw
Organic matter	88.0	87.2
Ash	12.0	12.8
Crude protein	19.3	3.2
Ether extract	3.5	3.3
Neutral detergent fibre	61.0	76.0
Acid detergent fibre	19.8	48.7

wheat straw and concentrate mixture is given in Table 1. Yeast culture (10 ml) was mixed with a small amount of concentrate mixture and offered to the animals at 10.00hr after the animals finished this, the balance amount of concentrate was fed. Animals were fed once daily at 10.00hr. Water was available *ad lib.* at 11.00 and 16.00 hr. Feed offered and residue left were recorded daily to calculate daily feed intake.

Growth and digestion trial

During the experimental period of 159 days, the calves were weighed fortnightly and consequent 2 days before the beginning and in the end of the digestion trial. At the end of the growth study a digestion trial of 6 days duration was conducted to determine the digestibility of the nutrients. The faeces and urine samples were collected after 2 days for the conditioning of the animals in the metabolism cages.

Sampling of rumen liquor

At the end of digestion trial, samples of rumen liquor were drawn by stomach tube after 4hr feeding for 2 consecutive days. Rumen fluid samples were strained through muslin cloth and pH was recorded immediately after sampling. A 30ml aliquot was stored under frozen condition after adding a few drops of diluted sulphuric acid for biochemical analysis. A 2ml aliquot was kept in a tube containing 2ml methyl formal saline reagent for protozoal counting. Another 30ml aliquot was sonicated in ice-bath immediately after collection for the estimation of cellular enzymes. Thereafter the samples were centrifuged at 27 000×g at 4°C for 30min. The clear supernatant was used as enzyme source.

Chemical analysis

The feed offered, residue left and faeces voided were analyzed for proximate principles as per AOAC (1980). The neutral detergent fibre and acid detergent fibre were analyzed following the method of van Soest *et al.* (1991). The rumen liquor samples were analyzed for TVFA (Barnett and Reid 1956), NH_3-N (AOAC 1980) and lactic acid as per Barker and Summerson (1941). Protozoa counting in the rumen liquor was done by the method of Kamra *et al.* (1991). The activities of carboxymethyl cellulase, amylase, xylanase, were estimated by using carboxymethyl cellulose, starch and xylan as substrate, respectively, and the reducing sugars released were quantified by the method of Miller (1959). Urease (Weatherburn 1967), β-glucosidase (Shewale and Sadana 1978), aspartic and alanine transaminases (Reitman and Frankel 1957) were also estimated.

Statistical analysis

Data were analyzed using t- test to find out the difference between means as per Snedecor and Cocharan (1967).

RESULTS AND DISCUSSION

Chemical composition of concentrate mixture and wheat straw is given in Table 1. The values were almost similar to those reported by Singh *et al.* (1998). The data on dry matter intake, growth performance and feed conversion efficiency are presented in Table 2. There were no differences in mean DMI and DMI/100kg body weight in control and yeast supplemented group. The DMI through concentrate and straw was same in the animals of both the groups. No change in DMI (Erdman and Sharma 1989), whereas, increased DMI (Putnam *et al.* 1997) by yeast supplementation was observed in dairy cows.

The calves of both the groups showed similar growth performance and feed conversion efficiency. Panda *et al.* (1995) reported higher live weight gain in the calves fed on diet supplemented with yeast along with culture medium. Since culture medium is a good source of nutrients and vitamins (Chaucheyras *et al.* 1995), hence it may be one of the reasons of higher weight gain in the later case. Higher weight gain in the yeast supplemented group was also reported by McLeod *et al.* (1991) in cattle whereas, Mutsvongwa *et al.* (1992) and Cabrera *et al.* (2000) observed no change in live weight gain and fibre digestibility by yeast supplementation. The variability in the reports on yeast feeding may be due to factors such as type of diet, physiological stage of animals, environmental conditions and strain of microbe used, etc.

The digestibilities of DM, OM, NDF, ADF and EE were similar between the groups (Table 2). Since the DMI and nutrient digestibility were same, the DCP and TDN contents of the ration were also same. Williams and Newbold (1990) did not observe any improvement in digestibility in yeast fed cows. Jouany *et al.* (1998) also reported no change in nitrogen digestibility by YC or *Aspergillus oryzae* supplementation in sheep, however, higher digestibility in yeast fed animals was reported by Wiedmeier *et al.* (1987) and Panda *et al.* (1995). According to Williams and Newbold (1990), the yeast cells may alter the site of digestion and hence the total digestive tract digestibility does not give an accurate picture of effect of yeast feeding in the rumen. No change in total tract digestibility was observed by yeast feeding (Newbold *et al.* 1995).

The activities of rumen enzymes, viz carboxymethyl cellulase, α-amylase, β-glucosidase, xylanase, protease, urease, aspartic and alanine transaminases are presented in

Table 2. Body weight gain and feed conversion efficiency (FCR), digestibility of nutrients and nutritive value of diet in calves

Item	YC	Control
Body weight gain		
Initial body weight (kg)	150.0±17.4	150.7±12.6
Final body weight (kg)	239.2±17.7	239.0±20.4
Average daily gain (g)	561±59	555±39
FCR (kg feed/kg gain)	9.41±0.30	9.70±0.27
Dry matter intake (kg/d)		
Concentrate	3.76±0.09	3.60±0.13
Roughage	2.17±0.21	2.05±0.23
Total	5.93±0.26	5.65±0.19
Digestibility of nutrients (%)		
Dry matter	65.3±1.9	67.0±1.5
Organic matter	64.7±2.8	66.3±2.2
Neutral detergent fibre	56.3±2.6	57.5±3.1
Acid detergent fibre	46.8±2.0	48.5±2.7
Crude protein	75.6±2.6	73.1±3.0
Ether extract	76.2±3.9	74.8±2.5
Nutritive value of ration (%)		
Digestible crude protein	10.1±0.3	10.0±0.2
Total digestible nutrients	66.4±3.6	68.0±2.9

Table 3. There were no differences in the enzyme activities between the 2 groups except protease which was significantly higher ($P<0.05$) in the rumen liquor of control than that in yeast supplemented group. The rumen enzymes are secreted by the microbes present in the rumen, thus representing the microbial status of the animal. No effect on enzyme activities thus may indicate no impact of yeast feeding on rumen microbial population and this may be the reason for similar nutrient digestibilities between the groups. The lower ($P<0.05$) protease activity in the yeast fed group gives some indication of shifting of site of protein degradation i.e. from rumen to lower tract.

Production of rumen metabolites is an indicator of microbial efficiency of nutrient degradation. In the present experiment, the production of VFA was similar in both groups. However, the ammonia nitrogen and lactic acid were higher ($P<0.05$) and pH was lower in the control group than in yeast supplemented group (Table 3). The higher ammonia nitrogen content in the control group may be due to higher protease activity. Significantly lower concentration of NH_3-N due to yeast feeding in buffalo fed high roughage diet was also reported by Kumar *et al.* (1997). Lower lactic acid concentration and higher pH of rumen liquor is a positive effect of yeast feeding which favours the efficiency of the rumen. It has been reported that by feeding yeast, the number of lactic acid utilizing bacteria increases in the rumen which resulted in the low concentration of lactic acid and high pH (Wallace 1996).

The ciliate protozoa as counted in the rumen liquor were in the range of 10^4 to 10^5 which was within the normal range as reported by Coleman (1988) and Kamra *et al.* (1991). Out of total protozoa, entodinomorphs were 94% and holotrichs

Table 3. Changes in hydrolytic enzymes, rumen metabolites and protozoa in the rumen liquor of animals fed *Saccharomyces cerevisiae* as a feed supplement

Item	YC	Control
Enzymes (units/100ml of rumen liquor)		
Carboxymethyl cellulase[a]	29.17±4.22	24.60±7.70
Amylase[a1]	22.67±5.07	26.29±4.11
Xylanase[b]	19.30±4.63	21.0±3.90
β-glucosidase[c]	45.07±1.01	45.60±0.64
Protease*[d]	53.50±5.33	76.8±7.00
Urease[e]	4.00±0.75	4.98±1.00
Aspartic transaminase[f]	0.57±0.05	0.63±0.03
Alanine transaminase[f]	0.43±0.06	0.49±0.07
Rumen metabolites		
TVFA (mmol/100 ml)	10.7±0.3	11.2±0.5
NH_3 N (mg/100ml)*	20.1±0.6	23.9±1.0
Lactic acid(mg/100ml)*	8.00±0.60	11.0±0.97
pH*	6.86±0.06	6.27±0.14
Ciliate protozoa (10^4/ml rumen liquor)		
Entodiniomorph		
Large	4.57±0.37	5.71±0.30
Small	59.3±2.58	54.26±3.79
Holotrich		
Large	2.10±0.54	4.87±1.19
Small	0.33±0.20	0.55±0.32
Total protozoa	68.25±2.53	65.41±5.59

Unit: a, µmol glucose/hr; a1, µmol glucose/min; b, µmol xylose/min; c, µmol p-nitrophenol /min; d, mg casein hydrolyzed/hr; e, µmol ammonia/min; f, µmol pyruvate/min; *significant $P<0.05$.

were 6%. The number of protozoa was same in both groups. An interaction between yeast feeding and rumen protozoa population was reported in sheep (Mathieu *et al.* 1996). Increased protozoal count by yeast feeding was also observed (Plata *et al.* 1994).

Effect of microbial feed additives on livestock production is still at a stage where it varies from no effect to positive effect, differing from species to species, strain to strain of the microbes. In the present study though there was no effect on performance of animal. Thus it is concluded that the strain of yeast used in the present experiment was not efficient enough to improve performance of animals under the set of conditions of this experiment.

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Influence of isobutyric acid supplementation on nutrient intake, its utilization and growth performance of crossbred calves fed low protein and urea containing rations

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ABSTRACT

The present experiment was conducted to observe the beneficial effect of dietary supplementation of sodium salt of isobutyric acid in low protein and urea containing wheat straw (WS) based diets. Eighteen crossbred growing calves, divided into 3 similar groups, were fed a basal diet consisting of wheat straw, concentrate mixture and green oat fodder in 40:40:20 proportion (T_1), basal diet + isobutyric acid (BCFA) @0.75% of basal diet (T_2) and basal diet +BCFA+ Urea to replace 1/3rd of dietary nitrogen (T_3) for 120 days. The average DMI (kg/100kg BW) was higher in T_3 (2.55) as compared to T_1 (1.95) and T_2 (1.95). The dietary supplementation of isobutyric acid improved the DM, OM and CF digestibilities by 8.6, 8.0 and 13.2% over T_1. The DCP content of experimental rations was similar in all the groups. However, the DCP intake (g/day and g/kg $W^{0.75}$) was higher in T3 (304.55 and 6.70) as compared to T1 (217.90 and 5.01) and T2 (226.94 and 5.52). Similarly TDN intake (g/day and g/kg $W^{0.75}$) was also higher ($P<0.05$) in T_3 (2.63 and 58.20) as compared to T_1 (1.80 and 41.80) and T_2 (1.90 and 43.95). However, the TDN content of both the BCFA supplemented rations was significantly ($P<0.01$) higher (64.35 and 63.23%) as compared to T_1 (59.60). The total gain in live weight in T_2 and T_3 was higher by 15.94 and 30.02%, respectively, over T_1. The average daily gain and efficiency of feed conversion were also higher in BCFA supplemented groups i.e., T_2 and T_3 calves by 13.38 and 26.71; and 12.48 and 22.16%, respectively, over T_1. The dietary supplementation of isobutyric acid improved the digestibility of nutrients and growth performance of calves.

Key words: Cattle, Growth, Isobutyric acid, Nutrient utilization, Volatile fatty acid

The present experiment was conducted to observe the beneficial effect of dietary supplementation of sodium salt of isobutyric acid in low protein and urea containing wheat straw (WS) based diets on nutrient utilization and growth performance in crossbred calves.

MATERIALS AND METHODS

Healthy crossbred male calves (18) of 1 year to 1 year and 6 months age were randomly divided in to 3 groups, based on body weight. Animals were fed composite diets consisting of WS, concentrate mixture (CM) and green oat fodder in 40:40:20 proportion, on dry matter (DM) basis (T_1), basal diet + isobutyric acid (BCFA) @0.75% of basal diet (T_2) and basal diet + BCFA + urea to replace 1/3rd of dietary nitrogen (T_3). The WS and CM were offered at 9:00 hr, while the green oat fodder was offered at 14:00 hr. Sodium salt of isobutyric acid was added @ 0.75% of total diet (on DM basis) in the form of aqueous solution in CM 2 and CM 3. All the diets were isonitrogenous (10% CP). The feeding experiment was

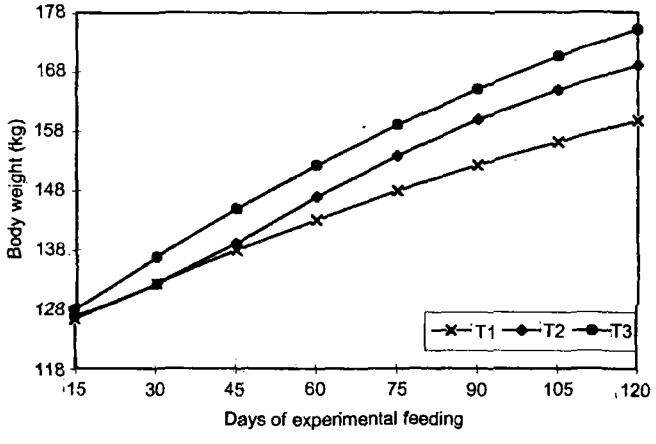

Fig. 1. Body weight changes in calves
Y T1 = 119.79 + 06.69X_1 - 0.34X_1^2 (r^2= 0.98 ; P<0.01)
Y T2= 109.96 + 11.10X_2 -0.46X_2^2 (r^2= 0.98 ; P<0.01)
Y T3 = 118.56 + 09.79X_3 - 0.34X_3^2 (r^2= 0.98 ; P<0.01)

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Table 1. Ingredient (%) and chemical composition (% of DM) of feeds and fodder

Components	Wheat straw	Green oat	CM-1 (No BCFA)	CM-2* (BCFA)	CM-3* (BCFA+urea)
Cereals					
Maize grain	-	-	65.00	65.00	75.00
Groundnut-cake	-	-	26.00	26.00	-
Wheat bran	-	-	6.00	6.00	8.50
Maize starch	-	-	-	-	10.50
Urea[1]	-	-	-	-	3.00
Minerals					
Mineral mixture	-	-	2.00	2.00	2.00
Common salt	-	-	1.00	1.00	1.00
Chemical composition					
DM	89.48	14.87	91.93	91.66	90.72
OM	88.40	89.05	89.70	89.30	92.50
CP	3.94	7.66	18.10	18.06	17.63
EE	1.33	1.33	6.00	5.89	2.33
CF	35.41	23.10	5.07	5.35	1.85
NFE	47.72	56.95	60.53	60.00	70.69
Ash	11.60	10.95	10.30	10.70	7.50

* Sodium salt of isobutyric acid (1.88 kg pure isobutyric acid, w/w per 100 kg DM of concentrate mixture) was added in aqueous form.
[1]Urea was used to replace 1/3rd of total dietary-N.

continued for 120 days. A metabolism trial of 7 days was conducted after 2 months of experimental feeding. Samples of feed offered, residue left, faeces and urine voided were analyzed for chemical composition AOAC (1984). Data were subjected to least square analysis of variance. Body weight changes of each animal were charted by fitting polynomial equations and the generated constants were subjected to analysis of variance to assess the treatment differences and significant group differences were compared by Duncan's multiple range test (Gomez and Gomez 1984). The pooled constants of the treatments were used for presentation of body weight changes (Fig. 1).

RESULTS AND DISCUSSION

The chemical composition of WS and oat fodder (Table 1)

Table 2. Dry matter intake and digestibility of nutrients in different groups

Parameter	T_1 (No BCFA)	T_2 (BCFA)	T_3 (BCFA +urea)
DM intake (g/day)*	3.02±0.02[a]	2.96±0.32[a]	4.16±0.09[b]
DM intake (kg/100 kg BW)*	1.99±0.16[a]	1.95±0.18[a]	2.55±0.12[b]
DM intake (g/kg $W^{0.75}$)*	70.32±5.75[a]	68.35±6.38[a]	91.86±3.37[b]
Nutrient digestibility (%)			
Dry matter*	60.25±0.41[a]	65.43±0.46[b]	65.08±0.18[b]
Organic matter*	62.99±0.55[a]	68.71±0.49[b]	67.93±0.20[b]
Crude protein*	70.63±1.30[a]	74.44±0.70[b]	69.53±0.35[a]
Ether extract	75.70±0.65	75.51±1.22	75.29±0.77
Crude fibre*	52.72±1.18[a]	59.67±0.50[b]	59.06±0.71[b]
Nitrogen free extract*	63.87±0.48[a]	69.80±0.70[b]	70.02±0.20[b]
Nutritive value and intake			
DCP intake (g/day)*	217.90±16.55[a]	226.94±32.49[a]	304.55±7.47[b]
DCP intake (g/kg $W^{0.75}$)*	5.07±0.41[a]	5.22±0.66[a]	6.70±0.21[b]
DCP (%)	7.19±0.13	7.51±0.30	7.31±0.17
TDN intake (kg/day)**	1.80±0.11[a]	1.90±0.20[a]	2.63±0.06[b]
TDN intake (g/kg $W^{0.75}$)*	41.80±2.82[a]	43.95±4.03[a]	58.20±2.00[b]
TDN (%)**	59.60±0.49[a]	64.35±0.45[b]	63.23±0.23[b]
Wheat straw in diet (%)*	44.57±1.20[a]	43.34±1.01[a]	46.68±0.98[b]
Concentrate mix. in diet (%)*	45.53±1.62[a]	41.40±1.31[b]	42.50±1.24[b]
Oat (%)*	14.90±0.91[a]	15.26±15.26[a]	10.82±0.56[b]

Different superscripts in a row indicated significant difference; *, P<0.05; **, P<0.01.

Table 3. Nitrogen intake and retention

Attributes	T_1 (No BCFA)	T_2 (BCFA)	T_3 (BCFA +urea)
Total N intake (g/day)*	49.67±4.40[a]	48.70±6.78[a]	70.07±1.58[b]
Faeces-N excreted (g/day)**	14.80±1.86[a]	12.39±1.63[a]	21.34±0.46[b]
Urinary-N excreted	19.75±1.49	18.05±2.42	23.25±1.13
Total-N excreted (g/day)*	34.56±3.23[a]	30.44±3.85[a]	44.59±1.67[b]
N-digested (g/day)*	34.86±2.65[a]	36.31±5.20[a]	48.73±1.20[b]
N-retention (g/day)*	15.11±1.26[a]	18.27±3.18[a]	25.48±0.95[b]
Faecal N as % of N intake**	29.80±1.30[a]	25.44±0.71[b]	30.47±0.33[a]
Urinary N as % of N intake	39.76±1.22	37.06±4.01	33.18±1.18
% retention of N intake	30.42±0.83	37.50±3.68	36.36±1.45
N absorbed as % of N intake*	70.63±1.30[a]	74.44±0.71[b]	69.53±0.33[a]
N retained as % of N absorbed*	43.34±1.07[a]	50.32±5.10[b]	52.29±2.00[b]

Different superscripts in a row indicate significant difference; *, P<0.05; **, P<0.01.

was within the range of normal values. The ether extract (EE) and crude fibre (CF) content of CM 3, was lower than that of the CM 1 and CM 2, due to the use of only starch and maize grain (low in fat contents) in CM 3 to replace groundnut-cake.

