MILK PROTEIN POLYMORPHISM AND MILK COMPOSITION IN VECHUR CATTLE

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THESIS

Submitted in partial fulfilment of the requirement for the degree

Master of Veterinary Science

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MANNUTHY, THRISSUR

1996

DECLARATION

*I hereby declare that this thesis entitled **Milk protein polymorphism and milk composition in Vechur cattle** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.*

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I am previlaged to acknowledge **DR. ABRAHAM VARKEY**, Professor, Department of Surgery, for his affectionate inspiration and constant encouragement.

My sincere thanks to **M.V. KUMARY**, Farm Assitant, staff and labourers of the Scheme on "Conservation of Germplasm of Vechur cattle" for their valuable help and co-operation.

I am also thankful to **DR. B. NANDAKUMARAN, DR.K.V. RAGHUNANDANAN, DR. P. NANDAKUMAR, DR. T.V. ARAVINDAKSHAN**, Ms. **VIJAYALAKSHMY** and other staff of the Department of Animal Breeding and Genetics.

In this juncture I thankfully remember my colleagues **DR. SHAJI, DR. MALARKANAN** and **DR.JACOB ABRAHAM**, for their help rendered during various srages of study.

I am grateful to the Dean for providing all the facilities for the study.

Lastly but not the least, I express extreme gratitude to all my sisters for the encouagement and confidence given to me especially by **JEYAMANY** and **DHANALAKSHMY**.

THIRUPATHY VENKATACHALAPATHY

Dedicated to my mother
R. Maruthayammal

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Introduction

INTRODUCTION

Livestock forms an integral part of our agricultural economy. India is a reservoir of vast and varied types of animal genetic resources. In terms of genetic diversity there are 27 breeds of cattle, 8 breeds of buffaloes, 42 breeds of sheep, 22 breeds of goats, 8 breeds of camels, 6 breeds of horses and 17 breeds of poultry. Thus India is the repository of 1/9th germplasm of cattle breeds of the world and almost all breeds of the riverine buffalo. The farm animals of India have unique characteristics of adaptations to withstand hostile climate, common epidermic pests and diseases of tropics. They can survive on inadequate quantities of feed, fodder and water. It is logical to assume that the genetic drift and natural selection, in isolated population of animals led to the evolution of different breeds which were given different names based on place of origin and on some well marked specific phenotypic characters. The discovery of artificial insemination in middle twentieth century opened new technologies of improvement. In India, crossbreeding was used as a tool to increase milk production by introducing exotic germplasm. However the process had other side effects, viz. loosing the distinctions of many breeds and over all reduction of genetic variation in the species. It simultaneously placed some of the breeds with lower productivity at risk. In this way some of native breeds eventually faced extinction prior to the thorough evaluation of their genetic potential.

The native cattle of Kerala have been evolved through several generations of natural selection against high humidity, heavy rainfall and hot climate. They have been considered as non-descript animals, but there has been a variety with characters distinguishing from others and known as "Vechur Cattle". The Vechur cattle had their origin in Vechur, a small place by the side of Vembanad lake near Vaikom in Kottayam district of Kerala. They were very popular four decades back. They had relatively higher milk production compared to other local cows (Velu Pillai, 1940). The extremely small size of the cow, low feed requirement, good adaptation and high disease resistance are the traits very much favoured by the farmers. Farmers preferred Vechur cattle for ploughing in marshy land because of the small size and weight. Kerala Agricultural University has succeeded in conserving this unique genetic resource with the financial aid from Indian Council of Agricultural Research. Genetic characterisation is in progress.

The present study has been taken up with the following objectives:

1. Identification of certain genetic markers in Vechur cattle

2. To study the milk components like fat, total solids, solids not fat as a part of characterisation of the breed

3. To study the fat globule size, distribution and Iodine value of milk fat

The studies on milk protein polymorphism play an important role in understanding the genetic characteristics of Vechur breed. The estimation of milk components is of high economic importance. It is useful to know the effect of crossbreeding on fat and solids not fat (SNF) content of milk which form the basis for the present pricing system in dairy industry. The size of fat globules is considered to be a breed characteristic and iodine value gives a measure of unsaturated fatty acids in the milk fat.

There is a belief that the Vechur cow milk has high thickness and medicinal value, so it was used to prepare medicated oils and ayurvedic medicines. This belief can not be set aside as baseless. The present studies play a vital role to characterise the Vechur cattle and help to find out the scientific basis behind the beliefs which were nurtured by the local people for several generation.

Review of Literature

REVIEW OF LITERATURE

2.1 Milk protein polymorphism

2.1.1 Casein

Cow's milk contains approximately 3.5 per cent protein and distributed as 2.9 per cent casein and 0.6 per cent whey proteins. The casein in milk exists in the form of a complex or micelle consisting of calcium caseinate plus phosphate, magnesium and citrate. The principal milk protein casein comprising of α-, β- and k-casein with notable difference in the mobility in an electric field which enables the identification of these proteins by an electrophoretic procedure.

2.1.1.1 α_{s1} - Casein

Kiddy *et al.* (1964) observed six casein phenotypes among 1378 cows. The distribution of phenotypes were 98 Ayrshires (all BB), 203 Brown Swiss (192BB and 11BC), 400 Guernseys (188 BB, 32 CC and 44 BC), 542 Holsteins (2 CC, 410 BB, 5 AC and 125BC), 68 Jerseys (44 BB, 2 CC and 21 BC) and 68 crossbred (67 BC and 1 BC). The reason for predominant of B was not apparent.