Animals consumed significantly (P<0.01) higher DM in T_3 when compared with T_2 and T_1 (Table 2). The proportion of WS in total diet was higher in T_3 (46.68%), followed by T_1 (44.57%) and T_2 (43.34%), whereas the proportion of CM was higher in T_1 (45.53%) than T_2 (41.40%) and T_3 (42.50%). Similarly the proportion of green fodder was less in T_3 (10.82%) than that in T_1 and T_2 (14.90 and 15.26%). The deviation in proportion of WS, CM and green fodder in all the 3 experimental diets from envisaged proportion (40:40:20), occurred primarily due to variable intake of WS, which was fed *ad lib.* while the other 2 dietary components, viz. CM and green fodder were fed in fixed quantities.

Dietary supplementation of BCFA (T_2 and T_3) improved the DM and OM digestibilities by 8.60 and 8.02% over T_1. The improvement in DMD and OMD might have occurred from increased utilization of crude fibre component of diet as its digestibility was significantly (P<0.05) higher (59.67 and 59.06%) in BCFA supplemented groups than T_1 (52.72%), which could be owing to the increased number and activity of cellulolytic microbes (Van Gijlswijk 1970).

Although the DCP was similar in all the experimental diets, the DCP intake, (g/day and g/kg $W^{0.75}$) was higher (P<0.05) in T_3 (304.55 and 6.70) as compared with T_1 (217.90 and 5.07) and T_2 (226.94 and 5.52). Similarly the TDN intake (kg/day and g/kg $W^{0.75}$) was higher (P<0.01) in T_3 (2.63 and 58.20) compared with T_1 (1.80 and 41.80) and T_2 (1.90 and 43.95) owing to improved utilization of dietary nutrients. As a result TDN content of BCFA supplemented diet (Table 2) was also higher (64.35 and 63.23%) than T_1 (59.60%).

The N-intake was higher (P<0.05) in T_3 compared with T_1 and T_2. Similarly, the total-N excreted through faeces was higher (P<0.01) in T_3 (21.34 g/day) compared to T_1 and T_2 (14.80 and 12.39 g/day). But on its expression as per cent of total-N intake, both the groups (T_1 and T_3) had excreted similar quantity of N in faeces (29.80 and 30.47%), while that in T_2, it was significantly (P<0.01) lower (25.44%) when compared with other two groups owing to higher (P<0.05) CP digestibility. Urinary N excretion was similar in all the groups. The N retained was higher (P<0.05) in T_3 (25.48 g/day), followed by T_2 (18.27 g/day) and lowest in T_1 (15.11 g/day). Improved N retention in the present investigation is in agreement with several reports available on BCFA supplementation (Umunna *et al.* 1975, Felix *et al.* 1980b, Klusmeyer *et al.* 1987, Gunter *et al.* 1990).

The total gain in live weight in T_2 and T_3 fed calves (BCFA supplemented groups) was higher by 15.94 and 30.02% over T_1. Similarly, the average daily gain (ADG) in T_2 and T_3 fed calves was 13.38 and 26.71% higher (P<0.01) over T_1. The generated constants pooled for indicated that the growth profile of T_2 and T_3 calves was significantly (P<0.01) better than T_1 and these calves grew faster by 25.75 and 41.20% over those of T_1 (Fig. 1). The positive influence of BCFA supplementation in the present growth trial, is substantiated by several other investigations (Lassiter *et al.* 1958, Felix *et al.* 1980a, Deetz *et al.* 1985). The average DMI per kg live weight gain was lower (P<0.01) in T_2 and T_3 than T_1. However, the differences between two BCFA supplemented groups (T_2 and T_3) were nonsignificant. The efficiency of feed conversion (FCE) was improved by 12.48 and 22.16% in T_2 and T_3 over that of T_1. The reason for higher FCE in T_2 and T_3 calves was that these calves gained 15.94 and 30.02% more live weight with 12.48 and 22.16% less DMI consumption for each kg gain as compared to T_1. The improvement FCE in calves fed BCFA supplemented diets may be due to improved digestibility of nutrients and N metabolism (Lassiter *et al.* 1958, Deetz *et al.* 1985).

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Effect of dietary supplementation of sodium salt of isobutyric acid on rumen fermentation and nutrient utilization in crossbred cattle fed wheat straw based low protein and urea containing diets

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ABSTRACT

The present experiment was conducted to observe the effect of dietary supplementation of sodium salt of isobutyric acid in low protein and urea containing wheat straw based diets on ruminal fermentation and nutrient utilization in crossbred cattle. Twelve crossbred rumen fistulated cattle, divided into 3 similar groups, were fed a basal diet consisting of wheat straw, concentrate mixture and green maize fodder in 40:40:20 proportion (G1), Basal diet+ isobutyric acid (BCFA) @0.75 % of basal diet (G2) and basal diet +BCFA+ urea to replace 1/3rd of dietary nitrogen (G3). The feed intake among different experimental groups did not differ significantly and the average total DMI (kg/100 kg B.W.) was 2.01. 2.28 and 2.24 kg/d. in G1, G2 and G3 , respectively. The dietary supplementation of isobutyrate improved the DM, OM, NDF and cellulose digestibility coefficients by 14.46,10.51, 10.70 and 11.31%, respectively, in BCFA and BCFA + urea fed groups over that fed diet without BCFA (G1). The total N retention on BCFA supplementation was improved mainly due to decreased urinary N excretion. The concentrations of ruminal total N (mg/100 ml SRL) were 37.07, 34.77 and 34.64 in G1, G2 and G3, respectively. Dietary supplementation of Na-salt of isobutyric acid significantly ($P<0.01$) reduced the ruminal ammonia N concentration as compared to G1 and the mean values (mg/100 ml) were 13.18, 9.42 and 11.15 in G1, G2 and G3. The TCA-N concentration (expressed as % of N intake) was higher ($P<0.01$) in BCFA fed groups. Similarly, ruminal TVFA concentrations were higher ($P<0.01$) in BCFA supplemented groups (101.14 and 95.62 mM/litre) than without BCFA (93.05 mM/litre). Among VFAs, the concentration of acetate was higher ($P<0.01$) in BCFA supplemented groups (71.07 and 69.73 mM/litre) as compared to G1 (64.98 mM/litre). However, the concentration of propionate and butyrate remained unchanged. The dietary supplementation of Na-salt of isobutyric acid in low protein and urea supplemented diets improved the nutrient utilization and rumen fermentation characteristics.

Key words: Cattle, Feeding, Isobutyric acid, Nutrient utilization, Rumen fermentation, Volatile fatty acid

The present experiment was conducted to observe the effect of dietary supplementation of sodium salt of isobutyric acid in low protein and urea containing wheat straw based diets on ruminal fermentation and nutrient utilization in crossbred cattle.

MATERIALS AND METHODS

Healthy crossbred young bulls (12) of 2 to 2.5 years with average body weight of 251±15.45 kg fitted with permanent rumen cannula were randomly divided into 3 groups on the basis of body weight. Animals were fed composite diets consisting of WS, concentrate mixture (CM) and green maize fodder in 40:40:20 proportion, on DM basis (G1), basal diet+ isobutyric acid (BCFA) at 0.75% of basal diet (G2) and basal diet + BCFA + urea to replace 1/3rd of dietary nitrogen (G3). The WS CM were offered at 9:00 hr , while the green maize fodder was offered at 14:00 hr. The sodium salt of isobutyric acid was added at 0.75% of total diet on dry matter (DM) basis in the form of aqueous solution in CM2 and CM3. All the diets were isonitrogenous (10% CP). Animals were offered clean drinking water twice a day i.e. 10:00 and 14:00 hr.

After 25 days of experimental feeding, a 7-day metabolism trial was conducted in individual metabolism stalls with facilities for quantitative collection of faeces and urine. Samples of feed offered, residue left and faeces and urine voided were collected daily and representative samples were drawn for further analysis. Pooled samples were dried at 60°C till constant weight and ground for chemical analysis. A separate set of samples of faeces and urine from the daily

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collection were preserved in dilute sulphuric acid for N estimation. Rumen metabolism study was carried out just after completion of metabolism trial. Feeds and drinking water were offered before 2 hr of sampling. Rumen liquor samples (50-60 ml) were collected for 3 consecutive days, starting from day 33 to 35 at 0,2,4,6,8,10 and 12 hr post-feeding through specially made perforated stainless steel probes covered with nylon cloth from 4 different sites in the rumen. The samples drawn from the rumen were acidified with 0.2 ml of 10N H_2SO_4 and stored in deep freeze at 5 °C for later analysis.

The DM, N, ether extract (EE) and ash contents were determined according to AOAC (1990), while, the cell-wall constituents (CWC) were estimated following the method of Goering and VanSoest (1970). Rumen liquor samples were analyzed for total-N (AOAC 1990), NH_3-N (Conway 1962), TCA-precipitable-N (Cline *et al.* 1958) and total volatile fatty acids (Barnett and Reid 1957). Individual VFA were estimated as per Ervin *et al.* (1961) using gas liquid chromatography fitted with flame ionization detector and a pair of stainless steel columns (200 cm long and 0.20 cm diameter) packed with chromosorb as stationary phase.

Data were subjected to test of significance (Snedecor and Cochran 1980) and significant differences were compared by Duncan's multiple range test (Gomez and Gomez 1984).

RESULTS AND DISCUSSION

The DM of CM 2 (91.80%) and CM 3 (89.93%) were comparatively lower than the CM 1 (92.45%) which may be attributed to addition of aqueous solution of sodium salt of isobutyrate (Table 1). All the 3 concentrate mixtures were isonitrogenous. The EE content of CM 3 was lower (2.33%) than the CM 1 (6.33%) and CM 2 (6.17%), because only maize grain and starch (low fat feeds) were used to replace groundnut-cake (GNC) in the CM 3. As a result of inclusion of maize grain and starch, the crude fibre (CF) and CWC were also lower in CM 3. However, the nitrogen free extract (NFE) was slightly higher in CM 3 (68.95%) than CM 1 (61.88%) and CM 2 (61.23%).

The differences in total dry matter intake (DMI), among the experimental groups were nonsignificant (Table 2). Similar to the results observed in the present experiment, no effect of BCFA supplementation on DMI was reported in other studies conducted on growing and lactating animals (Felix *et al.* 1980, Klusmeyer *et al.* 1987). Dietary supplementation of BCFA improved the DM digestibility (DMD) by 4.46 and 6.63%, respectively, in G2 and G3, over the unsupplemented group (Table 2). Similarly, the digestibility of organic matter (OMD) was also improved in BCFA supplemented groups, i.e., G2 and G3 (60.55 and 60.83%) compared to G1 (54.79%). Improvement in DMD and OMD, might have occurred from the increased utilization of CF of diets as the coefficients of fibre digestibility were higher in BCFA supplemented diets, G2 (59.59%) and G3 (59.05%), than G1 (54.42%). The increase in DMD and OMD, observed in the present study corroborated with the earlier findings (Cline *et al.* 1966). The

Table 1. Ingredient (%) and chemical composition (% of DM) of feeds and fodders

Ingredient/nutrient	Wheat straw	Maize fodder	CM-1 (No BCFA)	CM-2* (BCFA)	CM-3* (BCFA+Urea)
Cereals					
Maize grain	-	-	65.00	65.00	75.00
Groundnut cake	-	-	26.00	26.00	-
Wheat bran	-	-	6.00	6.00	8.50
Maize starch	-	-	-	-	10.50
Urea[1]	-	-	-	-	3.00
Minerals					
Mineral mixture	-	-	2.00	2.00	2.00
Common salt	-	-	1.00	1.00	1.00
Chemical composition					
DM	91.10	20.98	92.45	91.80	89.93
OM	87.43	86.04	91.00	89.89	90.63
CP	3.50	8.75	17.94	17.50	17.50
EE	1.67	2.50	6.33	6.17	2.33
CF	37.20	25.75	4.85	5.00	1.85
NFE	45.06	49.04	61.88	61.23	68.95
Total ash	12.75	13.96	9.00	10.11	9.37
NDF	83.00	68.00	22.00	21.50	17.00
ADF	50.50	36.00	6.50	6.00	4.00
Hemicellulose	32.50	32.00	15.50	15.50	13.00
Cellulose	44.00	30.50	5.50	5.00	3.90

* Sodium salt of isobutyric acid (1.88 kg pure isobutyric acid, w/w per 100 kg DM of concentrate mixture -CM) was added in aqueous form.[1] Urea was used to replace 1/3rd of total dietary-N.

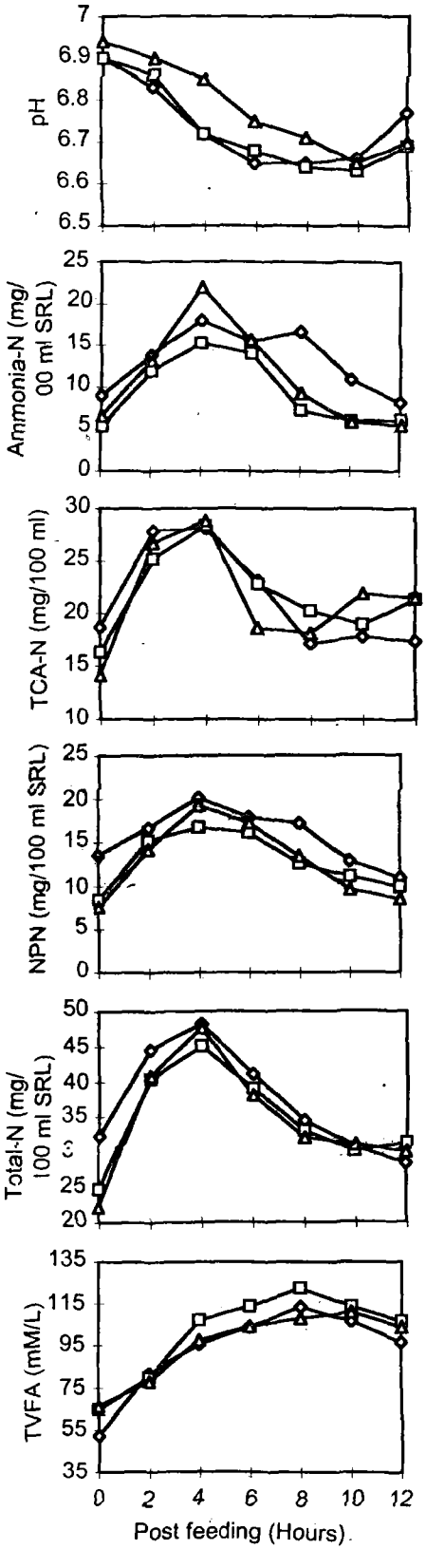

Fig. 1. Diurnal variations in ruminal metabolitis. No-□- BCFA; -◆- BCFA; -▽- BCFA + urea.

neutral detergent fibre (NDF) digestibility was 13.38 and 14.16% higher ($P<0.05$) in G2 (50.70%) and G3 (52.34%) over that of G1 (45.85%). Similarly, 11.31 and 8.56 increase in digestibility coefficients of cellulose were observed on G2 (61.90%) and G3 (60.37%) as compared with G1 (55.61%). However, the differences between BCFA supplemented groups were found to be nonsignificant. The improvement in NDF and cellulose digestibilities on BCFA supplementation observed in the present experiment, could be due to the increased number and activity of cellulolytic microbes (VanGylswyk 1970). These results corroborated with the findings of Cline *et al.* (1966) and Hefner *et al.* (1985), who also observed increased ruminal digestibility of NDF, ADF, *cellulose and hemicellulose on BCFA supplementation. Since* DMI and CP content of the ration and its digestibility remained unaffected due to BCFA supplementation, there was no variation in DCP intake on different diets (Table 2). The total digestible nutrient intake expressed as g/kg $W^{0.75}$, was significantly ($P<0.05$) higher in BCFA supplemented diets and this might be attributed to better digestibility of dietary components.

The differences in total N intake were nonsignificant (Table 2). The quantity of N excreted in urine was lower ($P<0.05$) in BCFA + urea supplemented group (41.62%) as compared to BCFA without urea (45.94%) and G1 (53.29%). The quantity of N retained (g/day) was maximum in G3 (14.88), followed by G2 (6.69) and lowest in G1 (1.17). These results suggested that dietary supplementation BCFA reduced the urinary-N excretion, thereby appreciable improvement in N retention was observed in G2 and G3 as compared with G1. These results are further confirmed by the findings of Van Gylswyk (1970) and Oltjen *et al.* (1971), where the dietary inclusion of BCFA positively affected the N metabolism primarily by reducing urinary-N excretion. Within the BCFA supplemented groups, improved N retention observed in animals fed BCFA + urea diet as compared with BCFA alone, might be ascribed to the readily available N and energy and consequently better synchronization in incorporation of NPN into microbial protein (Orskov 1982), since small amount of starch was added in the CM3 fed to BCFA + urea supplemented group.

Nonsignificant effect on rumen pH, in spite of higher TVFA production due to BCFA supplementation was observed in G2 and G3 (Table 3) might be due to the inclusion of isobutyric acid as Na-salt in the concentrate mixture. On hydrolysis of sodium isobutyrate, the release of Na molecule and subsequent attachment of H+ ions at the place of Na molecule on the fatty acids moiety, might have resulted in reducing the preponderance of H+ ions in the medium, *responsible for pH depression. The average pH values* recorded on dietary supplementation of BCFA in the present experiment were found to be optimal for fibre digestion (Mertens 1979) and cellulolytic bacterial growth (Orskov 1982). The results of the present study in which no effect of BCFA supplementation on ruminal pH was observed which

Table 2. Nutrient digestibility, retention and plane of nutrition

Parameter	G_1 (No BCFA)	G_2 (BCFA)	G_3 (BCFA +urea)	SEM
DM intake (g/day)	5.01±0.61	5.79±0.29	5.70±0.30	0.40
DM intake (kg/100 kg BW)	2.01±0.13	2.28±0.17	2.24±0.11	0.11
DM intake (g/kg $W^{0.75}$)	80.81±6.26	91.01±6.10	89.26±3.29	4.49
DCP intake (g/kg $W^{0.75}$)	4.35±0.17	4.46±0.16	4.86±0.11	0.16
TDN intake (g/kg $W^{0.75}$)*	44.50±1.58[a]	53.65±3.00[b]	51.12±1.72[b]	1.54
Total N intake (g/day)	79.29±6.94	84.69±2.55	83.83±3.90	1.32
Faeces-N excreted (g/day)	35.87±3.70	39.16±1.51	34.08±2.03	0.92
Urinary-N excreted	42.25±2.64	38.84±1.70	34.88±2.25	0.93
Total-N excreted (g/day)	78.12±6.02	78.00±2.77	68.95±4.26	1.28
N-digested (g/day)	43.42±3.54	45.53±1.14	49.70±2.68	1.03
N-retention (g/day)*	1.17±1.04[a]	6.69±2.09[b]	14.88±2.95[c]	0.93
Faecal N as % of N intake	45.24±3.69	46.34±0.60	40.67±1.59	0.97
Urinary N as % of N intake*	53.29±2.31[a]	45.94±2.16[b]	41.62±1.85[c]	0.77
% retention of N intake*	1.48±0.52[a]	7.85±0.42[a]	17.72±3.42[b]	1.04
N absorbed as % of N intake*	54.19±1.28[a]	53.79±0.54[a]	59.33±1.59[b]	0.67
N retained as % of N absorbed*	2.69±0.63[a]	14.62±5.05[b]	29.94±5.85[c]	1.41
Nutrient digestibility (%)				
Dry matter*	56.73±0.95[a]	59.26±1.48[b]	60.49±1.60[b]	0.54
Organic matter**	54.79±1.06[a]	60.55±1.44[b]	60.83±1.22[b]	0.53
Crude protein	54.91±1.28	54.04±0.41	59.33±1.60	1.39
Ether extract	77.02±4.24	77.64±3.63	71.37±1.04	1.18
Crude fibre*	54.42±1.97[a]	59.59±0.85[b]	59.05±1.50[b]	0.77
Nitrogen free extract	62.35±4.59	65.43±0.92	65.21±2.39	0.86
Neutral detergent fibre*	45.85±1.75[a]	50.70±0.80[b]	52.34±0.47[b]	0.58
Acid detergent fibre	41.56±2.07	47.44±0.52	45.11±0.76	0.48
Hemicellulose	50.26±2.42	57.75±1.21	58.83±1.20	0.88
Cellulose*	55.61±0.51[a]	61.90±0.86[b]	60.37±0.75[b]	0.72

Different superscripts in a row indicated significant difference; *, P<0.05; **, P<0.01.

corroborate with Hemsley and Moir (1963), Oltjen *et al.* (1971) and Hefner *et al.* (1985).

The dietary treatments did not influence the total-N concentration in SRL (Table 3). However, the ruminal NH_3-N concentration was significantly (P<0.01) reduced by 28.53 and 15.40% in G2 and G3, respectively, than G1. The differences in NH_3-N concentration between BCFA supplemented groups were nonsignificant, but on its expression as per cent of total-N concentration, it showed significantly (P<0.05) higher NH_3-N concentration (32.19) as compared with G2 (BCFA without urea, 27.09%). In spite of the inclusion of urea in the experimental diet 3 (BCFA + urea), the NH_3-N (% of total-N) was similar with G1 (without urea and BCFA, 35.55%). The NH_3-N contributed a major part in total NPN content present in SRL (Table 3). Both the treatment and sampling time had significant (P<0.01) effect on ruminal NH_3-N and NPN concentrations, however, interaction between treatment and interval was non-significant. The peak values across the treatments were noticed at 4 hr post-feeding and, thereafter, declined gradually to reach minimum (Fig.1). The reduction in NH_3-N and consequently the NPN, on BCFA supplementation have also been reported invariably by Hemsley and Moir (1963), Cline *et al.* (1966), and Oltjen *et al.* (1971). The depression in NH_3-N concentration in the present study, indicated that addition of BCFA in the diet, resulted in increased N assimilation by rumen microbes and more NH_3 was converted into microbial protein. The observation that the improvement in microbial protein synthesis (TCA-N as % of total-N) was observed to be associated with those treatment groups showing lower NH_3-N concentration in SRL. As a result TCA-N (as % of total-N) was highest (P<0.05) in G3 (63.31%), followed by G2 (63.00%) and G1 (57.92%).

There was significant effect (P<0.01) of dietary treatment and sampling period on TVFA concentration. The higher TVFA concentration on BCFA diets, occurred mainly owing to the differences observed in DM and fibre digestibilities. The increased TVFA levels in rumen liquor of BCFA supplemented diets have been reported to be associated with increased microbial activity and hence the digestibility (Hemsley and Moir 1963). The concentrations of individual VFA expressed as mM/litre, only acetate along with isobutyrate was found to be significantly (P<0.05) higher on BCFA supplementation (71.07 and 69.73 mM/litre) in G2 and G3 as compared with G1 (64.98 mM/litre). The increased acetate production with BCFA supplementation was observed

Table 3. Effect of BCFA supplementation on ruminal metabolites

Particulars	G_1 (No BCFA)	G_2 (BCFA)	G_3 (BCFA+urea)
pH	6.74±0.05	6.73±0.05	6.79±0.06
N fractions			
Total-N	37.07±1.80	34.77±1.38	36.64±0.35
NH_3-N**	13.18±1.22^a	9.42±0.40^b	11.15±0.55^b
NPN**	15.60±1.14^a	12.87±0.18^b	12.84±0.58^b
TCA-N	21.47±0.77	21.90±0.88	21.93±0.48
N fractions as % of total-N			
NH_3-N*	35.55±2.49^a	27.09±2.26^b	32.19±1.66^a
NPN*	42.08±1.27^a	37.00±1.38^b	37.07±1.49^b
TCA-N*	57.92±1.28^a	63.00±1.38^b	63.31±1.49^b
NH_3-N as % of NPN*	84.49±3.04^a	73.19±1.58^b	86.84±2.47^a
Total VFA (mM/l)**	93.05±0.63^a	101.14±1.79^b	95.62±1.18c
Molar concentration (mM/l)			
Acetate*	64.98±1.21^a	71.07±1.27^b	69.73±0.85^b
Propionate	18.98±1.34	19.18±1.17	18.06±2.11
Isobutyrate*	0.21±0.01^a	0.98±0.06^b	0.66±0.03^b
Butyrate	8.60±1.12	9.19±1.67	6.55±1.98
2-metyle butyrate	0.10±0.01	0.42±0.03	0.37±0.06
Isovalerate	0.18±0.04	0.30±0.04	0.25±0.02

Different superscripts in a row indicate significant difference; *, P<0.05; **, P<0.01.

in several studies reviewed by Cook and Towns (1987), however, their influence on other ruminal VFAs was rather inconsistent. In general, the increased acetate production could be correlated with increased cellulolytic activity as also reflected in improved fibre digestibility in the present study. The usefulness of supplementation of sodium salt of isobutyric acid in low protein and urea based diets as evident by the increased DM, OM, NDF and cellulose digestibilities, better N retention and rumen fermentation characteristics in crossbred cattle.

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Feasibility of duckweed as poultry feed—A review

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ABSTRACT

Duckweed (*Lemnaceae*), tiny free-floating vascular plants, have 4 common genera, *Spirodela*, *Lemna*, *Wolffia* and *Wolffiella*, and about 40 species are world wide distributed. It attracted considerable attention for utilization as protein supplement in poultry ration to minimize chronic scarcity and high cost of animal protein supplements. Crude protein (CP) content of duckweed (DW) is 7 to 40 % on dry matter basis depending on media where it was grown and the species involved. The essential amino acid (EAA) profile of DW is similar to other animal proteins with the exception of methionine. It contains 4g lysine/100g protein and it is comparable to other sources of protein. Their production rate of about 80 metric ton/ha/year of solid material is higher than other classic crops like alfalfa, soybean etc. Dried duckweed (DW) can be used in poultry diet as partial replacement of fish-meal, soybean-meal, alfalfa leaf-meal etc. It may be included as a part of protein concentrate mixture for layer and broiler. Its inclusion level is up to 15% in broiler ration and 40% in layer diet. Simultaneously, it may be useful for the treatment of waste-water and already being used in many developed countries. After reviewing these aspects it is suggested that DW could be used as an effective protein supplement in poultry diet.

Key words: Duckweed, Feed, Nutritive value, Poultry, Pollution control

The profit of a poultry enterprise mainly depends on economic feeding of ration, because feed accounts 70% of total cost involvement. The chronic scarcity and high cost of animal protein supplements of poultry has led to the research on alternative protein sources for feeding. Certain unconventional aquatic feed resources like duckweed (DW) has recently attracted considerable attention for utilization as a protein supplement in poultry ration.

Information on the feasibility of DW as poultry feed and its availability is limited. However, it is important to review the existing available information and highlight the areas of nutritional research for DW as poultry feed.

Duckweed and its distribution

Duckweeds are tiny free-floating monocotyledon vascular plants belonging to the family Lemnacea. They are classified as a floating group of aquatic weeds (Ali and Leeson 1994). This family consists of common genera, *Lemna, Spirodela, Wolffia* and *Wolffiella* among which about 40 species have been identified so far (Journey *et al.* 1993, Louis *et al.* 1980). The plants are of relatively simple morphology as they have no stems or true leaves and usually consist of a single or a few flat oval shaped fronds seldom exceeding 5mm length. Each frond may or may not have roots and the plants rarely flower (Ali and Leeson 1994). It grows in marshy lands particularly ponds, roadside ditches and low lying paddy fields with stagnant water bodies throughout the year. They are distributed throughout the world except in the extreme northern and southern regions where temperature drops below 0° during a part of the year. They are reared well in deserts and in extremely wet areas (Sarawak, Malaysia, Iceland, Clarkcity, Canada and others). Porath and Agami (1986) reported the occurrence of *Lemna gibba* and *Lemna minor* in some water cistern of the Jurdean desert. Biswas and Calder (1954) reported the occurrence of *L. minor* at an altitude of 3048m Western Tibet. Many species can survive temperate extremes, but grow faster under warm, sunny conditions. They spread by floods and aquatic birds.

In the Indian sub continent, 6 species of DW (5 species of *Lemna* and 1 species of *Wolffia*) are available (Biswas and Calder 1954). Subrahmaniyam (1962) reported 3 species, each from the genus of *Spirodela*, *Lemna* and *Wolffia*. The botanical survey carried out by Prain (1963) in the Britain and India and by Khan and Halim (1987) in Bangladesh mentioned the occurrence of 3 genera (*Lemna*, *Spirodela* and *Wolffia*) of DW. Two species for each of these genera have been reported in Bangladesh. These species are *Lemna perpusilla, L. trisulica; Spirodela polyrrhiza, S. punctata; Wolffia arrhiza, W. microscopica.* So, the worldwide distribution of different DW species makes it feasible to use for any probable purpose.

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Table 1. Proximate components (AOAC 1980) of duckweed (*Lemna minor*)[a]

CP	CF	EE	Ash	NFE	Reference
20	12	2	31	20	Islam *et al.* (1997)
15 to 25	15 to 30	ND	ND	ND	Mbagwu and Adeniji (1988)
5 to 45	5 to 15	ND	ND	ND	Mbagwa and Adenijii (1988)
25 to 37	9 to 11	ND	ND	ND	Rusoff *et al.* (1980)
14	10	2	25	50	Khan (1995)
up to 40	ND	ND	ND	ND	Porath *et al.* (1979)
18	12	2	2	67	Morrison (1961)
20 to 40	to 10	6	ND	ND	Culley (1976)

[a]All analyses are on dry matter basis (g/100g); ND- Not determined.

Potential source of protein and amino acids

The CP of DW obtained from natural waters (e.g., ponds, stream, lake, paddy field etc.) ranges from 7 to 20% (Tan 1970, Bhanthumnavin and McGarry 1971). However, in DW grown in enriched waters containing mineral media or effluent from agricultural and municipal waste lagoons, protein content greatly increases over 30-40%. However, DW from natural water has low concentrations of nutrients (Culley and Epps 1973). Culley (1976) indicated that dried DW was highly nutritive, containing 20-40% CP. It might be able to successfully compete with classical protein crops like alfalfa and soybean. According to Mazid and Akhter (1980) DW contained 20-23% CP on DM basis. This value ranged from 16.2 to 36.5% as reported by others (Rusoff *et al.* 1980, Porath *et al.* 1979, Louis *et al.* 1980) depending upon various factors.

The CP content of DW varies from 7 to 40% (Tables 1 and 2). The amount of proximate components depends upon different factors like (a) nutrient content of media where it is grown, (b) sunlight, (c) agitation of the DW, (d) overcrowding, (e) stage of maturity, and (f) species of DW etc.

The amino acid composition of few species of DW (*Lemna minor L*) collected from the natural waters of several countries (Canada-Muztar *et al.* 1978,Poland-Maciejewska-Potapzh *et al.* 1975, USSR-Tulagnov, 1973 *fide* Louis *et al.* 1980) was reported to contain all of the essential amino acids (EAA) in varying proportions. Mbagwu and Adeniji (1988) indicated that the DW protein has higher concentrations of EAAs, lysine and methionine than most of the plant protein and more closely resembles animal protein in this respect.

According to Rusoff *et al.* (1980) the EAA profile of the

Table 2. Dry matter production (t/ha/year) and CP content (g/100gDM) of different species of duckweed cultivated in different media

Species	Cultivation	DM production	CP (%)	References
S. polyrrhiza	Domestic waste water	36	-	Robson (1996)
S. polyrrhiza	Domestic sewage	18-32	30	*Gijzen *et al.* (1996)
	inorganic fertilizer	12-22	27	*Gijzen *et al.* (1996)
S. polyrrhiza	Domestic sewage inorganic fertilizer	17-32	-	Alaerts *et al.* (1996)
L. gibba	Pretreatment raw domestic sewage	55	30	Oron and Wildschut (1994)
L. gibba and *S.polyrrhiza*	Domestic waste water	11-55	30-40	Oron (1994)
S.polyrrhiza *L. perpusilla* and *W.arrhiza*	Septage from septic tank	9-21	24-28	Edward *et al.* (1992)
L. perpusilla	Septage from septic tank	11	-	Edward *et al.* (1990)
L. minor	Domestic waste water	27	37	Zirschky and Reed (1988)
L. gibba	Municipal waste	-	12-23	Oron *et al.* (1987)
L. minor	Domestic waste water	-	40	Logsdon (1989)
S. polyrrhiza	Sewage effluent	21	30	Sutton and Ornes (1975)

*Sultana (1998).

Table 3. Some essential amino acid content in duckweed (g/100g protein)

Amino acids	*Lemna minor*[a]	*Lemna gibba*[a]	Lemnacae family[b] (Mean ± sd)[c]
Lysine	6.90	7.03	4.01+_0.43
Methionine	1.57	2.64	0.90+_0.15
Histidine	2.30	2.79	1.78+_0.42
Argenine	9.26	6.31	4.54+_0.64
Threonine	4.78	4.95	3.12+_0.40
Glycine	5.51	5.77	3.68+_0.43
Valine	5.57	6.83	4.39+_0.64
Isoleucine	4.24	4.73	3.61+_0.37
Leucine	7.80	9.38	6.68+_0.58
Tyrosine	4.42	5.69	2.82+_0.44
Phenylalanine	5.02	7.24	4.16+_0.39

[a]Porath *et al.* (1979); [b]Rusoff *et al.* (1980) ; [c]mean of 4 species with standard deviation.

protein concentrate could be compared favourably with that of the FAO reference pattern (FAO 1973) with the exception of methionine (Table 4). Average values (g/100g of protein) for EAAs (Table 3) reflect the qualitative aspects of the CP of the DW. The quality and quantity of DW protein is encouraging to use in poultry diet.

generation of progeny over a period of 10 days to several weeks before dying. As the frond ages, its fibre and mineral contents increase and reproduction slows down (Journey *et al.* 1993). They also mentioned that the DW fronds can double their mass in 2 days under ideal conditions. This value is under aseptic, continuous light condition. It is usually 2.0 to 2.5 days (Porath *et al.* 1979) and faster than any other higher plants (Rejmankova 1975).

Under experimental conditions their production rate can approach the yield of 4 metric ton/ha/day of fresh plant biomass, or about 80 metric ton/ha/year of solid material. This growth pattern more closely resembles the exponential growth of unicellular algae and denotes an unusual higher biological potential feed supplement (Journey *et al.* 1989).

In the larger scale pond experiments, over 10 tons DW/ha (1.0kg/m^2/week) were harvested (Porath *et al.* 1979). Classical crops like maize and sunflowers may reach similar relative growth rate but only for a short time during their growth period (Evans 1972). Such high relative growth rate may be obtained in certain DW like *L. gibba* (Hurfeish clone) in aquaculture during the entire growing season.

Porath *et al.* (1979) evaluated 2 DW clones (*Lemnaceae*) for crop yield and relative growth rate (RGR) under conditions of increasing biomass density in manured and

Table 4. Essential amino acids in duckweed protein concentrate compared to FAO (1954) reference pattern, corn, rice, fish-meal and soybean-meal

Amino acid (g/100g protein)	*[a]Duckweed	[a]FAO	[a]Corn	[a]Rice	[b]Fish-meal	[b]Soyabean-meal
Lysine	4.0	4.2	2.3	3.2	11.1	6.8
Isoleucine	3.6	4.2	6.2	5.2	6.0	1.5
Leucine	6.7	4.8	5.0	8.2	10.4	8.2
Methionine	0.9	2.2	3.1	3.4	4.4	1.5
Phenylalanine	4.2	2.8	5.1	5.0	5.6	5.6
Threonine	3.1	2.8	3.7	3.8	6.0	4.3
Valine	4.4	4.2	5.3	6.2	4.7	4.8
Tryptophan	-	1.4	0.6	1.3	1.4	1.2

*Mean of 4 species; a Rusoff *et al.* (1980); b Calculated from Banerjee (1986).

Biomass yield and growth rate

Temperature, light intensity, wind and water current, humidity and rainfall, plant density, algae growth, depth of the lagoon, pH and nutrient content of media where it is grown and the DW species affect biomass yield and relative growth rate (Sultana 1998). The summarized dry matter production in various species grown in different media is shown in Tables 2, 5.

Reproduction of DW is primarily vegetative. Clones grown in natural (Rejmankova 1975) and laboratory (Landolt 1957) have shown quantitative variations in growth rate. An individual mature frond may produce daughter fronds bud from reproductive pockets on the side as many as 10

Table 5. Fresh duckweed yield (ton/ha/year) in inorganically and organically nourished ponds of PRISM (Gijzen *et al.* 1996)*

Year	DW yield in inorganically nourished pond (ton/ha/year)	Organically nourished pond (ton/ha/year)
1990	157	-
1991	248	145
1992	231	163
1993	270	192
1994	236	213
1995	222	260

PRISM- Projects in Agriculture Rural Industry Science and Medicine; **fide* by Sultana (1998).

unmanured ponds. Variation in amino acid composition was only negligible and greater crop yield and RGR at high density in the eutrophicated ponds. Because, it bioaccumulate as much as 99% of the nutrients contained in wastewater and produce valuable protein rich biomass as a byproduct (Journey *et al.* 1989).

Yields equivalent to 10 to 13 metric tons DM/ha/year were demonstrated on a small lagoon system, and in outdoor tanks maximum yield approached to 20 metric tons dry weight/ha/year (Said *et al.* 1979). Journey *et al.* (1993) indicated that the actual yield of fresh material from commercial scale cultivation of *Spirodela*, *Lemna* and *Wolffia* species at the Mirzapur experimental site in Bangladesh range from 0.5 to 1.5 metric ton/ha/day, which is equivalent to 13 to 38 metric tons/ha/year of solid material. Therefore, DM yields vary from 9 to 55 ton/ha/year (Table 2) depending upon the various factors.

Nutritional value for poultry

The nutritional value of DW as poultry feed has long been recognized (Lautner and Muller 1954, Boyd 1968, Muzaforon 1968, Abdulayef 1969, Hamid *et al.* 1993). Ali and Leeson (1994) indicated that the quality and nutritional value of aquatic weeds (including DW) could be improved by harvesting at the young, lush stage, by washing after harvest and applying gentle processing techniques. Truax *et al.* (1972) showed that dehydrated DW, when substituted for dried alfalfa-meal up to 5% of mixed poultry feeds, produced superior weight gain in chicks up to 3 weeks of age. This has been attributed to its balance amino acid profile. In another feeding trial the chickens fed diets containing 10% DW observed similar performance as chicken offered same amount of alfalfa meal containing diet (Muztar *et al.* 1976).

Haustein *et al.* (1994) indicated that the inclusion of 15% *Lemna* and 15% *Wolffia* in the diets of layers produced no significant difference in egg production, feed conversion or mean egg weights when compared with those of the control group. Comparison of the initial and final period (2wk vs 10wk) revealed that feed consumption decreased in control ($P>0.04$) over time, but in 15% *Lemna* group showed only a slightly decreased feed consumption ($P<0.07$). Egg number and egg weight parameters were not statistically different between 2 groups.

Haustein *et al.* (1990) used 3 isonitrogenous diets containing 25% *Lemna* (2 800 ME kcal/kg), 25% *Lemna* with a higher ME (2 900 kcal/kg) and 40% *Lemna* (2 800 ME kcal/kg). No significant differences were found between the control group and the hens fed 25% *Lemna* in egg production, feed consumption, feed conversion, mean egg weight, mean weight gain, number of eggs/hen per week, and yolk pigmentation. Pigmentation increased significantly ($P<0.01$) when 15% *Wolffia arrhiza* were included in the diets. Higher levels of *Lemna* species produced smaller, but still significant ($P<0.05$), incremental changes in yolk pigmentation when eggs from hens fed 25% *Lemna* species were compared with eggs from 40% group. No differences were found in the calcium concentration (43.0, 42.9 and 43.0%) of the shells among the groups fed with 0, 15 or 25% *Lemna*, respectively, in their diets.