Aschaffenburg (1968) reported that the A variant of α_{s}-casein was very rare and appeared to be confined to single blood line of Holstein originating in the state of Michigan. "B" variant of α_{s1}-casein predominated in Western breeds of cattle and frequency was lower in some of the African zebu breeds. The gene frequency of α_{s1}-casein B variant was exceeding 0.90 in many breeds and reached 1.00 in some breeds like Ayrshire.

The existence of polymorphism in the casein fractions of Indian cattle was first demonstrated by Aschaffenburg (1968). Milk samples from five breeds viz. Hariana, Desi, Sahiwal, Tharparkar and Red Sindhi were tested. Most of the variants were identical to those of Western breeds. Zebu breeds had very high gene frequency of α_{s1}-casein C unlike predominance of B variant in Western breeds.

Juneja and Chaudhary (1973) observed that the gene frequency of α_{s1}-casein C was 0.9 in Sahiwal and Rathi breeds.

Hussain (1974) reported that the α_{s1}-casein B allele predominated in all European breeds of cattle. African breeds like Boran (0.39) and Ankoli (0.40) had moderately high frequencies and some other African breeds like Neguni(0.86) and Pedi (0.92) had much higher frequency of B allele than that in Indian cattle.

Jairam and Nair (1983a) identified three types of α_{s1}-casein BB, CC and BC. The gene frequency of B allele of α_{s1}-casein was 0.09 in Sahiwal, 0.09 in Tharparkar, 0.03 in Red Sindhi and 0.51 in Brown Swiss x Sahiwal cross.

Ng-Kwai-Hang *et al.* (1984) phenotyped casein for 2045 Holstein cows by poly acrylamide gel electrophoresis. Gene frequencies observed were α_{s1}-casein A 0.003, B 0.970 and C 0.027. 94 percentage of animals were homozygous for α_{s1}-casein B variant. The A and C variants were rare and appeared only as heterozygous AB and BC.

Mclean *et al.* (1984) reported genetic variants at the α_{s1}-casein locus in Jersey and Holstein breeds. The gene frequency for α_{s1}-casein was 0.628 for B and 0.372 for C in Jersey cows and 0.963 and 0.037 for B and C variant in Friesian cows.

Lin *et al.* (1986) and Eenemaan *et al.* (1991) reported that the locus α_{s1}-casein was dominated by allele B (95.5 percentage) in Holstein Friesian, Ayrshire and Holstein Friesian/Ayrshire crossbred lines.

Madhavan (1995) observed that α_{s1}-casein locus consisted three phenotypes with two alleles B and C. The BC and BB phenotypes were higher in Jersey crossbred. The B allele had the highest frequency in all three genetic groups viz. Jersey crossbred (0.63), Brown Swiss crossbred (0.61) and Holstein Friesian crossbred (0.63).

2.1.1.2 β-casein

Genetic variants of β-casein was first reported by Aschaffenburg (1961) by using paper electrophoresis. The three variants β-casein A, B and C with different electrophoretic mobilities were detected in British breeds either singly (A, B or C) or in pairs (AB, BC and AC).

Thompson and Pepper (1964) reported that the occurrence of β-casein variant was breed specific, the β-casein A, B allele occur in Jersey and Holstein, A, B and C allele in Guernsey and Brown Swiss breeds and only A allele in Ayrshire breed.

Aschaffenburg (1968) reported a rare β-casein D variant in Indian desi and African Boran cows indicating link between Indian and African zebu cattle. The β-casein B variant in Indian cattle differs in its amino acid composition from Western breeds. The further electrophoresis of A variant of β-casein resolved into A1, A2 and A3. A3 allele was found to be rare in Holstein Friesian and Normande breeds. Frequency of A2 was found to be higher in Ayrshires and Guernseys and also in certain Indian breeds like Sahiwal, Tharparkar, Hariana, Red Sindhi and Desi. The frequency B variant of β-casein was very low in majority of the breeds. C variant of β-casein was not found in Jerseys but observed in Guernsey. C allele was found to be characteristic of Brown Swiss and other mountain breeds of cattle. The C variant was very low in Holstein Friesian cows.

Singh and Khanna (1972) reported β-casein variants in two Hariana populations maintained at Izatnagar and Haringhatta. They observed five phenotypes viz. A1A1, A2A2, BB, A1A2, A1B and A2B controlled by three alleles β-casein A1, A2 and B. The phenotype A2A2 was predominant in both populations. A1B was observed only in Izatnagar population and the frequency of β-casein A1, A2 and B were 0.051, 0.838 and 0.11 in Izatnagar and that for Haringhatta animals were 0.040, 0.804 and 0.156 respectively.

Juneja and Chaudhary (1973) observed two phenotypes at β-casein locus in Sahiwal, Rathi and Jersey X Sahiwal viz. AA, AB. The predominant phenotype was AA, though the frequency of heterozygotes AB was higher in Jersey X Sahiwal crossbred. The frequency of β-casein A allele was 0.94 in Sahiwal, 0.95 in Rathi and 0.81 in Jersey X Sahiwal. A new rare phenotype β-casein AD were observed in one of the Rathi cows. The casein D had slower mobility than that of β-casein B.

Frequency of B allele of β-casein was reported to be low in Indian zebu cattle (Jairam and Nair, 1983 b). The highly significant differences between the gene frequencies in zebu cattle (0.04- 0.07) and Brown Swiss X Sahiwal (0.16) indicated that gene frequency in exotic breed might be quite different from that in Indian cattle.

Ng-Kwai-Hang *et al.* (1984) observed that the gene frequency of β-casein A1 0.561, A2 0.421, A3 0.011 and β-casein B 0.007 in Holstein cows. The rare B gene was only in heterozygous combination with A1, A2 and A3.

Mclean *et al.* (1984) observed gene frequency of 0.074, 0.564 and 0.362 respectively for β-casein A, A2 and B in Jersey cows and 0.074, 0.564 and 0.362 for β-casein A1, A2 and B respectively in Friesian cows.

Lin *et al.* (1986) observed a gene frequency of 0.440 0.450, 0.07 and 0.04 for A1, A2, A3 and B allele in Ayrshire cattle.

Madhavan (1995) observed three phenotypes viz. AA, AB and BB at β-casein locus. The frequency of A allele was 0.81 in Brown Swiss crossbred, 0.75 in Jersey crossbred and 0.71 in Holstein crossbred. frequency of AA phenotype was highest in Brown Swiss crossbred (0.67) and AB was highest in Jersey crossbred.

2.1.1.3 k-casein

Aschaffenburg (1968) observed two variants of k-casein A and B. The A allele predominated in majority of cattle breeds except Jersey, Normande and some African zebu cattle.

Two phenotypes k-casein AA and AB were reported by Majumder and Ganguli (1970). The gene frequency of k-casein A was 0.63, 0.78 and 0.62 in Tharparkar, Sahiwal and Red Sindhi respectively. All the zebu breeds were predominantly of AA phenotype.

Juneja and Chaudhary (1973) reported two phenotypes AA and AB in zebu and three phenotypes AA, AB and BB in crossbred. The frequency of k-casein A was 0.92 in Sahiwal, 0.83 in Rathi and 0.54 in Jersey X Sahiwal.

Jairam and Nair (1983b) observed that the significantly higher gene frequency of 0.69 for B allele in crossbred cows while it was 0.26 in Sahiwal and Red Sindhi and 0.13 in Tharparkar breed.

Ng-Kwai-Hang *et al.* (1984) reported that the gene frequency of k-casein A and B were 0.744 and 0.256 in Holstein cows with 53 percent, four percent homozygous for A, B and 43 percent heterozygous for AB.

Lin *et al.* (1986) found three genotypes of k-casein AA, AB and BB in Holstein Friesian and Holstein Friesian X Ayrshire lines. k-casein locus was dominated by allele A (65.6%).

Madhavan (1995) observed two alleles A and B and three phenotypes viz. AA, AB, BB at k-casein locus. The frequency of AA was highest in Jersey crossbred (0.52) and AB phenotype in Brown Swiss crossbred (0.51). Allele A was predominant in all the populations.

2.1.2 Whey proteins

The proteins remaining after casein in the skim milk is known as whey protein which consists of mainly alpha-lactalbumin and β-lactoglobulin.

2.1.2.1 α-lactalbumin

Bhattacharya *et al.* (1963) found the frequency of α-lactalbumin A allele varying from 0.20 in crossbred to 0.37 in Sahiwal cows.

Mawal (1967) reported low frequency of A allele as 0.38 in Gir and 0.40 in Gavathi cows.

Aschaffenburg (1968) observed low gene frequency in some of the African breeds like Boran and Ankole, but other showed similar frequency as in Russian breeds.

Jairam and Nair (1983b) reported the frequency of A allele in Sahiwal, Tharparkar, Red Sindhi and Brown Swiss x Sahiwal crossbred cows as 0.18, 0.17, 0.16 and 0.08 respectively.

2.1.2.2 β- lactoglobulin

Aschaffenburg and Drewry (1955) observed β-lactoglobulin A and B variants in Short horn, Friesian, Ayrshire and Guernsey breeds.

Aschaffenburg (1968) reported that the zebu breeds had very low frequency of A variant compared to Western breeds. B allele was predominant in most cases and C variant was found only in South African Neguni cattle in heterozygous form.

Singh and Khanna (1972) observed β-lactoglobulin A, B and C variants in Hariana cattle. The gene frequency of B variant was reported to be 0.831 and 0.921 for Izatnagar and Haringhatta population.

Juneja and Chaudhary (1973) reported three phenotypes viz. AA, AB and BB at β-lactoglobulin locus in Sahiwal, Rathi and Jersey X Sahiwal cattle. The homozygous BB was predominant in all the genetic groups. The frequency of β-lactoglobulin A was 0.18, 0.21 and 0.32 in Sahiwal, Rathi and Jersey X Sahiwal crossbred.

Singh and Bhat (1980) phenotyped milk samples for β-lactoglobulin locus from cows of Hariana, Sahiwal, Kankrej, Ongole, Red Sindhi, Kangayam, Gir, Tharparkar, Holstein Friesian, crossbred of Holstein Friesian with Hariana and Sahiwal. Holstein cows exhibited A and B variant. Indigenous and crossbred cattle had three alleles Lg -A, Lg-B and Lg-C. Lg-B was the most common allele among indigenous breeds (0.44 to 0.94), Lg-A had relatively high frequency among Holsteins (0.57).