Haustein *et al.* (1990) concluded that sewage-grown DW could be successfully utilized as protein source in diets for Leghorn layer. The optimal level of *Lemna* in the diets of

Table 6. Duckweed utilization in poultry ration

Replacement of	Type of chicken	Results	References
Dried alfalfa-meal (up to 5%)	Chicks up to 3 weeks	Superior weight gain than control	Truax *et al.*(1972)
DW (up to 10%)	Outperformed chicken (mature)	Superior weight gain than control	Muztar *et al.* (1976)
Without replace 15% *Lemna* and 15% *Wolffia*	TOPAZ layer (Mature) FCR and mean egg wt.	Similiar egg production	Haustein *et al.* (1994)
Fish-meal or soybean-meal (up to 15%)	Leghorn Hyline (Mature)	Similar performance like control	Haustein *et al.* (1990)
Without replace (up to 10%)	Chick starter or broiler (up to 8 weeks)	Similiar weight gain and FCR	Johri and Sharma (1979)
Fish-meal (up to 5%)	Leghorn	Similar performance like control	Rojas and Bermy (1976)
Fish-meal with soybean meal (up to 9%)	Broiler chicks	Not promising	Islam *et al.* (1997)
Without replace (up to 15%)	Titan broiler (up to 28 days)	Not promising	Haustein (1994)
Fish-meal (up to 6%)	Growing ducklings	Similar performance	Hamid *et al.* (1993)

chickens was 15%, but even 40%, *Lemna* did not affect egg quality. Rojas and Bermy (1976) studied the use of *Lemna trisulaca*-meal as a partial replacement of the costly animal protein in the layer diets of same breed to reduce the proportions of fish-meal to 2% compared with 7% in the control diet, found good result.

Johri and Sharma (1979) reported that dried *Lemna minor* could be used in chick starter or broiler ration @100g/kg diet without affecting weight gain and feed efficiency. Haustein *et al.* (1994) reported that females given diets containing 5% DW increased final weights. The pigmentation of all chicken given DW increased significantly. Weight gain of broilers given 15% DW was similar to that of control. But, 25% DW significantly decreased feed intake and weight gain.

Islam *et al.* (1997) mentioned that total replacement of fish-meal with DW and soybean-meal may not be advisable but partial replacement of fish-meal with soybean-meal increases productivity. Haustein *et al.* (1990) concluded that up to 15% DW could be an important protein source for poultry in developing countries where soybean-meal or fish-meal is costly. Journey *et al.* (1993) demonstrated that DW could be substituted for soybean-meal and fish-meal in prepared poultry rations for broilers, layers and chicks. Acceptable levels of DW-meal in the diets of layers range up to 40% of total feed. In broilers up to 15% DW-meal produces growth rates equal to those produced by control feeds. Diets for chicks, consisting of up to 15% DW-meal are suitable for birds under 3 weeks of age. So, DW-meal may be suitable as a replacement of fish-meal and soybean-meal up to certain level in poultry diet.

Many scientists used different DW species as a partial replacement of protein supplements up to certain level for various types of poultry with positive results (Table 6).

Duckweed for pollution control

Municipal sewage treatment facilities remove considerable quantities of nutrients from wastewater, but the effluent still contains levels of nitrogen and phosphorus that are much higher than concentrations in natural water. Many industrial and agricultural effluents are not treated prior to release into the environment. In this case DW may be grown to uptake nitrogen (Boyd 1970). The DW culture using domestic waste is being practiced in Bangladesh since 1991. The pond had been designed as a plug flow channel, which is proceeded by a setting pond where human waste from a nearby hospital is being settled before use. Annual DW production under different production systems at the PRISM, Mirzapur demonstration farm, has shown encouraging result (Table 5). Keeping this view in mind, Bangladesh Livestock Research Institute, Savar, Dhaka, has an ongoing project entitled "Duckweed Research Project" funded by the Netherlands Government. It is giving emphasis to use DW for wastewater treatment and simultaneously use it as feed for livestock including poultry.

Duckweed based wastewater treatment system have demonstrated great efficiency in treating domestic wastewater and also have done so at a profit. Not enough is known, however, about the capability of duckweed to remove heavy metals and toxins from certain types of wastewater. Answers to these questions, as well as more precise information on nutrient uptake rates, are necessary to develop standardized engineering guideline for DW-based wastewater treatment facilities (Journey *et al.* 1989, Boyd 1970).

Considering distribution, availability, production, composition and nutritive value of DW for poultry, it is a good protein supplement in poultry feed. Intensive integrated DW production with wastewater treatment and processing for feed formulation may be helpful to improve the poultry production and ultimately increase the supply of animal protein with less polluted environment.

But, further research is important to make it conventional. Duckweed may be cultivated in an integrated farm with other aquatic plants and crops and also on animal shed waste and municipal waste. Different aspect of intensive DW production like fertilizer dozing, frequency of harvesting, best season of production etc. may be studied further.

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Biochemical studies on suckling vs nonsuckling buffalo calves in relation to performance of dams

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ABSTRACT

Effect of weaning on growth has been monitored through biochemical estimations of blood serum proteins, thyroid hormones and immunoglobulins in calves. Alterations in the levels of these biological entities have been considered as the variation due to differences in metabolic status of calves under 2 feeding management at the time of birth. These biochemical changes in serum indicate the metabolic compensation against energy drain due to weaning in the system. However, no differences in body weights were noted in suckling vs nonsuckling calves. The frequency of disease occurrence in nonsuckling calves was higher as compared to suckling calves.

Key words: Buffalo, Calves, Hormones, Immunity, Serum proteins, Thyroid hormones

An extended inter-calving period is a significant problem being detrimental to performance status of buffaloes. Suckling was identified as one of the major causes of long intercalving period (Jainudeen 1988, Janakiraman 1988). The practice of weaning is traditionally not accepted widely though a short-term removal of calf was found promising managerial practice to cut short the intercalving period. Information on effect of weaning on calf and dam's performance is scanty.

Present study is an attempt to study metabolism in relation to growth of suckling vs non-suckling calves and its impact on performance traits of dams.

MATERIALS AND METHODS

Calves (20) were randomly selected at the CIRB farm during routine calving period. Weaning was introduced in 10 calves at the time of birth and they were assisted to take colostrum in a pan. Other group of 10 calves was allowed to suckle their dams. All the calves were bled within 30 min after birth and on day 1, 4, 15, 60 and 90 thereafter. Total immunoglobulins, protein, thyroxin (T4) and triiodothyronin (T3) were estimated in blood serum using standard procedures. Thyroid hormones were estimated using ELISA kits.

Body weights of calves were recorded fortnightly. Data on performance traits of dams was recorded from farm records on calving interval, lactation length, dry period, service period and milk yield.

Test of significance was applied to the data of blood serum profile of the calves of 2 groups and the performance traits of respective dams of 2 groups of calves to study the effect of suckling vs nonsuckling behaviour of calves on the later performance of their dams of 2 groups of calves.

RESULTS AND DISCUSSION

Health status of weaned calves was studied in comparison to suckling ones. Blood profile of serum was analyzed to differentiate the metabolic status of calves while suckling and/or weaned. Body weights of calves were compared in 2 groups of calves. Impact of weaning on their dams' performance was studied through monitoring the significance in differences of performance traits of respective dams of calves in 2 groups.

Total immunoglobulins

Total immunoglobulins in blood serum of calves were quite comparable with earlier reports (Sikka *et al.* 1997). The levels of these proteins in the 2 groups of calves are comparable (Table 1). The average serum Ig levels at the age of 24 hr after birth were not significantly different in calves of two types of feeding management. The range of absorbed Ig levels at the age of 24 hr was 23 - 81 mg% which showed a significant individual variation in absorption of Ig from colostrums as reported earlier (Sikka *et al.* 1998). It showed that the individual capacity of Ig absorption by calves circumvents the Ig uptake mode keeping the same overall immunity status in 2 groups of calves. Hence suckling did not have any additive effect over nonsuckling mode of

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Table 1. Average ± SE serum immunolglobulins levels (mg %) in suckling vs non-suckling buffalo calves

Groups	At birth	Day 1	Day 4	Day 15	Day 30	Day 60	Day 90
Suckling	9.6±0.5	47±1.6	53±1.3	54±1.2	44 ±1	49 ± 1	43 ±0.6
	(29 ± 2)	(37 ±1)	(42±0.7)	(44±0.9)	(47 ±1)	(47 ±1)	(61±1.2)
Nonsuckling	14.6±1.1	53 ±2	52 ±2	43 ±1.6	42 ±1.3	46±1.7	50 ±1.4
	(28±0.7)	(39±0.6)	(42±0.9)	(43±0.9)	(46±0.9)	(50 ±1)	(56±1.3)

Values in parenthesis are the body weights in kg of calves.

colostrums uptake in calves as explained earlier (Besser *et al.* 1991).

Birth weight of calves was highly correlated with the basal immunoglobulin levels in blood serum of calves (n=0.68). Lower immunoglobulin levels at the age of 24 hr of calves was well associated with lower gain in body weights at the age of 90 days in both the categories of calves. Total gain in body weights were 27.4 ± 0.9 and 28.1± 1.2 kg, respectively, in suckling and nonsuckling calves during 90 days from birth. No significant variation in body weights was noted up to 3 months of age in 2 groups of calves (Table 1).

Total proteins

Total proteins in suckling and non-suckling calves were shown in Table 2. Levels are slightly higher than the earlier values (Kumar *et al.* 1888). Average serum protein levels remained higher in calves of suckling group from day I to 15 days of age, however, the levels remained low in nonsuckling group of calves for first 4 days. Probably, at the initial stage proteins were utilized to meet the energy requirement in non-suckling group of calves.

Thyroxin and triiodothyronin

The triiodothyronin (T_3) and thyroxin (T_4) levels in blood serum of calves are shown in Table 2. The values seem to be lower than reported T3 levels of calves by Madan (1984). The blood serum thyroxin levels were 120, 96 and 74 ng/ml on these days. Values reported in earlier studies (Madan 1984) were 112, 69 and 82 ng/ml respectively.It seems that behaviour of thyroid gland differs in non-suckling vs suckling calves. Higher levels of circulatory T_3 hormone was found associated with lower dietary or nutritious status of an individual by Baishya *et al.* (1996). Elevation of triiodothyronin in blood serum indicates the metabolic compensation to fight out the nutritional imbalances in body (Wichtel *et al.* 1996). In general, the T_3 levels are built-up by deiodination of thyroxin to regulate the biological activity of thyroid hormone, especially, during the active growth period (Ratcliff 1988). Such nutritional imbalances are equilibrated by the breakdown of complex biomolecules like proteins in nonsuckling calves as in present study.

Incidence of disease

Frequency of occurrence of diarrhoea in nonsuckling calves was significantly higher than the suckling calves. It occurred during first 7 days of life in nonsuckling calves in comparison to suckling calves where this period was extended up to 25-60 days after birth. Average rate of diarrhoea occurrence in non-suckling calves was 2-3 times higher than in suckling calves. Non-suckling calves are probably, devoid of appropriate immunity and so the lesser degree of disease fighting ability. Such immunity could be improved in non-suckling calves through appropriate management to reduce mortality.

Performance traits of dams

Effect of long and short-term suckling on performance of dams was studied (Table 3). The service period was reduced by 72 days in dams of non-suckling calves. The difference in service period of dams of 2 groups of calves was not significant statistically but was in agreement with the reports of Jainuddin (1988) who suggested that weaning reduces the incidence of postpartum anestrus. Service period extended due to the lack of symptomatic estrus in spite of the ovulation (Hanumantha Rao *et al.* 1988, Janakiraman1988).

Calving interval of dams of suckling group was higher in comparison to non-suckling calves' dams. This finding is in agreement with the earlier work of Jasiorowski (1980). Dry

Table 2. Average ± SE of biochemical estimates in blood serum of suckling vs non-suckling buffalo calves

Blood profile	At birth	At birth	Day 1	Day 1	Day 4	Day 4	Day 15	Day 15	Overall average
	Suc	Nsuc	Suc	Nsuc	Suc	Nsuc	Suc	Nsuc	
Protein(g%)	6.1±3	10 ±3	10±1	6.7±1	10±2	5.2 ±1	8.5±1	9.6 ±2	8.4 ±1
Serum T_4(ng/ml)	62±15	46 ±12	125±40	116±16	114±14	78±17	95±15	52 ±6	90 ±12
Serum T_3 (ng/ml)	0.8±0.1	0.7±0	0.9±0.1	0.7±0.2	0.8±0.3	1.5±0.6	0.5±0	0.3±0	1.2 ±0

n =10.

Suc, Suckling; Nsuc, non-suckling.

Table 3. Average ± SE of some performance traits of dams of suckling vs non-suckling calves

Dams of calves	Calving interval (CI)	Lactation length (LL)	Dry period (DP)	Service period (SP)	Milk yield (MY)
Suckling	512 ± 52	306 ± 31	207 ± 46	260 ± 5.3	1478 ± 206
Non-suckling	480 ± 5.1	323 ±24	168 ± 40	187± 4.3	1669 ± 180

n =10.

period was reduced in the dams of non-suckling calves (Table 3). Suckling of dams for longer interval in suckling calves dams could increase the service period which in turn enhanced the dry period. Differences in lactation yield and lactation length of dams of 2 groups of calves were not significant. The test of significance of these traits might be more meaningful on higher population.

The early weaning of calves seems to be beneficial to dam's performance with no damage to calves health.

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Resource usage efficiency in sheep breeding enterprises in Konya province and effects of different production strategies on profitability

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ABSTRACT

General characteristics of sheep breeding in Konya region of Turkey, and resource usage efficiency level were determined by using Cobb-Douglas type production function. Geometric means of 1998/99 and 1999/00, which were surveyed from 51 enterprises that were determined by using randomly layer sampling method, were used. The percentage sheep breeding enterprises income was determined as lamb sales, milk sales, inventory value increases, wool sales and manure revenues, which were 70.09%, 23.49%, 4.28%, 1.53% and 0.77% respectively. Sheep breeding enterprises expenditure distribution were found on feed, labour, veterinary services, depreciation cost and other expenditure, and the values were 53.03 %, 31.33 %, 3.41%, 7.91 % and 4.33 % respectively. Financial ratio and rantability factor were estimated to be 18.55 and 25.76 respectively. The marginal value productivity of input factors at feed, labour, veterinary services, depreciation cost and other expenditure were 1.78 TL, 1.74 TL, -9.25 TL, 5.88 TL and -2.80 TL respectively. Return to scale in sheep breeding enterprises in Konya was 0.91.

Key words: Breeding, Economic analysis, Profitability, Sheep

Nabradi *et al.* (1998) reported that the main point determining the income in sheep and goat breeding in Hungary was production cost and product prices, and that production cost resulted from breeding shed animal and feed cost.

Between 1982-1986 the number of sheep in Hungarian reduced 31.0 %. In this country, the characteristic of sheep breeding enterprises are small scale enterprises and 92 % of these enterprises have less than 300 sheep in their flock (Javar *et al.* 1997). Margetin *et al.* (1998) suggested that the profitability of sheep enterprises could be improved by financial support.

Soedjane *et al.* (1988) reported that in Indonesia, small ruminant breeding was in small scale, that the enterprises had 13-400 sheep and that there was no significant correlation between flock management and productivity, but intensive production was found more profitable than the other from the result of economic analysis.

Chauhan (1992) found that larger farms enjoy considerable economics of scale, the average number of sheep kept by farmers was over and above by the minimum indicated by the break even point; and that crossbreeding was needed to increase wool and mutton yields in India.

Raina *et al.* (1993) indicated that in India production costs were higher on small farms as they tended to be more labour intensive; the large farms were more capital intensive. The study of resource utilisation indicated that profitability of wool production could be increased by raising investment in all factors of production on all sizes of farms. Cevger (1997) determined marginal value product of input factors and reported return to scale 1.06.

Rodney and Schroeder (1998) reported that number of sheep had reduced 77.0 % between 1961-1996 and average reduction percentage in a year was 4.0 %. According to the economic analysis done by author the main product in sheep breeding was meat and wool. However, they suggested that the production structure could be diverted according to the target market demand and the relative production percentage, and economic alterations have mainly affected the range of lamb and wool sales income. They concluded that rough and hired grazing license costs were more important than the other cost factors with the help of econometric model.

MATERIALS AND METHODS

In this study panel data 1998/99-1999/00 taken from 51 enterprises by survey were used to analyze resource usage efficiency and profitability analysis. Randomly selected enterprises were classified into 3 groups; small scale (flock size less than 50), medium scale group (flock size between 51-100), and large scale group (>101) (Ray 1993).

Enterprises examined on this study cost factors and income factors were determined according to national accounting

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system (Akdogan 1994). Cost factors were determined as feed cost (including purchase of grass license feed), labour cost (including family cost, hired labour cost and herdsmen), veterinary services and drug, deprecation cost and miscellaneous cost which are energy, repair and maintain, inventory value decrease /actual decline in book value of animal due to age and number available (if inventory value decrease occurs), interest cost and management cost. Income factors for sheep breeding enterprises were determined as milk sales value, lamb sales, inventory value increase/actual increase in book value of animals (if it occurs), wool (fleece) sales value and manure incomes.

In this research, financial ratio, rantability factors ratio and output/input (O/I) ratio were used to determine profitability of enterprises (Aral 1977).

The resource usage efficiency in sheep breeding production was studied through Cobb-Douglas production function

$$Y=f(x_1^{b1}, x_2^{b2}, x_3^{b3}, x_4^{b4}, \ldots x_n^{bn})$$

$$\text{Log } Y_i=\text{Loga}+b_1.\text{Log}X_1+b_2.\text{Log}X_2+b_3.\text{Log}X_3+\ldots+b_n.\text{Log}X_n$$

where,

Y= total income (output) Turkish Lira (TL); X_1= feed cost; X_2= labour cost; X_3 = eterinary services and drug cost (TL); X_4 =depression cost (TL); X_5 = miscellaneous cost (TL).

According to multiply regression analysis results obtained by econometric model and basis on regression analysis results marginal value product (MVP) was calculated (Heady and Dillion 1961).

RESULTS AND DISCUSSION

Sheep breeding enterprises total revenue and total expenditure and their percentage value (%) in total are shown in Table 1.

Capital structure, financial ratio, rantability ratio and output/input ratio were presented in Table 2, and production and marketing strategies and estimated sheep production are given in Tables 3 and 4 respectively.

Mainly income proportion taken from sheep breeding yield was lamb sales (Table 1). Others were provided by milk sales and the others were inventory value increase, wool and manure incomes. The percentage of income factors changed in small, medium and large scale enterprises when enterprises income were evaluated according to average value of sheep breeding enterprises. However, the percentage distribution of income factors resembled to average value of sheep breeding enterprises in Konya region. First aim of sheep breeding was mainly lamb production. Milk production in sheep breeding was abandoned because of 2 factors. One of the reasons was lack of efficient milk marketing organization and/or co-operative and the other one was that there was no sufficient milk market price in milk production in the villages. It was determined that milking machinery could not be used in ewe milk production at all. The reason of this was that the owner of enterprises did not accept this innovation.

The other point we have to focus on income factors percentage is that inventory value increase was low compared with average value and small scale enterprises in Konya. This might have been resulted from insufficient management and lack of their control on sheep flock in Konya province. Inventory value increase percentage in large scale enterprises was low according to average value and small scale enterprises.

Wool sales income was highly low (Table 1). Margetin *et al.*(1998) reported that wool sales income was low in Slovakia. This value was highly low in results of Shah *et al.* (1991)

Table 1. Enterprises revenue and expenditure and their percentage (%) in total (TL, Turkish Lira)

	Average	1-50 (small)	51-100 (medium)	101 (large)
		Revenue		
Revenue (TL)	4771087647	1199643000	3224030000	6642782069
(%) Milk sales	23.49	23.02	26.82	22.56
(%) Lamb sales	70.9	67.50	66.04	72.66
(%) Invantor value increase	4.28	6.88	4.87	3.14
(%) Wool sales	1.53	1.47	1.52	1.55
(%) Manure	0.77	1.2	0.91	0.57
		Expenditure		
Total expenditure (TL)	3390082157	1104409100	2119732500	4726113448
(%) Feed	53.03	45.49	51.27	56.36
(%) Labour	31.33	34.59	35.07	28.65
(%) Veterinary services and drug	3.41	4.69	3.61	2.87
(%) Depressions cost	7.91	8.19	6.05	8.59
(%) Miscellaneous	4.33	7.06	3.99	3.52

also. The main cause for low wool income is due to poor wool quality, and textile industry did not need that poor quality wool anymore. There was no co-operative or organization for wool marketing in both Turkey as a whole and particularly in Konya province.

More higher cost factor in sheep breeding in Konya province was feed cost. The other important cost factor was labour cost, depression cost, miscellaneous cost and veterinary services and drug cost respectively. This finding was similar to Nabradi (1998) result in sheep breeding. Labour cost percentage for medium and small scale enterprises were higher than average value and small scale enterprises. Depreciation cost was higher than the others because of investment cost. Sheep breeding enterprises conducted their activities with networth (initial capital) and that enterprises using loan capital (liabilities) was far less. In this condition, government has to regulate its policies for profitable and productive sheep breeding in both Turkey and Konya. Average financial rantability ratio was 25.57% and for small, medium and large scale were 7.12%, 23.57% and 20.415 respectively (Table 2). Rantability factor ratio was 25.76%, 12.95%, 34.47% and 26.57%, respectively on the above sequence.

Output/input ratio showed that the input, which the enterprises used in production process and the output, increased when the enterprises scale 1 went up. Result of both rantability ratios and output/input ratios showed that medium scale enterprises were more profitable than the others. Most profitable enterprises was medium scale enterprises. The results taken from this study were similar to study of Cevger (1997).

According to utilizing lambs there were 3 production systems (selling post weaning, selling on autumn, selling after fattening) in sheep breeding enterprises in Konya province.

Table 2. Capital structure and ratios in sheep breeding enterprises

	Total assets	Net revenue	Financial rantability	Rantability factor ratio	Output/input
Average	6977077451	1540587451	18.55	25.76	1.41
1-50 (Small)	2265050000	150284000	7.12	12.95	1.15
51-100 (Medium)	5341416667	1166470833	23.57	34.47	1.59
101 (large)	9278739655	2174809310	20.41	26.57	1.43

Table 3. Production and marketing strategies applied to post weaning and their effects on rantability of enterprises

	Financial rantability	Rantability factor	O/I
Selling post weaning	9.40	16.77	1.20
Selling on autumn	18.95	22.64	1.40
Selling after fattening	21.19	30.98	1.50

These production systems were evaluated with rantability ratios and output/input ratios (Table 3). Sheep breeding enterprises could increase their incomes by lamb fattening. For this purpose, financial support of government is needed. The main factor for early lamb selling is insufficient financial structure of enterprises. With financial support, early lamb slaughtering can be prevented.

It is argued that one of the different approaches to improve productivity is to examine whether the use of resource is efficient or inefficient. If it is found that the use of resource is inefficient, productivity may be increased by making proper adjustment and coordination in the use of factors of production among different categories of farms in an optimal direction.

Table 4 represents the result of Cobb-Douglas production function. The adjusted coefficient of multiple determination (R^2) was statistically significant ($P<0.01$). Farms results can be interpreted as follows: R^2 90.0 % of the variations in sheep breeding can be attributed to feed cost (X_1), labour cost (X_2),

Table 4. Estimated sheep production in Konya province, average value productivity (AVP) and marginal value producivity (MVP)

	Elasticities±Sx	Geometric	Mean	AVP	MVP	R^2	F
		Log	Anti Log			0.90	95.53***
Revenue (Y)		9.579	3793149850				
Feed (X_1)	0.585±0.078**	9.155	1428893959	2.79	1.78		
Labour (X_2)	0.286±0.070**	8.902	797994687	5.61	1.74		
Veterinary services and drug (X_3)	-0.148±0.065*	7.920	83368118	57.50	-9.25		
Depression (X_4)	0.242±0.062**	8.288	190985326	22.29	5.88		
Miscellaneous (X_5)	-0.046±0.051	7.968	92896639	56.03	-2.80		
Return to scale	0919						
Constant	1.210±0.471						

n=51, *P<0.05; **P<0.01, P<0.001

veterinary services and drug cost (X_3), depreciation cost (X_4) and miscellaneous cost (X_5).

The elasticity coefficient associated with (X_1) indicates that keeping X_2, X_3, X_4 and X_5 constant at their geometric mean levels 1% increase in X_1 will, on an average, increase the output of sheep breeding production by about 0.585 %.

Return to scale was found 0.91. This means that there was reduction return to scale in sheep production in Konya province. This value was lower than that reported earlier review done (Cevger 1997, Aral 1977, Chauhan 1992, Raina *et al.* 1993, Sakarya 1990), but this finding was similar to that reported by Yasankul (1974).

In this study average value product was found in veterinary services and drug cost and the least in feed cost. Marginal value product, an important factor for determining resource usage level, calculated for feed cost as 1.78 TL ($P<0.01$). This value showed that marginal 1 TL for feed cost had marginal 1.76 TL income for enterprises in sheep breeding. This finding was similar to Cevger's result.

Marginal value product for labour cost was calculated to be 1.74 ($P< 0.01$) TL. Marginal 1 TL had marginal 1.74 TL revenue for enterprises. This is because family labour was widespread in sheep production enterprises and hired labour price was low due to unemployment in urban areas. Marginal value product was -9.25 TL ($P<0.05$) in veterinary services and drug cost. Most efficient factor for this value was unconscious drug using in sheep production. Especially parasiticide drugs, vitamins and premixes have been used widespread by farmers. To reduce drug costs, the owner of enterprises must be educated.

Marginal value product was 5.88 ($P<0.01$) in depression cost. This factor includes investment depression cost and livestock depression. Therefore, this finding indicated that as the ewe number of flock in these enterprises increase the profitability would increase in sheep breeding also. So return to scale must be arrived.

For the miscellaneous cost factor marginal value product was -2.80, but not statistically significant ($P>0.05$). The most important factor for this cost was inventory value decrease and interest cost. It was necessary for enterprise owners to reduce death percentage in flocks so that profitability taken from sheep breeding could be raised to obtain positive marginal revenue. At the same time, interest policy on sheep breeding implicated by government has to reduce according to low profitability of livestock sector

It is concluded that sheep breeding is the best for meat production. Sheep breeding have been done extensively by primitive methods. Efficient cooperative or organization is necessary for enterprises.

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Interaction of temperature and stocking rate on growth and meat yield of broilers

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ABSTRACT

Male (1440) and female (1440), 21-day-old, broiler chicks were housed in 8 environmentally controlled rooms kept at 20.3°C (3 rooms) and 23.7°C, 25.8°C or 28.7°C (3 rooms) to determine the interaction of rearing temperature and stocking density on growth and meat yield of broilers. There were 2 pens of each sex in each room and were given a floor space of either 515cm² or 1030cm²/bird. From each pen 2 birds were killed and dissected when they reached average live weights of 1.50, 2.00, 2.50 and 2.75kg. The carcasses were dissected for meat content.

For male and female broilers, within the achieved temperature range, feed intake and growth rate declined linearly with the rise of temperature regardless of stocking density. The rate of decline in both feed intake and growth rate appeared to be slightly greater for males than for females and greater at high stocking density than at low stocking density. Feed conversion efficiency declined and mortality increased significantly with the increase of rearing temperature was found only at high stocking density, but at low stocking density feed conversion efficiency and mortality had no relationship with temperature.

Rise in rearing temperature increased eviscerated yield, dark meat and, therefore, the total meat content in the carcass regardless of sex and stocking density, but breast meat content had little relation with rearing temperature. The ratio of breast to dark meat yield, however, was reduced linearly with rising temperature in both sexes and at both stocking density. The breast: dark meat yeild was slightly higher in females than that in males and higher at low stocking density than at high stocking density at all temperatures.

Key words: Broiler, Growth, Interaction, Meat yield, Stocking density, Temperature

The adverse effects of elevated environmental temperature on the performance of broilers are well documented (Charles 1986, Howlider and Rose 1987, Howlider and Rose 1992). Bray (1983) and Howlider and Rose (1989) showed that high ambient temperature not only diminishes growth rate, but may also reduce the breast: dark meat yield of broilers. Stocking rate has a profound effect on broiler growth and meat yeild (Shanawany 1988, Proudfoot *et al.* 1979). Wathes (1978) reported that the rate of sensible heat loss from a bird in a group is only 30-60% of that from birds outside the cluster. Therefore, birds housed at a high stocking rate are more likely to be affected by an increase of environmental temperature than those loosely stocked. The metabolic heat production from a bird increases and surface area relative to metabolic mass decreases with increasing live weight or age (Bohren *et al.* 1982). So birds at heavier live weights are expected to be more sensitive to increasing rearing temperature than those at lower live weights.

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Charles *et al.* (1978) reported similar performance of broilers at 20°C or 25°C with a stocking density of 18 or 20 birds/m² with no apparent interaction between temperature and stocking density. Deaton *et al.* (1968), growing birds in cycling temperature environment with a wider range of temperature (21.1-37.8°C), also found no differences in feed conversion and mortality between temperatures and stocking densities with no interaction between temperature and stocking rate when stocking density was 11 or 15 birds/m².

Live weight differences between temperatures, between stocking densities tested may be the reasons why results of those experiments contradict the observation of Wathes (1978) on sensible heat loss. It is possible that comparison of broiler performance at different temperatures and with different stocking densities made to a given live weight rather than to a fixed age might give a better understanding of interaction of temperature and stocking density on broilers.Broiler producers must grow their broilers to some given live weights to satisfy market requirement, which signifies that information on the temperature and stocking density interaction may be more meaningful if conducted until a fixed weight is achieved. The aim of this study was to investigate the interaction of

Table 1. Regressions (Y=bx) of gains of meat (y) of male (M) and female (F) broilers at high (HSR) vs low stocking (LSR) rate on live weight gain (x)

Y variable (1)	Temp°C (2)	Sex (3)	Stocking rate (4)	b (5)	±SE (6)	Variance accounted %
Gain of eviscerated yield (kg)	20.3	M	High	0.670	±0.0090	95.9
			Low	0.675	±0.0048	98.9
		F	High	0.673	±0.0051	98.4
			Low	0.677	±0.0066	97.3
	23.7	M	High	0.694	±0.0096	98.7
			Low	0.704	±0.0102	96.8
		F	High	0.709	±0.0091	98.7
			Low	0.682	±0.0047	99.6
	25.8	M	High	0.704	±0.0134	98.0
			Low	0.680	±0.0134	97.3
		F	High	0.709	±0.0133	97.5
			Low	0.685	±0.0111	97.7
	28.7	M	High	0.730	±0.0087	96.9
			Low	0.691	±0.0054	98.5
		F	High	0.747	±00061	98.4
		M	Low	0.697	±0.0094	95.8
Gain of bresat meat (kg)	20.3	M	High	0.142	±0.0039	84.3
			Low	0.148	±0.0036	90.0
		F	High	0.142	±0.0035	84.8
			Low	0.153	±0.0023	94.0
	23.7	M	High	0.145	±0.0067	89.3
			Low	0.164	±0.0088	87.7
		F	High	0.152	±0.0051	93.0
			Low	0.141	±0.0050	88.2
	25.8	M	High	0.136	±0.0064	82.9
			Low	0.137	±0.0070	82.7
		F	High	0.151	±0.0067	89.5
			Low	0.152	±0.0047	91.8
	28.7	M	High	0.142	±0.0032	88.7
			Low	0.143	0.0036	88.4
		F	High	0.152	±0.0031	91.1
			Low	0.143	±0.0036	84.8
Gain of dark meat # (kg)	20.3	M	High	0.210	±0.0037	93.9
			Low	0.207	±0.0045	91.0
		F	High	0.209	±0.0024	95.7
			Low	0.205	±0.0026	94.3
	23.7	M	High	0.214	±0.0045	96.7
			Low	0.228	±0.0070	93.0
		F	High	0.219	±0.0049	95.8
			Low	0.226	±0.0049	96.7
	25.8	M	High	0.229	±0.0094	84.5
			Low	0.209	±0.0090	85.5
		F	High	0.227	±0.0052	96.1
			Low	0.213	±0.0066	90.3
	28.7	M	High	0.229	±0.0057	91.2
			Low	0.224	±0.0031	95.7
		F	High	0.256	±0.0037	95.6
			Low	0.223	±0.0055	87.5
Gain of total meat (kg)	20.3	M	High	0.352	±0.006	92.8
			Low	0.355	±0.0061	94.1
		F	High	0.351	±0.0039	96.1
			Low	0.357	±0.0041	96.0
	23.7	M	High	0.360	±0.0109	94.4
			Low	0.393	±0.0162	95.6

(*Table 1 continued*)

1	2	3	4	5	6	7
		F	High	0.371	±0.0076	96.8
			Low	0.367	±0.0049	98.5
	25.8	M	High	0.364	±0.0137	87.5
			Low	0.346	±0.0142	88.9
		F	High	0.378	±0.0100	95.4
			Low	0.365	±0.0086	94.5
	28.7	M	High	0.386	±0.0072	93.5
			Low	0.367	±0.0047	96.5
		F	High	0.408	±0.0051	96.5
			Low	0.366	±0.0080	89.3
Gain of thigh meat (kg)	20.3	M	High	0.094	±0.0016	94.1
			Low	0.091	±0.0020	89.7
		F	High	0.093	±0.0015	92.2
			Low	0.093	±0.0014	91.2
	23.7	M	High	0.098	±0.0028	94.5
			Low	0.101	±0.0036	89.8
		F	High	0.099	±0.0021	95.7
			Low	0.101	±0.0039	91.4
	25.8	M	High	0.103	±0.0039	89.0
			Low	0.093	±0.0037	86.8
		F	High	0.101	±0.0017	97.4
			Low	0.095	±0.0035	84.2
	28.7	M	High	0.144	±0.0034	87.0
			Low	0.101	±0.0017	95.3
		F	High	0.119	±0.0021	93.8
			Low	0.100	±0.0029	82.2
Gain of drumstick meat (kg)	20.3	M	High	0.066	±0.0015	90.6
			Low	0.063	±0.0016	89.0
		F	High	0.064	0.0012	89.0
			Low	0.063	±0.0010	91.2
	23.7	M	High	0.064	±0.0017	94.6
			Low	0.070	±0.0039	76.1
		F	High	0.069	±0.0020	93.7
			Low	0.070	±0.0020	93.7
	25.8	M	High	0.069	±0.0053	67.0
			Low	0.068	±0.0029	90.4
		F	High	0.072	±0.0025	90.8
			Low	0.066	±0.0018	92.1
	28.7	M	High	0.068	±0.0014	91.3
			Low	0.070	±0.0012	93.6
		F	High	0.071	±0.0017	87.8
			Low	0.069	±0.0017	86.6
Gain of wing meat (kg)	20.3	M	High	0.027	±0.0010	83.4
			Low	0.025	±0.0009	83.7
		F	High	0.024	±0.006	87.3
			Low	0.023	±0.0006	88.9
	23.7	M	High	0.026	±0.0010	92.0
			Low	0.029	±0.0019	77.0
		F	High	0.026	±0.0016	84.9
			Low	0.027	±0.0015	86.0
	25.8	M	High	0.028	±0.0014	78.4
			Low	0.028	±0.0019	83.2
		F	High	0.28	±0.0016	84.9
			Low	0.025	±0.0010	90.6
	28.7	M	High	0.028	±0.0010	84.9
			Low	0.029	±0.0007	89.4

(*Table I concluded*)

1	2	3	4	5	6	7
		F	High	0.027	±0.0010	80.9
			Low	0.026	±0.0010	79.2
Gain of trimmings (kg)	20.3	M	High	0.023	±0.0009	76.0
			Low	0.023	±0.0012	66.4
		F	High	0.020	±0.0010	59.3
			Low	0.020	±0.0009	67.7
	23.7	M	High	0.022	±0.0011	86.8
			Low	0.023	±0.0027	59.1
		F	High	0.020	±0.0014	75.0
			Low	0.020	±0.0008	90.7
	25.8	M	High	0.023	±0.0023	42.8
			Low	0.020	±0.0020	70.8
		F	High	0.022	±0.0022	68.3
			Low	0.020	±0.0014	72.9
	28.7	M	High	0.026	±0.0012	75.6
			Low	0.021	±0.0009	71.2
		F	High	0.027	±0.0010	79.9
			Low	0.022	±0.0010	74.2
Gain of abdominal fat plus skin (kg)	20.3	M	High	0.106	±0.0034	72.6
			Low	0.113	±0.0035	82.5
		F	High	0.144	±0.0041	85.6
			Low	0.134	±0.0041	81.7
	23.7	M	High	0.117	±0.0047	89.0
			Low	0.112	±0.0032	94.1
		F	High	0.156	±0.0072	85.6
			Low	0.135	±0.0054	88.4
	25.8	M	High	0.199	±0.0066	81.1
			Low	0.111	±0.0067	84.0
		F	High	0.169	±0.0104	79.1
			Low	0.144	±0.0139	66.0
	28.7	M	High	0.122	±0.0043	76.6
			Low	0.113	±0.0026	83.6
		F	High	0.153	±0.0050	78.5
			Low	0.150	±0.0053	81.3
Gain of hind bone length (cm)	20.3	M	High	12.9	±0.27	82.1
			Low	12.6	±0.26	87.7
		F	High	13.1	±0.22	90.7
			Low	12.8	±0.23	87.2
	23.7	M	High	12.9	±0.72	58.9
			Low	12.5	±0.36	92.5
		F	High	14.1	±0.53	79.1
	25.8		Low	12.8	±0.62	66.0
		M	High	15.2	±0.33	89.2
			Low	14.1	±0.68	78.3
		F	High	12.8	±0.46	83.3
			Low	13.1	±0.56	74.6
	28.7	M	High	14.1	±0.29	88.6
			Low	13.1	±0.29	86.1
		F	High	14.9	±0.67	60.0
			Low	13.6	±0.37	72.8

temperature and stocking density on growth, meat yield and meat composition of male and female broilers while growing them to some given live weights allowing rearing period to vary.