Jairam and Nair (1983 b) reported significant differences between Tharparkar and Sahiwal or Red Sindhi in the β-Lg. A gene frequencies. The gene frequency of A allele in Sahiwal, Tharparkar, Red Sindhi and Brown Swiss x Sahiwal crossbred cows were 0.23, 0.13, 0.23 and 0.31 respectively.

Ng-Kwai-Hang *et al.* (1984) observed gene frequency of β-lactoglobulin A 0.387 and β-Lg B 0.613 in Holstein cows. The phenotypic frequencies were 0.135, 0.505 and 0.360 for AA, AB and BB.

Lin *et al.* (1986) observed AA, AB or BB variant in β-Lg locus was dominated by allele B (78.4 per cent). Madhavan (1995) observed A and B allele at β-Lg locus. The frequency of A allele was 0.46 in Holstein Friesian crossbred, 0.40 in Brown Swiss crossbred and 0.43 in Jersey crossbred.

2.2 Milk composition

The milk yield and milk composition in the cow are influenced by many environmental and hereditary factors. This may vary between individuals, herds and breeds. Indian breeds of cattle in contrast to exotic dairy breeds yield lower quantity of milk, but with higher percentage of fat and solids not fat.(Singh *et al*,1961).

2.2.1 Milk fat percentage

2.2.1.1 Effect of species

The effect due to species on milk fat percentage was found to be statistically significant by different authors, Byron *et al.* (1972); Singh *et al.* 1961; Balwant Rai Puri *et al.* 1961; Ghosh and Anantakrishnan (1963).

2.2.1.2 Effect of breed

Several breeds of cattle, as a result of long continued segregation and inbreeding had characteristic differences in the composition of milk they produce. The fat percentage of recognised Indian breeds of cattle and their crosses reported by several workers given in Table 2.1.

Table 2.1 Average milk fat percentage of recognised Indian breeds of cattle and their crosses

Author(s)	Breeds of cattle	Fat percentage
Annual Report NDRI (1948)	Sindhi	4.90
	Gir	4.73
	Tharparkar	4.55
	Sahiwal	4.55
Balwant Rai Puri *et al.* (1961)	Red Sindhi	4.50
	Tharparkar	4.70
Kohli *et al.* (1961)	Hariana	4.40
Singh *et al.* (1961)	Sahiwal	5.30
	Hariana	5.90
	Local	4.30
Ghosh and Ananthakrishnan (1963)	Sindhi	5.15
	Gir	5.10
	Crossbred	4.83
Arora and Gupta (1969)	Nimari	4.43
Tilakaratne *et al.* (1975)	Jersey x Sindhi	5.51
Babu Rao and Jayaramakrishna (1983)	Brown Swiss x Ongole	4.19
	HF x Ongole	3.66
	Ongole	5.20
Naikare *et al.* (1992)	Friesian x Gir	3.93
	Jersey x Gir	4.36
	Brown Swiss x Gir	3.94
Iype *et al.* (1994)	Crossbred	3.40

Among exotic cattle Jersey was found to be having a high fat percentage (5.05) compared to Brown Swiss, Holstein Friesian, Guernsey, Ayrshire and Simmental breeds of cattle (Byron et al., 1972; Belyaev and Kislewa, 1966; Tilakaratne *et al.*, 1975). Meat breeds like Angus, Hereford and Charolais had milk fat percentage of less than three (Melton *et al.*, 1967).

2.2.1.3 Effect of stage of lactation

Various workers reported that the significant effect of stage of lactation on the fat percentage of cow milk. The major constituents tended to decline nearly in the fourth week of lactation after which there was a significant increase as lactation advanced (Bayoumi, 1959; Singh *et al.*, 1961; Ghosh and Anantakrishnan, 1964; Arora and Gupta, 1969; Sadana *et al.*, 1978).

Iype *et al.* (1994) observed a significant difference in fat percentage of early, middle and late lactation in crossbred cows of Kerala.

2.2.1.4 Season

Bayoumi (1959); Ghosh and Ananthakrishnan (1963); Sommerfeldt *et al.* (1985); Mohran and Fahmy (1992) observed a significant effect of season on fat percentage of both cow and buffalo. It was higher during monsoon seasons.

Arora and Gupta (1969) revealed that the fat percentage of cow was not influenced by season and they explained that the slight changes was due to availability of fodder and high temperature stress in summer months.

2.2.1.5 Parity

Though changes were erratic, the effect of lactation number was significant on fat percentage (Sadana *et al.*, 1978; Naikare *et al.*, 1992).

2.2.1.6 Pregnancy

Parekh and Gangwar (1968) observed an increase and decrease trend in fat percentage during mid and late pregnancy. Sharma *et al.* (1990) noticed significant variation in milk constituent due to stage of pregnancy.

2.2.1.7 Ration

Several authors reported that significant effect of ration on fat percentage of cows (Taparia, 1969; Sathian, 1992).

2.2.2 Total solids percentage

2.2.2.1 Species

The total solids content of the milk varies depends upon the species. Singh *et al.* (1961); Ghosh and Anantakrishnan (1963); Mohran and Fahmy (1992) observed a significant difference in total solids content of cow and buffalo milk.

2.2.2.2 Breed

The variation in total solids content of milk due to breeds was significant. The breed wise total solids content reported by various authors is given in Table 2.2. Exotic breeds showed the trend of total solids same as that of fat percentage. Guernsey and Jersey had total solids percentage of above 14 compared to other breeds like Brown Swiss, Ayrshire, Holstein Friesian, Short horn, Red Danish and Simmental.

Table 2.2 Average total soilds percentage of milk in recognised Indian breeds of cattle and their crosses

Author(s)	Breeds of cattle	Total solids percentage
Annual Report NDRI (1948)	Sindhi	13.93
	Gir	13.56
	Tharparkar	13.42
	Sahiwal	13.58
Balwant Rai Puri *et al.* (1961)	Red Sindhi	13.11
	Tharparkar	13.00
Arora and Gupta (1969)	Nimari	13.69
Tilakaratne *et al.* (1975)	Jersey x Sindhi	14.55
Babu Rao and Jayaramakrishna (1983)	Ongole	14.51
	Ongole x Brown Swiss	13.29
	Ongole x HF	12.26
Johnson (1995)	Crossbred	12.99

2.2.2.3 Stage of lactation

Darshanlal and Narayanan (1991) reported that the total solids content of the milk was significantly affected by the stages of lactation, (Bayoumi, 1959; Mohran and Fahmy, 1992) but not by the lactation numbers in cows. But in buffaloes both number and lactation stage had significant effect on total solids content.

Ghosh and Anantakrishnan (1963) observed a period of decline for nearly in the fourth week of lactation in cows and seventh week in buffaloes (Parekh and Gangwar 1968) after which there was a significant rise as lactation advanced.

2.2.2.4 Season

Ghosh and Anantakrishnan (1963) as well as Sommerfeldt (1985) observed significant effect of season on total solids content of milk.

Barbano and Dellavalle (1985) studied biweekly variation of total solids content of pooled cow milk samples and reported the non significant effect of season.

2.2.2.5 Pregnancy

Parekh and Gangwar (1968) reported an increase and decrease trend in total solids percentage during mid and late stage of pregnancy in cow.

Sharma *et al.* (1990) reported that the stage of pregnancy accounted for a significant effect on total solids content of milk.

2.2.3 Solids Not Fat percentage

2.2.3.1 Species

The solids not fat content differed significantly due to species. Ghosh and Anantakrishnan (1964): Mohran and Fahmy (1992) found significant difference in solids not fat content of cow and buffalo milk.

2.2.3.2 Breed

The difference in solids not fat percentage due to breed is given in Table 2.3.

Solids not fat percentage of exotic breeds of cattle showed lesser variation compared to fat and total solids percentage. Byron *et al.* (1972) reported solids not fat content of above nine percent in Guernsey, Jersey, Brown Swiss, Ayrshire breeds of cattle.

Table 2.3 Average soilds not fat percentage of milk in recognised Indian breeds of cattle and their crosses

Author(s)	Breeds of cattle	Solids not fat percentage
Annual Report NDRI (1948)	Sindhi	9.03
	Gir	8.83
	Tharparkar	8.87
	Sahiwal	9.03
Ghosh and Ananthakrishnan (1963)	Sindhi	8.86
	Gir	8.91
	Crossbred	8.78
Arora and Gupta (1969)	Nimari	9.26
Babu Rao and Jayaramakrishna (1983)	Ongole	9.30
	Ongole x Brown Swiss	9.09
	Ongole x HF	8.68

2.2.3.3 Stage of lactation

The stage of lactation and age of the cow had a significant effect on solids not fat content of the milk (Bayoumi, 1959); Singh *et al.*, 1961)

Ghosh and Anantakrishnan (1964); Arora and Gupta (1969) observed no definite trend and concluded that the stage of lactation was not having significant effect on SNF content of the milk in cows.

2.2.3.4 Season

Several authors reported that the significant effect of season on the SNF content of the milk of cattle (Ghosh and Anantakrishnan, 1964; Arora and Gupta, 1969; Sommerfeldt *et al.*, 1985; Mohran and Fahmy, 1992).

2.2.4 Correlation among constituents

Non-significant correlations between fat and SNF percentages were reported by Bayoumi (1959); Arora and Gupta (1972). Babu Rao and Jayaramakrishna (1983) found significant correlation between fat and SNF percentages in Ongole and their crosses and Sommerfeldt *et al.* (1985) observed positive significant correlation.

Significant correlation values (Babu Rao and Jayaramakrishna, 1983) were obtained between fat and total solids percentages among Ongole and their crossbred cows and by Belyaev and Kiseleva (1966) in Black Pied and Simmental cows.

Babu Rao and Jayaramakrishna (1983) reported significant positive correlation between SNF and Total solids percentages in Ongole and their crossbred cows.

2.3 Fat globule

The lipids exist in milk in the form of spherical droplets or globules (Jenness and Patton 1959). They are very small, but vary considerably in size (2 to 20μ). The milk phospholipids are present in the fat globule membrane and found to be an important factor in the development of brain and nervous tissues and also paly a vital role in the fat digestion and absorption due to its emulsifying action (Norman, 1978). An increase in the number of smaller size of fat globule is always associated with greater surface area and higher phospholipid content (Kuchroo and Narayanan, 1977a & b). The small size fat globules are digested more readily by lipases because of the greater surface exposed (Jenness, 1980). The size of fat globule influenced by species, breed, stage of lactation, but the influence of environmental factors such as feed, climate were less definitive (Jenness and Patton, 1959).

2.3.1 Effect of species

Fahmi *et al.* (1956) reported that the average size of fat globules were 3.30μ, 3.49μ, 4.55μ and 5.92μ for sheep, goat, cow and buffalo milk.