MATERIALS AND METHODS

Procedure

Day-old Cobb broiler chicks (1 500 males and 1 500 females) after toe punching (differentiating sex) were brooded

Table 2. Regressions of daily feed intake (g), daily weight gain (g), feed conversion ratio (feed intake: live weight gain) and mortality of male (M) and female (F) broilers at high (HSR) vs low stocking (LSR) rate on rearing temperature (°C)

Parameters	Intercept	±SE	slope	±SE	Variance accounted
Feed intake g/day (Y)					
M/HSR	207.60	±11.009	-3.540	±0.4433X	65.8
M/LSR	181.18	±14.696	-1.941	±0.5833X	25.6
F/HSR	194.13	±13.251	-3.207	±0.5364X	53.7
F/LSR	162.64	±16.641	-1.583	±0.6776	13.3
Live weight gain g/day (Y)					
M/HSR	131.88	±8877	-2.912	±0.3574	67.8
M/LSR	100.68	±4.742	-1.180	±0.1899	55.6
F/HSR	108.18	±5.960	-2.317	±0.2413	75.3
F/LSR	85.05	±6.460	-1.096	±0.2615	35.6
Feed conversion ratio (Y)					
M/HSR	0.85	±0.326	+0.051	±0.0131	31.0
M/LSR	1.80	±0.177	+0.003	±0.0071	0.0*
F/HSR	1.17	±0.331	+0.046	±0.0134	26.4
F/LSR	1.69	±0.362	±0.019	±0.0148	2.1
Mortality % (Y)					
M/HSR	-21.81	±5.241	+1.104	±0.2110	46.0
M/LSR	409	±2.777	-0.058	±0.1112	0.0*
F/HSR	-19.47	±6.286	+1.001	±0.2545	33.0
F/LSR	2.85	±1.908	-0.032	±0.0772	0.0*

together and fed on commercial broiler crumbs up to 21 days of age. Thereafter, 2 880 (1 440 males and 1 440 females) broilers were randomly selected and distributed on floor pens in 8 rooms in an environmentally controlled house. The rooms were intended to be kept at constant temperatures of 21°C (3 rooms), and 23.8°C, 26.7°C or 29.5°C (3 rooms). A data logger was used to record temperature every 3 hr in each room. The humidity was measured using a dry and wet bulb hygrometer. The achieved temperatures were 20.8, 23.7, 25.8 and 28.7°C and corresponding relative humidity levels were 82.3, 82.8, 80.7 and 75.4%. Three electric bulbs of 25 watt(s) were provided in each room that produced an average lighting intensity of 2.42lux at the feeder level. The birds were subjected to a continuous photoperiod of 23 hr and 30 min and dark period of 30 min each day. The average ammonia concentration in air of the rooms was within the range of 1.5-2.5ppm. There were 2 pens (90 broilers/pen) of each sex in each room given a floor space of 515cm^2 or 1 030cm^2/bird. Wood shavings were used as litter for all pens. Throughout the experiment all broilers were fed *ad lib.* on a conventional pelleted feed containing 13MJME and 209g of protein/kg. Hanging tube feeders (2) and 1 drinker were provided for the birds in each pen. Male (6) and female (6) broilers at the age of 21 days were dissected (Jones 1984) to establish meat content of their carcasses.

The weight of birds in each pen was monitored on alternate days throughout the experiment by weighing 20 birds at random. The feed intake and mortality in each pen were recorded when the average weight reached 1.50, 2.00, 2.50 and 2.75kg/bird. When the birds reached above mentioned slaughter weights 2 birds weighing average of the pen weight were randomly selected for dissection. The birds were fasted for 2 hr and then killed by cervical dislocation. The birds were then plucked and eviscerated. The meat (breast meat, thigh meat, drumstick meat, wing meat and trimmings) was stripped from the carcass (Jones 1984). The gain of meat (breast meat and dark meat) were calculated as the difference between weight of meat at slaughter weight and the average meat content of male and female broiler carcasses at 21 days of age.

Statistical analysis

All meat yield (gains of breast meat, thigh meat, drumstick meat, wing meat and trimmings) parameters of male and female broilers at different temperatures with high vs low stocking density were regressed on live weight gains from 21 days of age to give estimates of meat gains for each kg live weight gain (Table 1). Daily feed intake (g) and live weight gain and feed conversion ratio (feed intake: live weight gain) of male and female broilers at high vs low stocking density are regressed on rearing temperature (independent variable). The respective estimates of meat gains for each kg live weight change from 21 days of age are also regressed on rearing temperature to show the effect of temperature on meat yield. The data were analysed using Genstat statistical package (Alvey 1977).

RESULTS AND DISCUSSION

The weights of male and female broilers at 21 days of age

Table 3. Regressions (y=a+bx) of gains of meat (y) for each kg live weight gain for male (M) and female (F) broilers at high (HSR) vs low (LSR) stocking rate on rearing temperature (°C) (x)

Parameter	Intercept	±	SE	Slope	±	SE		Variance accounted %
Gains of breast meat g								
M/HSR	147.69	±	18.151	-0.262	±	0.7315	x	0.0*
M/LSR	179.55	±	52.831	-1.281	±	2.1290	X	0.0*
F/HSR	121.66	±	13.912	+1.121	±	0.5606	X	50.0
F/LSR	167.47	±	26.778	-0.821	±	1.0791	X	0.0*
Gain of dark meat g #								
M/HSR	121.17	±	22.695	+4.186	±	0.9146	x	86.9
M/LSR	182.76	±	46.300	+1.390	±	1.8658	X	0.0*
F/HSR	94.34	±	31.969	+5.418	±	1.2885	x	84.8
F/LSR	175.22	±	37.331	+1.686	±	1.5044	X	7.9
Gain of total meat g								
M/HSR	270.24	±	24.485	+3.868	±	0.9867	X	82.7
M/LSR	363.17	±	101.181	+0.085	±	0.4077	X	0.0*
F/HSR	215.99	±	23.757	+6.538	±	0.9574	x	93.8
F/LSR	339.59	±	14.778	+0.981	±	0.5955	X	36.3
Gain of eviscerated weight g								
M/HSR	527.51	±	12.586	+6.984	±	0.5072	x	98.4
M/LSR	658.48	±	60.397	+1.178	±	2.4339	x	0.0*
F/HSR	505.83	±	37.387	+8.270	±	0.4965	x	87.2
F/LSR	628.67	±	12.320	+2.298	±	0.4965	x	87.2
Ratio of breast: dark meat								
M/HSR	0.847	±	0.0760	-0.0081	±	0.00306	x	8.8
M/LSR	0.956	±	0.0836	-0.0114	±	0.00333	x	14.7
F/HSR	0.856	±	0.0703	-0.0091	±	0.00284	x	13.0
F/LSR	0.934	±	0.0681	-0.0091	±	0.00274	x	13.8
Gain of abdominal fat plus skin g								
M/HSR	69.89	±	0.858	+1.872	±	0.4380	x	85.2
M/LSR	113.18	±	4.708	-0.038	±	0.1895	x	0.0*
F/HSR	119.46	±	44.4716	+1.463	±	1.7899	x	0.0*
F/LSR	90.89	±	13.063	+2.025	±	0.5265	x	82.1
Gain of thigh meat g								
M/HSR	44.29	±	11.717	+2.354	±	04722	x	88.8
M/LSR	77.39	±	20.154	+0.786	±	0.8122	x	0.0*
F/HSR	30.99	±	21.220	+2.924	±	0.8551	x	78.1
F/LSR	81.85	±	15.703	+0.625	±	0.6328	x	0.0*
Gain of drumstick meat g								
M/HSR	57.88	±	9.000	+0.360	±	0.3627	x	0.0*
M/LSR	49.44	±	9.912	+0.744	±	0.3994	x	45.1
F/HSR	47.16	±	8.332	+0.887	±	0.3358	x	66.6
F/LSR	52.90	±	12.044	+0.573	±	0.4853	x	11.6
Gain of wing meat g								
M/HSR	23.20	±	3.771	+0.164	±	0.1520	x	5.4
M/LSR	17.17	±	5.600	+0.430	±	0.2267	x	46.6
F/HSR	16.36	±	4.703	+0.401	±	0.1895	x	53.8
F/LSR	18.18	±	6.777	+0.280	±	0.2731	x	4.0
Gain of trimmings g								
M/HSR	14.88	±	6.003	+0.350	±	0.2419	x	26.8
M/LSR	29.41	±	5.058	-0.311	±	0.2038	x	30.7
F/HSR	2.00	±	7.769	+0.822	±	0.3131	x	66.3
F/LSR	15.15	±	3.177	+0.217	±	0.1280	x	38.5
Gain in length of hind limb g$								
M/HSR	87.96	±	41.814	+2.022	±	1.6850	x	12.8
M/LSR	105.22	±	31.421	+1.037	±	1.2262	x	0.0*
F/HSR	97.52	±	38.314	+1.613	±	1.5440	x	3.0
F/LSR	107.06	±	8.096	+0.962	±	0.3263	x	72.0

*The residual variation exceeds the variance of y variate; S The sum of length of thigh bone, drumstick bone and feet; #The sum of thigh meat, drumstick meat, wing meat and trimmings.

were 647.9g and 585.8g respectively. The carcasses of male and female broilers at 21 days of age contained 11.09% and 11.16% breast meat, 8.07% and 7.77% thigh meat, 5.72% and 4.96% drumstick meat, 2.31% and 2.15% wing meat, 1.28% and 1.77% trimmings, 1.22% and 1.71% abdominal fat and 6.96% and 7.73% skin respectively. Eviscerated yields of male and female broilers were 62.61% and 56.78% respectively.

Growth performance

In both male and female broilers within the achieved temperature range, feed intake and growth rate declined linearly with the rise of temperature regardless of stocking density. The rate of decline in both feed intake and growth rate appeared to be slightly greater for male than for females and greater at high stocking density than at low stocking density. A significant decline in feed conversion efficiency and increased mortality with increasing rearing temperature was found only at high stocking rate, and at low stocking rate feed conversion and mortality had little relation with rearing temperature.

The linear decline in feed intake, growth rate and feed conversion efficiency with increasing rearing temperature at high stocking density confirms, but similar feed conversion at different temperatures at low stocking density contradicts other experimental evidence (Howlider and Rose 1987). Similar feed conversion at different temperatures is, however, supported by Howlider and Rose (1989) where birds in individual cages ate similar amounts of feed to a given live weight at 6 different temperatures (21.7-31.5°C). Increased feed intake at high stocking density with increasing rearing temperature may partially be explained by the energy expenditure in struggling (e.g. panting, crowding around waterers, wing lifting, wing spreading etc) to dissipate heat (Griffin and Vardaman 1971). A linear increase in mortality with increasing temperature at high stocking density coincides with Deaton *et al.* (1983) and Brown (1986). The interaction of stocking density and temperature obtained is in agreement with Wathes (1978) and Husseini *et al.* (1987), but disagrees with Charles *et al.* (1978) and Deaton *et al.* (1968) who failed to establish interaction of stocking density and temperature on broiler growth. In this study, stocking density considerably influenced broiler growth.

Meat yield

Rise in rearing temperature increased eviscerated yield, dark meat and therefore, the total meat content of the carcass linearly in both male and female broilers, but little variation was found between the breast meat gains at different temperatures (Table 3). The increase in dark meat yield at higher temperatures was greater at high stocking density than a low stocking density. However, the ratio of breast to dark meat yield declined linearly with increasing rearing temperature almost equally in both sexes regardless of stocking density. The regression equations showed that the breast: dark meat was slightly greater in females than in males and greater at low stocking density than at high stocking density at all temperatures. There was an accelerated deposition of abdominal fat plus skin with increasing rearing temperature, which differed between male and female broilers with respect to stocking density. The hind limb bones (Thigh + drumstick bone + feet) also tended to increase in length with increasing temperature in both sexes and with both stocking density.

As in this study, increased eviscerated yield (therefore, increased total meat) at high temperatures has also been reported by Howes *et al.* (1962). Accelerated gain of dark meat was mainly responsible for increased total meat at high temperatures. The reduction of breast to dark meat ratio with increasing temperature, however, is quite consistent with Bray (1983) and Howlider and Rose (1989). Linear decline of breast: dark meat from 20.3°C onward in both sexes regardless of stocking density signifies that the optimum temperature for getting maximum breast meat may either be 20.3°C or even below. The tendency for an increase in length of hind limb bones with increasing rearing temperature suggests that the shape of birds perhaps changed which may be related to the increased need for heat loss through their extremities to the hot surroundings. Change in shape at elevated temperatures is supported by Lamoreaux (1943) and Fuller (1965). Lamoreaux (1943) reported larger combs in birds reared at higher temperatures and Fuller (1965) observed larger ears of pigs reared at higher temperatures than that at lower temperatures.

The demand for cut-up parts and further processed broiler meat in rising throughout world (Perreault and Leeson 1987, Watts 1988, Grey and Richardson 1988), its being greater for breast (white) than for dark meat (Grey and Richardson 1988). Grey and Richardson (1988) also reported that proportion of meat increases at heavier live weights. Therefore broiler producers may be interested to grow their broilers to higher live weights to satisfy the demand for cut-up parts and further processed meat. Results of this experiment showed that the proportion of total meat and ratio of breast: dark meat is not only the function of live weight, but it can be altered by rearing temperature. Reduction of breast meat at elevated temperatures is likely to be higher at heavier live weights. High environmental temperature is common in the tropics. The high temperatures may affect financial returns not only by extending the rearing period and increasing the feed required to reach given live weights, but also by reducing the breast: dark meat ratio of salable meat.

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Factors affecting body weight at different ages, age at first fertile service and age at first calving in Karan Fries cattle

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The study was carried out in the Karan Fries cattle developed at the National Dairy Research Institute, Karnal. Since this herd has completed 6-7 generations as closed breeding system, there was likelihood of increase in inbreeding level and subsequent ill effects on performances of the animals. In view of above the present study was undertaken to estimate the effect of genetic and non-genetic factors on body weight at different ages and early reproductive traits. Data were collected on 1 349 daughters sired by 52 bulls during the period of 12 years (1980-1991). A pedigree chart was prepared for every animal and the pedigree of each animal was traced back up to foundation stock. The coefficient of inbreeding for every animal was estimated by method of path coefficient (Wright 1922). The abnormal records resulted from premature birth and incurable diseases were excluded from this study. The data were classified into 4 periods of 3 year each to overcome the differences in managemental practices on the basis of temperature, rain and relative humidity. Each calendar year was divided into 5 seasons, i.e. winter (Dec-Jan), spring (Feb-March), summer (April-June), rainy (July-Sep) and autumn (Oct-Nov). The animals were classified into 4 groups based on their coefficient of inbreeding (Fx). These were non inbred (Fx=0), lowly inbred (Fx $\leq$6%), marginally inbred (Fx >6$\leq$12) and highly inbred (Fx >12%).

The effect of various factors on body weights at different ages, AFS and AFC was analysed by least-squares analysis of variance (Harvey, 1975) using following model.

$$Y_{ijklm} = \mu + I_i + S_j + P_k + S_l + e_{ijklm}$$

where,

Y_{ijklm} is performance of mth female belonging to ith inbreeding group, jth sire, kth period of birth and lth season of birth, μ is population mean, Ii is effect of level of inbreeding, S_j is effect of sire, P_k is period of birth, S_l is season of birth, and e_{ijklm} is residual random error assumed to be normally and independently distributed with 0 mean and variance σ^2 (NID) ($0,\sigma^2$). DMRT was used to test the differences among sub classes (Kramer 1957). The heritability was estimated on corrected data for non-genetic effects by paternal halfsib correlation method (Becker 1985). The traits studied were body weight at birth, 3- month, 6- month, 12-month, weight at first fertile service (WFS), weight at first calving (WFC), age at first fertile service (AFS) and age at first calving (AFC).

The least-squares means for body weight at different ages, age at first fertile services and age at first calving with respect to different effects are presented in Table 1. The observed body weight at different ages were in agreement with those reported by Shrivastav *et al.* (1985), Nautiyal and Bhat (1989) and Joshi *et al.* (1991) in crossbred cattle. The estimate for AFC was in agreement with Raheja (1994). The effect of sire was found to be significant on birth weight, 3 month and 12 month weight, WFS, WFC, AFS and AFC. There was a high variation with respect to sire on body weight at different ages, which decreased with the advancement of age. Narayanswamy *et al.*(1984), Gour and Dave (1986), and Singh (1995) in body weight at different ages and Raheja (1994) in AFC also found significant effect of sire in crossbreds.

The average inbreeding coefficient of inbred females was 6.65 % and the proportions of inbreds were 20.96 %. The average annual increase in inbred and their inbreeding coefficient were 1.10 and 0.37 % respectively. Regression of body weight at different ages on inbreeding co-efficient was found to be significant and negative on birth weight, 3month, 6 month, 12 month weight, WFS and WFC. However, the effect was smaller (-0.023 ± 0.00 for WFS to -0.132 ± 0.059 for birth weight) in its magnitudes for above mentioned traits. Least-square analysis, however showed significant effect of inbreeding on birth weight, AFS and AFC (Table 1). Inbred calves (F>12%) had significantly lower birth weight. In this herd low to moderate level of inbreeding have either negligible or favourable effects on body weight at birth and on subsequent ages though the effect was nonsignificant. Srinivas and Gurnani (1981) and Reddy and Sampath (1989), in different zebu breeds, Ali (1997) and Thompson *et al.* (2000) in exotic cattle and Singh (1995) in Karan Swiss reported

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Table1. Least-squares means of body weight at different ages, age at first fertile service and age at first calving in Karan Fries

Traits	Body weight (kg)						AFS (day) Mean±SE	AFC (day) Mean±SE
	Birth Wt. Mean±SE	3M Wt. Mean±SE	6M Wt. Mean±SE	12M Wt. Mean±SE	WFS Mean±SE	sFC Mean±SE		
Overall Mean	27.3±0.3 (1302)	57.9±0.6 (1178)	110.9±1.2 (1136)	178.1±1.4 (1034)	306.6±2.8 (668)	397.9±3.6 (645)	697.6±9.8 (668)	977.9±9.8 (645)
Inbreeding Groups								
1	27.2a±0.2 (1040)	57.6±0.5 (943)	110.8±0.8 (914)	179.1±0.9 (835)	303.5±1.9 (544)	396.3±2.4 (527)	671.6a±6.6 (544)	952.2±6.7a (527)
2	28.5b±0.5 (124)	59.9±1.1 (111)	115.3±2.0 (103)	182.0±2.4 (92)	312.8±4.9 (60)	405.4±6.3 (57)	694.0ab±16.8 (60)	975.3±16.7ab (57)
3	27.5ab±0.6 (60)	57.6±1.5 (54)	109.3±2.7 (51)	178.3±3.3 (46)	305.3±6.5 (32)	399.1±8.6 (29)	729.8b ±23. (32)	1011.5±23.4b (29)
4	25.8c±0.6 (78)	56.5±1.3 (70)	107.9±2.5 (68)	172.8±2.9 (61)	304.8±6.5 (32)	390.9±8.2 (32)	695.1ab±22.8 (32)	972.7±22.8ab (32)
Seasons								
1	28.2ac±0.4 (392)	59.6a±0.8 (347)	111.8a±1.5 (337)	167.5a±0.9 (296)	300.2a±3.5 (181)	393.5a±4.6 (175)	700.4±12.5 (181)	979.3±12.5 (175)
2	28.4a±0.4 (202)	57.8a±1.0 (181)	106.0b±1.8 (169)	180.6b±2.2 (147)	294.8a±4.4 (109)	385.0a±5.5 (105)	696.5±15.3 (109)	980.6±15.3 (105)
3	27.3cd±0.4 (174)	54.2b±1.0 (160)	105.9b±1.9 (150)	189.5c±2.3 (143)	322.6b±4.6 (95)	408.5b±5.8 (94)	718.2±15.7 (95)	995.5±15.8 (94)
4	26.8b±0.4 (352)	59.1a±0.8 (322)	112.5a±1.5 (315)	182.6b±1.8 (297)	313.2b±3.7 (191)	404.5b±4.8 (182)	699.8±12.9 (191)	979.6±12.9 (182)
5	25.6d±0.4 (182)	59.0a±0.9 (168)	118.1c±1.7 (165)	169.9a±2.1 (151)	302.4a±4.3 (92)	398.3ab±5.4 (89)	673.3± 14.6 (92)	954.7±14.7 (89)
Periods								
1	24.4a±0.8 (329)	56.3ab±1.9 (308)	107.0±3.5 (307)	164.9a ±4.1 (299)	300.0±9.1 (177)	390.2±11.5 (175)	668.2a±30.9 (177)	943.2±31.0a (175)
2	27.5b±0.5 (376)	59.9a±1.1 (328)	114. ±2.1 (317)	183.3bc ±2.5 (286)	309.6±4.4 (190)	393.4±5.6 (185)	666.7a±15.1 (190)	945.8±15.2a (185)
3	27.8b±0.5 (283)	56.7b±1.2 (268)	110.3±2.2 (264)	186.2b ±2.6 (249)	304.0±4.5 (209)	394.9±5.8 (198)	698.3a±15.8 (209)	976.4 ±15.9a (198)
4	29.4c±0.5 (314)	58.8ab±1.2 (274)	111.3±2.2 (248)	177.6c±2.8 (200)	313.0±6.0 (92)	413.3±7.5 (88)	757.3b±20.9 (92)	1046.3± 0.7b (88)

Value in parenthesis are number of observations, Means with different superscript in a row differ significantly.

adverse effect of high level (>10%) of inbreeding on growth and age at calving. The least square averages of AFS and AFC for different level of inbreeding varied from 671.6 ± 6.6 and 952.2 ± 6.7 days (non inbred) to 729.8 ± 23.0 and 1011.5 ± 3.4 days (marginally inbred) respectively. The average AFS and AFC for different levels of inbreeding did not differ significantly from non-inbred except for the group of marginally inbred (F>6≤12%). The results showed that inbreeding have greater effect at early age both on growth and reproduction. The use of small number of sires and mating among relatives has contributed to the increase in inbreeding.