Balwant Rai Puri *et al.* (1961) observed a range in the size of fat globules in different samples of goat, cow and buffalo milk as 2.06 to 3.53μ, 3.26 to 3.99μ and 3.68 to 5.05μ respectively.

Kuchroo and Narayanan (1977b) reported the average size of fat globule in cow and buffalo milk were 3.5μ, 4.6μ.

George (1981) studied size of fat globules in Alpine X Malabari and Saanan X Malabari crossbred goats as 2.56μ and 2.70μ respectively. The size was found to be decreasing with the advancement of lactation.

2.3.2 Effect of Breed

The distribution of fat globule size was influenced by breed, the cows of Channel Island breeds produce milk containing more of larger globules than those of other breeds. The fat globule size distribution for four breeds of cattle (Jenness and Patton, 1959) is given in Table 2.4.

Table 2.4 Per cent distribution and mean size of fat globules in milk of Four breeds of cattle

Breed	Range in microns						
	0-2.4	2.4-4.8	4.8-7.2	7.2-9.6	9.6-12	12-14.4	Mean
Jersey	8.1	38.3	32.1	18.1	5.3	1.1	5.48
Gurnsey	6.5	38.9	35.0	14.4	4.4	0.7	5.36
Ayrshire	14.6	54.0	23.4	6.2	1.6	0.2	4.24
Holstein	14.5	54.6	24.5	5.1	1.1	0.2	4.18

2.3.3 Effect of stage of lactation

The size of fat globules was affected by stage of lactation. It was reported by Upadhyaya *et al.* (1973) in buffaloes: Katiyar *et al.* (1973) in Sahiwal cows. Kuchroo and Narayanan (1977b) observed an increase in the proportion of smaller size fat globules and decrease in bigger size fat globules as the lactation progressed.

2.3.4 Effect of milking interval

The relative size of fat globules in the morning and evening milk were not similar (Byron *et al.,* 1972) unless the milking intervals were equal.

2.3.5 Effect of fractional milking

Whittlestone (1958) observed no difference in size of fat globules between middle milk and strippings. Kuchroo and Narayanan (1977a) reported that the size of fat globules have a negative trend towards the termination of milking corresponding to increase in the fat content of milk.

2.3.6 Correlation coefficient between fat globule size and fat percentage

Kuchroo and Narayanan reported (1977a) significant negative correlation of fat globule size with fat percentage as -0.7056 in buffalo and -0.8648 in cow milk. The correlation coefficient for early, middle and late lactation was - 0.0963, - 0.1606 and + 0.4033 in buffalo and - 0.4236, -0.0984 and - 0.1373 in cow's milk respectively.

Kuchroo and Narayanan (1977b) observed significant negative correlation coefficient between fat globule size and phospholipid content in fore milk and strippings of cow and buffalo.

2.4 Iodine Value

Iodine value gives a measure of the unsaturated fatty acids present in the fat (Jenness and Patton, 1959). The saturated fatty acids are digested easily by β-oxidation than unsaturated fatty acids and found to be very useful in malabsoption syndromes (Vasudevan and Sreekumari,1995).

Basu (1962) reported that the iodine value for milk fat from different breeds of goats and cows varied from 32.8 to 33.9, while buffalo milk fat showed a lower value of 29.4.

Fahmi and Fahmy (1972) found that with advancing stage of lactation iodine value of both cows and buffaloes tended to decrease. Iodine value at the beginning of lactation and the relative rates of increase during the lactation period were higher in cows than in buffaloes. They reported that iodine value were not affected by change in ration.

Ramamurthy *et al.* (1978) reported variation in iodine number from 21.6 to 31.6 in cow's milk fat.

George (1981) reported iodine number for the milk fat of Alpine x Malabari and Saanen x Malabari goats as 24.95, 25.09 respectively and found that it was increased with the advancement of lactation in crossbred goats. Henry and Newlander (1987) reported a range in the iodine number of cow milk fat as 30 to 34. They observed a sharp increase during pasture season and when oily feeds were fed. It also increased during last few weeks of lactation period and to some extent during fasting.

Materials and Methods

MATERIALS AND METHODS

Fourteen Vechur cows maintained under ICAR Scheme on "Conservation of Germplasm of Vechur cattle of the coastal area and other dwarf cattle of high ranges of Kerala" were used for this study (Fig. 1).

Collection of milk samples

Milk samples of 50 ml from individual cows were collected mixing at weekly intervals in the morning and evening for one lactation. Samples were analysed for percentage of fat, total solids, solids not fat and fat globule size. The sample for milk protein polymorphism were collected in a sterile plastic tube directly from the teats. Half litre of milk was used for the estimation of iodine number at fortnightly intervals for four months.

Management of cows in the farm

The cows in milk in the farm were fed with adlibitum roughage and one kg. of concentrate mixture having following composition.

Fig.1 Vechur cattle of Kerala

Yellow maize	:	20 parts
Ground nut oil cake	:	28 parts
Wheat bran	:	17 parts
Rice polish	:	32 parts
Mineral mixture	:	1.5 parts
Salt	:	1.5 parts

The animals were vaccinated against foot and mouth and Haemorrhagic septicemia as routine.

Weaning is not practised and letting down of milk is done by suckling of calves. Samples were collected from individual cows in the morning and evening.

The traits studied were:

1. Milk protein polymorphism:

2. Percentage of fat, total solids and solids not fat in milk

3. Fat globules size and distribution

4. Iodine value

3.1 Milk protein polymorphism

3.1.1 Procedure of Separation of casein

Fractionation of casein was started within an hour of sample collection by using the method suggested by Thompson and Kiddy (1964) with slight modification.

Five ml of milk sample was centrifuged for 15 minutes at 3000 rpm. The skim milk under the fat layer was aspirated with a long hypodermic needle and syringe to fresh centrifuge tubes. 1N hydrochloric acid were added to the skim milk samples till the pH was adjusted to 4.6. The samples were again centrifuged at 3000 rpm for 15 minutes and the whey portion was removed. The casein obtained in the tubes were washed for four to five times with distilled water.

3.1.2 Separation of Genetic variants of Casein

The variants of casein were separated by poly acrylamide gel electrophoresis. The genetic variants were identified by their electrophoretic mobility as per the nomenclature of major and minor milk proteins of cow's milk established by Eigel *et al.* (1984).

3.1.3 Buffers and solutions: Electrophoretic buffer

Tris	-	12 g
Glycerine	-	57.6 g
Distilled water	-	2.0 l
pH	-	8.3

Acrylamide stock solution (A)

Acrylamide (Sisco)	-	32 g
N, N, Methylene Bisacrylamide	-	0.8 g

The two reagents were dissolved in 100 ml distilled water and filtered.

Gel buffer (B)

Tris	:	9 g
Urea	:	24.4 g
Citric acid	:	0.9 g
Distilled water	:	100 ml
Beta Mercapto Ethanol	:	4 drops

Ammonium persulphate (C)

50 mg of ammonium persulphate was freshly dissolved in 50 ml distilled water and used.

Working gel solution

Composition for the 8 % acrylamide gel

Acrylamide (A)	:	6.25 ml
Gel buffer (B)	:	8 ml
Distilled water	:	2.75 ml
TEMED	:	0.03 ml
Ammonium persulphate (C)	:	8 ml

Staining solution

Amido black 10 B	:	1g
Distilled water	:	930 ml
Acetic acid	:	70 ml

Destaining solution

Methanol	:	2500 ml
Glacial acetic acid	:	500 ml
Distilled water	:	2500 ml

Preserving solution

Acetic acid	:	70 ml
Distilled water	:	930 ml

3.1.4 Procedure

3.1.4.1 Preparation of gel

A continuous buffer system of 8 per cent acrylamide solution at pH 8.3 was used.

Two plates of equal size, one made up of acrylic sheet and the other one the glass plate was used for the preparation of gel. The acrylic sheet was having 1.5 mm high frame on three sides which formed the thickness of the gel. On the free side without the frame the acrylic sheet was having projection to form well on the gel. The glass plate was kept in opposition by an application of vacuum grease on the frame. Paper clips were applied on all sides. Freshly prepared working gel solution was filled into the gap between the plates. Care was taken to avoid the formation of air bubbles in the gel. Polymerisation was completed within 20 minutes.

Preparation of casein sample for electrophoresis

The casein was dissolved in 6M urea solution for the application of the samples for electrophoresis.

3.1.4 2 Electrophoresis

Acrylic sheet was carefully removed and gel with glass plate was gently placed in the electrophoretic chamber containing the electrode buffer. Whatman filter paper No.1 was used as the wick for the completion of circuit connecting the gel and electrode buffer. Enough numbers of filter papers of equal sizes and same level at the edges were used for uniform voltage gradient. The wicks were wetted well and placed gently on either side of the gel.

Twenty microlitres of casein sample dissolved in 6 M urea solution was charged into the wells. Bromophenol blue was used as marker on two wells as indicator. An initial current of 200 V at 10 MA for 10 minutes followed by 200 V at 25 MA for 5 Hours was applied.

3.1.4.3 Staining, destaining and preservation

The gel was removed from the glass plate and put in destaining solution for 2 hours. Excess stain was removed by keeping the gel in destaining solution for 12 hours. Then it was preserved in the preserving solution.

The genetic variants were identified by their relative mobility on the gel.

3.1.2 Whey proteins

Whey protein variants was separated by the method of Ng-Kwai-Hang *et al.* (1984). The same method for casein with the following modifications was applied for whey proteins. Gel buffer (B) devoid of urea with same composition was used. Crystaline urea 0.4 g/ml was added to the supernatant left after casein precipitation, twenty microlitres of the samples were applied to the wells. Twelve percentage acrylamide gel was used.

3.1.3 Analysis of data

The gene frequencies at different loci were calculated by the direct counting method and expressed in percentage.

3.2 Milk composition

3.2.1 Fat percentage

Fat percentage of milk was estimated by Gerber's method as per the procedure described in IS : 1224 Part I (1977).

3.2.2 Total solids percentage

Total solids percentage of milk was estimated by gravimetric method (IS : 1479 - Part II 1961).

3.2.3 Solids not fat percentage

Solids not fat (SNF) content of milk was determined by finding the difference between total solids content and fat content of milk.

3.2.4 Statistical Analysis

Mean, Standard error and coefficient of variation were estimated for each parameter. Correlation between traits was estimated by the method described by Snedecor and Cochran (1967).