The effect of season of birth was found to be significant in body weight at all ages (Table 1). The highest birth weight was observed for spring born calves and lowest for autumn born but at the age of 12 month and at first calving (32.4 month), maximum weight was found in summer born calves and lowest for winter and spring born calves, respectively. The results obtained in the present study are in agreement with those reported by Josi *et al.* (1991), Dhangar and Patel (1992) and Nagare and Kulkarni (2000). The effect of period of birth was found to be significant on birth weight, 3 month, 12 month weight, AFS and AFC. The fluctuations in body weight and AFS and AFC might be partly due to use of different set of sires and partly due to changes occurred in managemental practices and climatic factors over the periods. The results obtained in the study are in agreement with those reported by Shrivastava *et al.* (1985) and Nautiyal and Bhat (1989) and Raheja (1994). The heritability (h^2) for birth weight, 3 M, 6 M, 12 M body weight, WFS, WFC, AFS and AFC were 0.50 ± 0.11, 0.21 ± 0.07, 0.17 ± 0.07, 0.38 ± 0.10, 0.11 ± 0.07, 0.19 ± 0.09, 0.86 ± 0.21 and 0.82 ± 0.21, respectively. These results indicate higher additive genetic variability at early ages and their effect is relatively lower as compared to that of non-genetic factors with increase in the age.

The results inferred that higher level of inbreeding have poor effect on growth and reproduction at early ages. Mating among relatives should be avoided unless the genetic potential is great.

SUMMARY

Performance of Karan Fries cattle born during 1980-91 under closed breeding were analysed for body weight at different ages, AFS and AFC. Among the genetic factors sires were major source of variation for body weight at different ages and in both the reproductive traits. Variability among sires was higher for age than weight at first fertile service and weight at first calving. Genetic variability for growth traits gradually decreases as the age advances. Regression of body weight at different ages on breeding coefficient was significantly negative and magnitude of inbreeding was more at early ages. However, least squares analysis showed significant effect of inbreeding on birth weight, age at first fertile service and first calving. Therefore, level of inbreeding especially of bulls should be accounted for in the selection programmes. Season of birth significantly affected body weight at birth and at later ages. Effect of periods was significant on body weight at birth, 3 months, 12 months, AFS and AFC. Increase in mean values was estimated for body weights, AFS and AFC over the periods.

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Effect of feeding mineral mixture on plasma manganese (Mn++) concentration in cattle

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Experiment was conducted to study the circulating level of manganese (Mn++) in crossbred (H × F) non-cycling heifers, cycling heifers and cycling lactating cow. Six lactating crossbred (Friesian 75% × Hariana 25%) cows (second to fourth lactation, 3.5 to 7 years of age), 6 crossbred (Friesian 87.5% × Hariana 12.5%) cycling heifers (30 to 36 months) and 6 non-cycling heifers at each age group of 10 to 13 months, 18 to 21 months and 27 to 30 months were selected from animal herd of Animal Production Research Institute, Pusa, Bihar. All the animals were kept confined providing sufficient space. Green fodder (sorghum) of known Mn content (32.5 ± 3.23 ppm) and freshwater *ad lib.* for 1 month were made available to them. Prostaglandin PGF2α (15 mg, prosolvin, intervet/intercare) was injected intramuscularly (designated as day 0). Mineral mixture having 0.12% Mn was fed to each non-cycling heifers of above age groups @ 30 g bid from day 7 to day 42. The cycling heifers were provided only green fodder (sorghum) *ad lib.* Cycling lactating cows were provided green fodder *ad lib.* supplemented with concentrate @ 1 kg/ 3 kg of milk. Blood samples were collected by jugular venipuncture from each non-cycling heifer on day 0 (before treatment), 7, 14, 21, 28, 35 and 42 in heparinzied test-tubes. Blood sample from each cycling heifers and lactating cycling cows were also collected on the day they exhibited estrus symptom (day 0) and thereafter on day 7, 14, 21, 35 and 42. Plasma were separated within 2 hr of blood collection by centrifuging at 3000 rpm for 20 min and stored at refrigerated temperature. Mn++ was estimated in plasma within 24 to 48 hr of blood collection by atomic absorption spectrophotometer.

The plasma sample for estimation of Mn++ was prepared by mixing 1 ml plasma with 4ml in triple distilled water and filtered in clean vial as per partial modification of the method used by Saxena and Gupta (1993). Data were analyzed for averages and standard error. Two-way analysis of variance was used to illucidate the differences among the individual groups and that of pretreatment period over other samples (Snedecor and Cochran 1967).

The magnitude of plasma Mn++ concentration (mean ± SE) recorded during present experiment in non-cycling heifers of 10 to 13, 18 to 21 and 27 to 30 months, cycling heifers and lactating cycling cows is presented in Table 1. The plasma Mn++ concentration of both cycling heifers and lactating cycling cow in initial samples was significantly ($P<0.01$) higher than the pretreatment (day 0) plasma Mn++ concentration of 10 to 13 months, 18 to 21 months and 27 to 30 months heifers. The injection of $PGF_2\alpha$ did not have any effect on estrous cyclicity or plasma Mn++ concentration. However, the supplementation of mineral mixture had significant effect on plasma Mn++ concentration in non-cycling heifers of all age groups. The plasma Mn++ concentration increased significantly on day 14 ($P<0.05$) in 27 to 30 months heifers while heifers of 10 to 13 months and 18 to 21 months exhibited significantly ($P<0.01$) higher concentration of plasma Mn++ on day 21. After gradual increase the plasma Mn++ concentration in 27 to 30 months non-cycling heifers resumed almost the similar concentration by day 42. Two out of six non-cycling heifers of 27 to 30 months exhibited estrus for shorter duration of 12 hr at the end of experiment.

The lower Mn++ concentration in non-cycling heifers than cycling heifers might be one of the factors for depressed reproductive function. As it has been substantiated that the Mn++ deficiency in cattle is related to suppression of estrous, poor follicular development and weak estrus (Wilson 1966, Bourne 1967). Higher concentration of Mn++ stimulate the mechanism for synthesis of steroid hormones in the gonads. The exhibition of estrus and suppression of follicular development in cattle fed low Mn++ diet was because of reduced availability of Mn++ for proliferation of granulosa cell population (Parmar *et al.* 1986).

The present observation also agreed with report of higher concentration of Mn++ at estrus than anoestrus period and repeat breeding cows (Parmar *et al.* 1986). Besides, the role

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Table 1. Plasma (mean ± SE) concentration (PPM) of manganese (Mn^{++}) in crossbred heifers and cows

Blood sampling/ treatment schedule	Treatment group non-cycling heifer				Control group	
	10 to 13 months	18 to 21 months	27 to 30 months	Over all mean±SE	Cycling heifers (30 to 36 months)	Lactating cycling cow (3.5 to 7 years)
0 day (pretreatment level) PGF2α (15 ml, I/M)	0.30^{a}±0.015	0.32^{a}±0.014	0.35^{a}±0.014	0.32+0.01	1.01^{k}±0.012	0.99^{k}±0.031
7th day mineral mixture (30 g bid)	0.30^{a}±0.014	0.33^{a}±0.010	0.36^{abc}±0.020	0.33+0.01	0.97^{k}±0.011	0.98^{k}±0.032
14th day	0.32^{a}±0.024	0.36^{abc}±0.022	0.42^{bcd}±0.027	0.37+0.02	0.99^{k}±0.014	1.00^{k}±0.030
21st day	0.41^{bcd}±0.010	0.43^{cd}±0.016	0.47^{de}±0.019	0.44+0.01	1.01^{k}±0.014	0.96^{k}±0.029
28th day	0.47^{de}±0.013	0.51^{ef}±0.023	0.65^{hi}±0.033	0.54+0.02	1.00^{k}±0.013	0.98^{k}±0.034
35th day	0.55^{fg}±0.018	0.60^{gh}±0.020	0.74^{j}±0.043	0.63+0.03	1.00^{k}±0.011	0.97^{k}±0.031
42nd day	0.71^{ij}±0.015	0.71^{ij}±0.022	0.92^{k}±0.037	0.78+0.03	1.01^{k}±0.010	0.98^{k}±0.033

Means with different superscripts both row- and column-wise differ significantly (P<0.05).

of Mn++ was also observed in the synthesis of sterols and its participation of gonadal hormone synthesis (Benedict *et al.* 1965). On the other hand gonadotropins was considered to influence Mn++ transport and modify its availability to different tissues of reproductive organs. Besides the direct role in the enzymatic and other tissue metabolic function Mn++ is considered necessary for normal fertility in ruminants and deficiency of Mn++ depresses the reproductive efficiency of all farm animals (Hidiroglou *et al.* 1977).

The values of Mn++ recorded during present experiment among non-cycling heifers fall within the range of Mn++ reported in anaestrous cows (Prasad and Rao 1997, Tambe *et al.* 1998, Parmar *et al.* 1986). The significantly increased concentration of Mn++ in plasma on day 14 (27 to 30 months) and day 21 (10 to 13 months and 18 to 21 months heifer) was in line with the identical elevation of other trace minerals (Fe, Cu, Zn and Co) in the same plasma samples (Kumar 2000). The significantly higher concentration of Mn++ in cycling heifers and cycling lactating cows than non-cycling heifers as well as step-wise increase in Mn++ concentration during advancing age of prepubertal period seems to be associated with stages of hypothalamohypophyseal and gonadal maturation.

SUMMARY

An experiment was conducted on crossbred (Friesian 87.5% × Harridan 12.5%) non-cycling heifers of 10 to 13 months, 18 to 21 months and 27 to 30 months, cycling heifers (27 to 30 months) and cycling lactating cows to estimate circulating plasma manganese concentration before and after feeding mineral mixture. The plasma manganese concentration in cycling heifers (1.01 ± 0.012 ppm) and cycling lactating cows (0.99 ± 0.031 ppm) was significantly (P<0.01) higher than non-cycling heifers of 10 to 13 months (0.30 ± 0.015 ppm), 18 to 21 months (0.32 ± 0.14 ppm) and 27 to 30 months (0.35 ± 0.014ppm). Feeding of mineral mixture increased circulating plasma Mn++ concentration in all age groups of non-cycling heifers within 21 days and reached similar to cycling animal in heifers of only 27 to 30 months by day 35.

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The efficacy of phytase in broiler diets containing low phosphorus, calcium and crude protein

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Phytic acid in plants can ionically bind minerals and proteins. It has the ability to bind proteins at acidic, alkaline and neutral pH (Sebastian *et al.*1997). Because of its chelating ability, phytate may reduce the availability of essential minerals (Morris 1986). Phytate also has the ability to bind protein and to form a complex, protein-cation-phytic acid (Campagne *et al.*1990). This complex may reduce the protein utilization. Phytate in plant feedstuffs has the potential to bind proteins and negatively affect protein digestibility (Carmovale *et al.* 1988). Phytase supplementation may release the phytate bound protein for utilization. Dietary supplementation with microbial phytase can increase the phosphorus availability and decrease excretion (Van Der Klis *et al.*1997). Farrell *et al.* (1993) have demonstrated that supplemental phytase improved nitrogen retention in broiler chicks. Lei *et al.* (1993) have indicated that in pigs, the microbial phytase might influence the utilization of dry matter, and other nutrients such as calcium and crude protein. An improvement in the utilization of phytate bound minerals and amino acids will reduce the level of inorganic phosphorus sources in diets. Calcium and crude protein levels in diets may be reduced by supplementing phytase. The objective of this experiment was to investigate the efficacy of phytase supplementation on growth performance, toe ash and excreta phosphorus in broiler chickens fed similar phytate-phosphorus containing phosphorus, calcium and crude protein-deficient diets.

Seven-day-old Ross male broiler chicks (70) were weighed and distributed to individual wire cages. There were 14 chicks per treatment. All birds were exposed to continuous fluorescent light. Feed and water were provided *ad lib.*

Table 1. Ingredient and nutrient composition of experimental diets

Ingredient composition	Basal	Basal+ phytase	Low P+ phytase	Low P-Ca+ phytase	Low P-Ca-CP+ phytase
Corn	51.25	51.25	51.58	53.24	56.48
Soybean-meal (44% CP)	30.92	30.92	31.16	30.84	28.93
Wheat	4.52	4.52	4.51	4.50	4.63
Fish-meal	4.71	4.71	4.50	4.50	2.74
Soybean oil	5.78	5.78	5.68	5.21	5.10
Ground limestone	0.97	0.97	1.51	0.66	0.72
Dicalcium phosphate	1.18	1.18	0.38	0.37	0.66
Salt	0.25	0.25	0.25	0.25	0.25
Vitamin + mineral premix	0.25	0.25	0.25	0.25	0.25
DL-methionine	0.17	0.17	0.17	0.17	0.22
L-lysine	–	–	0.01	0.01	0.02
Calculated and analyzed nutrient composition					
Metabolizable energy (kcal/kg)	3075	3075	3075	3075	3075
Crude protein	21.00	21.00	21.00	21.00	19.50
Available phosphorus	0.45	0.45	0.30	0.30	0.30
Phytate phosphorus	0.21	0.21	0.21	0.22	0.22
Calcium	0.96	0.96	0.96	0.64	0.64
Methionine + cystine	0.86	0.86	0.86	0.86	0.86
Lysine	1.21	1.21	1.21	1.21	1.21

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Table 2. Effects of phytase supplementation on performance, toe ash, excreta dry matter, ash and phosphorus of broiler chickens (8-38 days of age)

Diet	Weight gain (g)	Feed intake (g)	Feed: gain (g:g)	Excreta DM (%)	Toe ash (wet basis) (%)	Excreta ash (%/DM)	Excreta P (%/DM)
Basal	1680^{b}	2941^{b}	1.75	22.75	29.79^{a}	3.39^{a}	1.15^{a}
Basal+phytase	1730^{ab}	3034^{ab}	1.76	22.45	26.73^{ab}	3.14^{b}	1.10^{a}
Low P+phytase	1772^{ab}	3024^{ab}	1.71	21.48	28.89^{ab}	3.08^{bc}	0.94^{b}
Low P-Ca+phytase	1821^{a}	3148^{a}	1.74	21.98	24.57^{ab}	2.71^{c}	0.96^{b}
Low P-Ca-CP+phytase	1800^{ab}	3144^{a}	1.75	22.90	26.39^{ab}	2.92^{c}	1.04^{b}
Pooled SE	19.74	28.36	0.01	1.32	0.69	0.05	0.001

^{a,b,c}Means witnin columns with no common superscript differ significantly ($P<0.05$).

throughout the 31-days trial. The experimental design was completely randomized arrangement. The levels of phosphorus (P), calcium (Ca) and crude protein (CP) in five experimental diets were basal, basal + phytase, low P + phytase, low P-Ca + phytase and low P-Ca-CP + phytase, respectively. Supplemental phytase (1200 U/kg diet) was added to the diets except control diet. Ingredient and nutrient composition of diets given to broiler chickens from 7 to 38 days were shown in Table 1. The crude protein level in low P-Ca-CP + phytase diet was 18.5 % and the remaining diets contained 19.5% CP between 29 and 38 days. The energy levels in diets were 3 125 metabolizable energy kcal/ kg diet from 29 to 38 days.

Feed intake and weight gain were determined individually on days 14, 21, 28 and 38. Excreta was collected from 7 chicks in each treatment at 14, 21, 28 and 35 days of age. Pooled excreta samples were analyzed for dry matter, phosphorus and ash. Total P concentration in diets and excreta were determined colorimetrically using vanadomolybdate procedure (AOAC 1984). The phytate P content of diets was analyzed using the iron precipition method of Oshima *et al.* (1964). On day 38, seven birds from each dietary treatment were slaughtered. Toe samples were obtained by severing the middle toe through the joint between the second and third tarsal bones from the distal end. The fat free toes were ashed at 600 °C for 4 hr (Potter 1988). Data obtained from experiment were subjected to the analysis of variance and differences in means were compared by the procedure of Duncan.

The results obtained from this experiment are summarized in Table 2. Phytase supplementation resulted in no significant differences in feed:gain values of chicks when the diets were either deficient or adequate in phosphorus, calcium and crude protein. However, low P-Ca + phytase diet increased ($P<0.05$) body weight gain by 8.4 % and feed intake by 7.03% compared to basal diet. Broilers given low P-Ca or low P-Ca-CP supplemented diets with phytase consumed more food than those fed basal diet. These results are in accordance with findings of Mitchell and Edwards, (1996), Yi *et al.* (1996), Sohail and Roland (1999) and Gippert *et al.* (2000).

The phytin-P levels in experimental diets were the same (0.21 % for 8 to 28 days and 0.22% for 29 to 38 days). The dry matter content of excreta was not significantly influenced by the treatments. The percentage ash of wet toe in broilers fed supplemented experimental diets with phytase were similar to broilers given unsupplemented basal diet. The excreta ash was decreased by supplementing phytase to diets ($P<0.05$). The reduction in excreta ash can be explained by the effect of phytase on the utilization of other phytate-bound minerals in diets. The excreta P in broilers fed both basal and basal + phytase diets were higher than those given low P, low P-Ca and low P-Ca-CP supplemented diets with phytase ($P<0.05$). Compared with the basal diet, excreta phosphorus was decreased by 9.56 to 18.26 % by adding phytase in diets containing 0.45 and 0.30 % available phosphorus. These results have also been supported by findings of Mitchell and Edwards (1996), Yi *et al.* (1996) and Sohail and Roland (1999). They have demonstrated that addition of phytase to the diet containing low phosphorus resulted in 18 to 25 % reduction in the phosphorus excretion when compared with diet containing adequate phosphorus. Phytase can hydrolyze phytate bound phosphorus effectively at lower dietary calcium levels. Excreted phosphorus in excreta can be decreased by adding phytase in diets have low phosphorus levels. The reduction in dietary phosphorus, calcium and crude protein levels to decrease the cost of broiler diets by adding phytase did not affect broiler performance.

SUMMARY

The efficacy of phytase supplementation on growth performance, toe ash and excreta phosphorus in broiler chicken fed similar phytase-phosphorus containing phosphorus, calcium and crude-protein deficient diets was studied. Feed: gain values and toe ash did not differ in chicks on different diets. Excreta ash decreased in broilers fed on phytase-supplemented diet.

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Growth performance of broiler rabbits under different levels of protein

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Key words: Crude protein, Dry matter,Economics, Feed efficiency,Growth, Rabbit

Broiler rabbits are capable of thriving well in almost all the climatic zones of India. Rabbits are most suitable for meat, fur, and wool, as they have small body size, short generation interval, high productive potential, rapid growth rate, genetic diversity and ability to utilize by-products of forages efficiently. Soviet Chinchilla (SC) breed is the most suitable for rearing in semi-arid region (Gulyani *et al.*2000), temperate region (Sastry and Mahajan 1981) and North Eastern Hill region (Rohilla and Bujarbaruah 1999) of our country.

Soviet Chinchilla (SC) kits (40) weaned at the age of 35 days were randomly alloted to groups (T1, T2, T3 and T4) as per the experimental design (RBD) and maintained on 4 rations containing 12, 14, 16 and 18% crude protein (CP), respectively. The kits were fed *ad lib.* twice daily (morning and evening) and the leftovers of the previous day were recorded before offering fresh feed. Clean drinking water was made available to all the experimental kits 24 hr. Daily feed intake and weekly body weights of kits were recorded up to the end of the experiment (56 days). Digestion trial was conducted for 7 days and the samples of faeces, urine and experimental feeds were analysed (AOAC 1984). Total weight gain, DM intake, feed conversion efficiency and economics of rearing was worked out. Data were analysed as per the standard statistical techniques (Snedecor and Cochran 1980).

Treatment had a highly significant ($P<0.01$) effect on the growth of broiler kits, feed efficiency and nutrient digestibility (Table 1). The final body weights of the rabbits at the end of the experiment (91 days) were significantly ($P<0.01$) higher in T3 than that in T1, T2 and T4 groups, respectively. Similarly the average daily gain was also significantly ($P<0.01$) higher in T3 than in T1, T2 and T3. The linear increase in body weight with the increased level of protein up to T3 group indicated that the rabbits kept on 16% diet converted most of the nutrients towards the growth and fattening after meeting their requirement. Similar findings were published by Prasad *et al.* (1996) and Narute *et al.* (2000).

Table-1. Means±SE of growth performance of broiler rabbits under different levels of crude protein

Particulars	T1 (12%CP)	T2 (14%CP)	T3 (16%CP)	T4 (18%CP)	Significance
Initial body weight (g)	572.25^{a}±1.70	570.00^{a}±1.45	570.30^{a}±1.68	571.08^{a}±1.55	NS
Final body weight (g)	1697.75^{a}±30.68	1843.20^{b}±22.54	2005.80^{c}±43.70	1936.30^{c}±55.45	**
Total weight gain (g)	1125.50^{a}±23.45	1273.20^{b}±45.60	1435.50^{c}±57.55	1365.22^{c}±32.25	**
Average daily gain (g)	20.09^{a}±1.36	22.73^{b}±1.58	25.63^{c}±1.65	24.37^{c}±1.28	**
Total DM intake (g)	5076.40^{a}±25.63	5178.88^{b}±48.75	5280.80^{c}±66.72	5335.12^{d}±82.35	**
DM-intake (g/day)	90.65^{a}	92.48^{b}	94.30^{c}	95.27^{c}	**
CP- intake (g/day)	10.87^{a}	12.94^{b}	15.08^{c}	17.15^{d}	**
Feed conversion ratio	4.51^{a}	4.06^{a}	3.68^{b}	3.90^{b}	**
Feed efficiency	0.22^{a}	0.24^{b}	0.27^{c}	0.25^{b}	**
Protein efficiency	1.85^{a}	1.75^{b}	1.70^{c}	1.42^{d}	**
Total feed cost (Rs)	39.40^{a}	41.13^{b}	43.92^{c}	45.35^{d}	*
Cost/kg gain in wt (Rs)	35.02^{a}	32.30^{b}	30.60^{c}	33.22^{b}	*

Figures with different superscripts differ significantly in a column.

The dry matter intake (DMI)/ kg body weight was significantly ($P<0.01$) higher in T1 followed by T2, T3 and T4, which indicated that at lower levels of crude protein, animals consumed more dry matter, so as to meet the protein

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demand for different physiological functions. The feed conversion ratio and feed efficiency was significantly ($P<0.01$) superior in T4 than rest of the 3 groups under study. However, the protein efficiency was higher in T1 than T2, T3 and T4 treatments but the total weight gain was very low, which clearly indicated that it is uneconomical for commercial broiler rabbit production. These findings are in close confirmation with those reported by Ayyat (1994) and Narute *et al.* (2000).The digestibility of CP significantly ($P<0.01$) increased with the increasing level of crude protein. Similar results were obtained by Deshmukh *et al.* (1990) and Narute *et al.* (2000). The digestibility of EE, CF and NFE was significantly ($P<0.01$) different among the 4 treatments.

The total feed cost was significantly ($P<0.05$) higher in T4 than that in T1 (Rs 39.40), whereas, the cost per kg body weight gain was significantly higher in T1 group followed by T4, T2 and T3. No mortality and any type of illness was observed in any of the treatment groups throughout the experimental period. These findings are in congruence to earlier reports by Narute *et al.* (2000).

Based on the present findings it may therefore be concluded that 16% (T3) crude protein level was appropriate and economical for getting better growth in commercial broiler rabbit production. To ascertain the best suitable protein level in the rabbit diet at various growth stages, further such more studies are required.

SUMMARY

An experiment on 40 broiler kits (Soviet Chinchilla) maintained on 4 different crude protein levels (12, 14, 16 and 18%) up to the age of 13 weeks was conducted. Average daily weight gain was recorded maximum (25.63g) in T3 followed by T4 (24.37g), T2 (22.73g) and T1 (20.09g), respectively. However, the cost per kg gain was significantly ($P<0.05$) lower in T3 in comparison to T2 and T4. Hence it may be concluded that 16% crude protein level in broiler rabbit ration is an economic and better protein supplement.

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Performance of female guineapigs fed gram replaced economic diet

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The guineapigs (*Cavia porcellus*) are being reared for different experimental purposes, and one of them is to test the potency of vaccines or drugs at laboratory scale. For this purpose healthy and good nutritional status of animals is indispensibly required for getting an accurate drug or vaccine response. Guineapigs are presently being reared on costly feed ingredients like gram and wheat, which are primarily consumed by human beings. In developing countries like India, it is difficult to spare these feed ingredients for the feeding of laboratory animals. Therefore, present study was undertaken to assess the performance of female guneapigs on gram replaced diet.

Female guineapigs (40) just weaned, were procured from laboratory Animal Section and were randomly divided into 2 groups of 20 each. In both the groups, concentrate mixture and available green fodder was fed *ad lib.* to meet the requirements of the animals. The concentrate mixture of group 1 consisted of wheat bran, crushed maize, fish-meal and crushed gram @ of 35, 30, 10 and 25%, respectively. Whereas, concentrate mixture of group 2 contained wheat bran, crushed maize, fish-meal, deoiled GNC, minerals and vitamins supplement, common salt and ascorbic acid @ of 35,45, 10, 8, 1.45, 0.50 and 0.05%, respectively. After 10 weeks of growth study a digestion trial was conducted to assess the nutrient intake and digestibility.

Representative samples of concentrate mixtures, green fodder and faeces were preserved as per Schneider and Flatt (1975) for further chemical analysis. The proximate principles in feeds and faeces were done as per AOAC (1995) and fibre fraction as per Van Soest *et al.* (1991). Thereafter, female guineapigs were placed for breeding trial to assess the litter size. For this purpose 1 adult guinea pig was placed in each cage having 5 female guineapigs. The new guineapigs were delivered after 60-65 days of gestation period. The statistical analysis of data was carried as per Snedecor and Cochran (1968).

Chemical composition (Table 1) of concentrate mixtures and green grass data showed that the diets were isonitrogenous. Mean DM intake through concentrate mixture in group 1 and 2 was 24.8 and 24.4 g/day, respectively, and through green fodder was 19.5 and 19.6 g/day. Total dry matter intake (g/day) in groups 1 and 2 was 44.3 and 44.0 (Table 2), respectively, and was comparable between 2 groups. The DMI as % of body weight was also found to be comparable between the groups. The intake of DCP and TDN was 4.96 and 32.86 g/day in group 1 and 5.12 and 32.29 in group 2, respectively, and did not differ significantly between 2 groups. The intake of nutrients was comparable to the NRC (1972). Digestibility of nutrients corroborated well with the findings of Meyer *et al.* (1996) who studied the digestibility of various feeds and also the food tolerance limit. During 10 weeks of experimental feeding, total body weight changes between groups were similar and the average daily gain was 3.23±1.12 and 3.19±0.11g in groups 1 and 2, respectively, which also did not differ significantly and corroborated well with the findings of Sakaguch *et al.* (1957). The mortality (15.0 and 20.0%) and litter size (4.0 and 3.5) in respective groups were comparable. On current market price of feed ingredients, the cost of concentrate mixtures 1 and 2 was Rs 11.2 and Rs 09.6/ kg, respectively, which clearly indicated that gram replaced

Table 1. Physical and chemical composition of two diets fed to the female guineapigs (% DM basis)

Particulars	Group 1	Group 2	Mixed green grass
OM	87.70	86.20	87.2
CP	19.30	19.50	8.0
EE	3.46	3.18	2.65
NDF	19.10	20.20	50.50
ADF	13.20	10.80	38.10

Maize 30 and 45, crushed gram 25 and 00, wheat bran 35 and 35, deoiled groundnut-cake 00 and 8, fish-meal 10 and 1.0, mineral and vitamin mix, 00 and 1.45, common salt 00 and 0.50 and ascorbic acid 00 and 0.05% in concentrate mixtures 1 and 2, respectively.

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Table 2. Mean body weight changes, feed intake and digestibility of nutrients, mortality and litter size in female guineapigs

Particulars	Group 1	Group 2
Initial body weight (g)	168.0±11.25	168.7±14.53
Final body weight (g)	394.1±18.19	392.3±16.79
Average daily gain (g)	3.23±0.12	3.19±0.11
Dry matter intake (g/day)		
Concentrate mixture	24.8±0.08	24.4±0.3
Green fodder	19.5±1.25	19.6±1.26
Total	44.3±1.18	44.0±1.05
DMI as% of body weight	11.2±0.26	11.2±0.24
Intake of nutrients (g/day)		
DM	44.3±1.18	44.0±1.05
OM	39.7±1.08	39.3±0.76
CP	6.32±0.1	6.26±0.07
DCP	4.96±0.16	5.12±0.08
TDN	32.86±1.02	32.29±1.13
Intake of nutrients (% of body weight)		
DM	11.25±0.26	11.21±0.24
OM	10.07±0.18	10.03±0.21
CP	1.60±0.02	1.60±0.03
DCP	1.26±0.01	1.30±0.01
TDN	8.34±0.19	8.23±0.18
Digestibility (%)		
DM	75.55±2.04	76.22±1.18
OM	78.45±1.25	78.84±1.16
CP	78.35±1.49	81.74±0.86
EE	75.63±1.68	76.18±1.52
NDF	60.64±2.41	60.81±1.94
ADF	42.20±1.04	43.64±0.52
Average daily gain (g)	3.23±0.12	3.19±0.11
Mortality (%)	15.0	20.0
Litter size	4.0±0.44	3.5±0.34

concentrate mixture is cheaper (Rs 1.6/kg) and economical. From the results it may be deduced that female guineapigs can be reared on gram replaced diet without any ill affect on their performance.

SUMMARY

A study was undertaken to assess the performance of female guneapigs fed on gram replaced diet. It was found that female guineapigs could be reared on this economic diet without affecting their performance.

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Effect of seasons on daily herd production performance of Frieswal and Sahiwal cattle

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The effect and magnitude of variation in the production and reproduction performance due to season and periods varies with species, breed, genetic make up of the animal, geographical and agro-climatic location of the farm etc. Study of these non-genetic factors help to develop the farm and location specific management pointeers to avoid the seasonal fluctuations in the farm produce and for ensuring steady and smooth supply of output to the consumers. Thus, the present study was done about the trend and extent of variation due to season and period in daily herd production performance of Frieswal and Sahiwal cattle maintained at Military Farm, Meerut.

Daily herd production performance records as wet average (kg), herd average (kg), total milk yield of the herd (kg), number of milch and dry cows, total lactating herd size, percentage of milch and dry cows in the herd were recorded in Frieswal and Sahiwal cows maintained at Military Farm, Meerut, from 1 April 1995 to 31 March 1999. Frieswal, a crossbred cattle, is on its way to be evolved as a breed, having 5/8 Holstein-Friesian inheritance with native Sahiwal and the females are now being bred in successive generations with 5/8 HF × Sahiwal bulls. The period of study was divided into 4 years and each year was further subdivided into 3 seasons i.e. summer (April-June), rainy (July-October) and winter (November-March). The percentile data were transformed by arc-sine transformation technique (Snedecor and Cochran 1994). Data were subjected to statistical analysis by mixed model least squares and maximum likelihood computer program, PC-2 version (Harvey 1990).

Year and season showed significant effects ($P<0.01$) in most of the traits, except the percentage of milch and dry Frieswal cows (Table 1). Changes in management and feeding are the attributable factors for variable trends in herd performance. The significant effect of year and season of calving on milk yield were reported by many research workers (Rao and Nagarcenkar 1979, Kakran and Joshi 1990, Dhangar *et al.* 1991, Shettar and Govindaiah 1999).

Results indicated that the highest daily wet average, herd average and total daily milk yield of the herd were obtained during summer and lowest during rainy season in Frieswal cattle (Table 1). Although, there were no significant variation in the percentage of milch cows over the seasons, the decline in production performance from July onwards indicated that the herd's yield was worst affected by rainy season that is hot

Table 1. Least-squares means of herd productivity performance for Frieswal cattle

Particulars	No. milch cow	No. dry cow	Lactating herd size	Cow %		Total herd yield (kg/day)	Wet average (kg/day)	Herd average (kg/day)
				Milch	Dry			
Over all	392.11±0.80	144.10±0.46	536.21 ±0.60	73.28 ±0.06	26.27 ±0.06	3675.73 ±13.29	9.38 ±0.02	6.85 ±0.02
Years								
1	348.49±1.58[a]	123.17±0.91[a]	471.67 ±1.17[a]	73.90 ±0.12[a]	26.06 ±0.12[a]	3410.64 ±26.12[a]	9.73 ±0.04[a]	7.20 ±0.04[a]
2	384.65±1.58[b]	129.48±0.91[b]	514.13 ±1.17[b]	74.92 ±0.12[b]	25.06 ±0.12[b]	3518.28 ±26.15[a]	9.10 ±0.04[b]	6.83 ±0.04[b]
3	435.97±1.58[c]	147.47±0.91[c]	583.71 ±1.17[c]	74.73 ±0.12[b]	25.28 ±0.12[b]	3745.60 ±26.15[b]	8.62 ±0.04[c]	6.40 ±0.04[c]
4	399.34±1.58[d]	176.00±0.91[d]	575.34 ±1.17[d]	69.44 ±0.12[c]	30.60 ±0.12[c]	4028.41 ±26.15[c]	10.07 ±0.04[d]	6.98 ±0.04[d]
Seasons								
Summer	391.86±1.58[a]	144.73±0.9[a]	536.59 ±1.17[a]	73.45 ±0.12	26.59 ±0.12	3945.20 ±26.08[a]	10.08 ±0.04[a]	7.39 ±0.03[a]
Rainy	383.83±1.36[b]	142.46±0.78[b]	526.29 ±1.00[b]	73.01 ±0.11	26 99 ±0.10	3254.97 ±22.38[b]	8.53 ±0.04[b]	6.18 ±0.04[b]
Winter	400.64±1.22[c]	145.10±0.70[c]	545.75 ±0.91[c]	73.38 ±0.09	26.59 ±0.09	3827.02 ±20.20[c]	9.53 ±0.03[c]	6.99 ±0.03[c]

Means with similar and no superscripts in a row under the sub-heads of years and seasons do not differ significantly ($P<0.01$).

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Table 2. Least squares means of herd productivity performance of Sahiwal cattle

Particulars	No. milch cow	No. dry cow	Lactating herd size	Cow %		Total herd yield (kg/day)	Wet average (kg/day)	Herd average (kg/day)
				Milch	Dry			
Over all	67.57 ±0.09	35.09±0.10	102.67 ±0.06	65.88 ±0.05	34.08 ±0.05	496.73 ±1.25	7.36 ±0.02	4.84 ±0.01
Years								
1	62.36 ±0.18^a	38.59±0.20^a	100.95 ±0.12^a	61.75 ±0.11^a	38.12 ±0.11^a	467.71 ±2.46^a	7.47 ±0.03^a	4.63 ±0.02^a
2	72.80 ±0.18^b	30.72±0.20^b	103.52 ±0.12^b	70.43 ±0.11^b	29.57 ±0.11^b	512.77 ±2.46^b	7.04 ±0.03^b	4.95 ±0.02^b
3	68.08 ±0.18^c	35.15±0.20^c	103.22 ±0.12^{bc}	66.04 ±0.11^c	33 95 ±0.11^c	485.18 ±2.46^c	7.15 ±0.03^c	4.70 ±0.02^c
4	67.05±0.18^d	35.91±0.20^d	102.96±0.12^c	65.17±0.11^d	34.81±0.11^d	521.26±2.46^d	7.79±0.03^d	5.07±0.02^d
Seasons								
Summer	67.36±0.18^a	35.90±0.20^a	103.26±0.12^a	65.34±0.11^a	34.66±0.11^a	538.83±2.45^a	8.00±0.03^a	5.22±0.02^a
Rainy	69.30±0.15^b	32.12±0.17^b	101.42±0.10^b	68.29±0.09^b	31.61±0.09^b	485.85±2.11^b	7.03±0.03^b	4.80±0.02^b
Winter	66.04±0.14^c	37.26±0.15^c	103.31±0.09^c	63.98±0.08^c	36.01±0.08^c	465.52±1.92^c	7.06±0.03^b	4.50±0.02^c

Means with similar and no superscripts in a row under the sub-heads of years and seasons do not differ significantly ($P<0.01$).

and humid. Similar reports of poor performance in hot-humid weather conditions are agreeable with Sindhe *et al.* (1990) and Dhangar *et al.* (1991) in various breeds of dairy cattle. Better performance during summer season indicated that high temperature might not be that much limiting factor for milk production as that of hot and humid conditions during rainy season. Besides, the availability and quality of fodder played major role for seasonal fluctuations in milk yield.

In Sahiwal herd almost similar trend was obtained. Like Frieswal, the wet average was the lowest in rainy season and highest in summer in Sahiwal cows. However, the variation between winter and rainy season was nonsignificant (Table 2). The highest herd average was observed during summer for both the groups i.e. Sahiwal and Frieswal. Unlike Frieswal, the lowest herd average in Sahiwal cattle was obtained during winter. The lowest herd average during winter might be due to significantly higher number / per cent of dry cows during this season as compared to other seasons. Higher total herd yield during rainy season as compared to that in winter might be due to more number of cows in milk during rainy season (Table 2). The magnitude of reduction in wet and herd average from March (highest average yield) to October (lowest average yield) was 23.39 and 26.67%, respectively, in Frieswal cows. In Sahiwal herd the highest and lowest average yield were obtained during April and November, respectively. The magnitude of reduction in wet and herd average in Sahiwal cows (21.81 and 23.52%, respectively) was little lower as compared to Frieswal, which might be attributed to the better withstanding capabilities of Sahiwal cattle in adverse climatic conditions i.e. hot-humid. The similar trend in herd performance of both Sahiwal and Frieswal cows indicated that maximum attention is to be paid during rainy season (July-October) to avoid seasonal imbalance of milk production. Although, there was slight reduction in herd yield during May-June, the drastic reduction from July onwards necessitates reduction of humidity stress on priority basis as compared to heat stress and feeding of good quality fodder like dried berseem and oats might prove helpful under the existing condition. Alternatively, inclusion of more high protein concentrate mixture along with non-leguminous kharif fodder could prove useful to maintain the desired level of milk production during rainy season.

SUMMARY

Herd production performance from April 1995 to March 1999 of Frieswal and Sahiwal cows maintained at Military Farm, Meerut, was analyzed to find out the seasonal variation in milk production. Year and season showed significant effect on daily herd and wet averages of both Frieswal and Sahiwal cows. During hot humid season milk production was worst affected both in Frieswal and Sahiwal herds. There were slight reduction in milk yield during May and June and drastic reduction was observed from July onwards reaching to the lowest levels in October and November. The magnitude of reduction in wet and herd average was less in Sahiwal as compared to Frieswal cows.

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