The influence of stage of lactation ie weeks 1-44 and time of milking were worked out using least squares analysis of variance as described by Harvey (1986) for non orthogonal data. Model used was:

$$Yijk = u + Ti + Sj + eijk$$

where,

Yijk	=	k^{th} observation of j^{th} week (stage) of the i^{th} time milking
u	=	General mean
Ti	=	Effect of i^{th} time of milking (t = 1 -2)
Sj	=	Effect of j^{th} stage of lactation (S = 1 - 44)
eijk	=	Random error

3.3 Fat globule

3.3.1 Measuring the size of fat globules

The technique of Rangappa (1964) with modifications in dilution of milk was used to estimate the size of fat globules. One ml of milk sample was made up to 10 ml with water. The microscopic examination was carried out by placing one drop of diluted sample on a slide on to which a cover slip was placed. The diameter of the fat globules were then measured under the microscope by fitting an eye piece micrometer to the eye piece of the microscope, the scale of which being previously determined by a 1/100 stage micrometer.

Milk samples were taken from cows individually. The size of atleast 100 fat globules were measured in each sample. To ensure that all the globules viewed in the field were recorded, the microscopic field was divided into sections by full rotation of the eye piece. The diameter of every fat globule present in the circular space made by rotating half of the graduation of the eye piece a full rotation were noted. In this way repeated measurements of the same fat globules were avoided.

3.3.2 Method of analysis

Fat globule size was determined by the method of Kuchroo and Narayanan (1977a). To estimate the average diameter, the fat globules measured were grouped according to their diameter. The average diameter was then obtained by multiplying the number of globules in each group by its average diameter, summing the products and dividing it by the total number of globules in all groups. Pooled samples of milk from goats, crossbred cattle and buffaloes were taken as controls and also for comparison as against individual samples from Vechur animals.

3.4 Iodine value

The iodine value is the number of grams of iodine absorbed by 100 g of oil or fat. The softness of fat is directly related to iodine number. iodine number was estimated by Wiji's method (IS : 3508-1966 part I 1980)

3.4.1 Procedure

Weighed accurately 0.40 - 0.45g of the clean ghee from individual cows in a dry iodine value flask. The fat was dissolved in 15 ml of carbon tetrachloride and by means of a burette exactly 25 ml of Wijis reagents (Iodine mono chloride 5ml; Glacial acetic acid 500 ml) was added. The flask was closed well and mixed carefully and left for one hour in the dark. Then 20 ml of 10 percentage potassium iodide solution and approximately 150 ml of distilled water were added and mixed well. The contents were titrated with 0.1 N sodium thiosulphate solution swirling the liquid constantly. Shortly before the end of the titration 2 ml of the starch solution was added and the contents were shaken vigorously. A blank test was carried out by using the same quantity of the reagents. Then number was calculated as follows.

$$\text{Iodine value} = \frac{12.69\ (B - S)\ N}{W}$$

Where

B - Volume in ml of standard sodium thiosulphate required for the blank

S - Volume in ml of standard sodium thiosulphate required for the sample

N - Normality of the standard sodium thiosulphate solution

W - Weight in gram of the material taken for the test

The iodine value is expressed as a number.

Results

RESULTS

4.1 Milk protein polymorphism

Fourteen animals were typed for milk protein variants such as α_{s1}-casein, β-casein, κ-casein and whey proteins α-lactalbumin and β-lactoglobulin. The phenotype and gene frequency are presented in Table 4.1.

4.1.1 Casein

4.1.1.1 α_{s1}-casein

Three phenotypes (Fig.2) were observed in α_{s1}-casein locus viz., BB, BC and CC with alleles B and C. The Phenotype BB was fast moving and CC slow moving. The diagrammatic representation is given in Fig. 3.

4.1.1.2 β-casein

Two phenotypes AA and AB with two alleles A and B were observed at β-casein locus (Fig. 2). The diagrammatic representation is given in Fig. 3.

Table 4.1 Phenotype and gene frequency of α_{s1}-, β and k-caseins, α-Lactalbumin and β-Lactoglobulin types in Vechur cattle.

Variant	Phenotype frequencies			Gene frequency	
α-casein	BB	BC	CC	B	C
	0.14 (2)	0.22 (3)	0.64 (9)	0.25	0.75
β-casein	AA	AB	BB	A	B
	0.29 (4)	0.57 (8)	0.14 (2)	0.58	0.42
k-casein	AA	AB		A	B
	0.17 (1)	0.83 (5)		0.59	0.41
α-lactalbumin	AA	AB		A	B
	0.57 (4)	0.43 (3)		0.78	0.22
β-lactoglobulin	AA	AB	BB	A	B
	0.57 (8)	0.29 (4)	0.14 (2)	0.71	0.29

* Numbers in parenthesis denotes number of observation

4.1.1.3 κ-casein

Two phenotypes (Fig.2) AA, AB were observed in k-casein locus. The diagrammatic representation is given in Fig.3. The phenotype AA was fast moving in β-casein and k-casein.

4.1.2 Whey proteins

4.1.2.1 α-Lactalbumin

Two phenotypes AA (Fig. 4) and AB with two allele A and B were observed at α-Lactalbumin locus. The AA was fast moving. The diagrammatic representation is given in Fig. 5.

4.1.2.2 β-Lactoglobulin

β-Lactoglobulin had three phenotypes viz., AA, AB and BB. The AA was the fast moving (Fig.4). The diagrammatic representation is shown in Fig.5.

4.2 Milk Composition

4.2.1 Milk fat percentage

The milk fat percentage of the morning and evening milk samples was estimated at weekly intervals. The mean fat percentage with standard error and coefficient

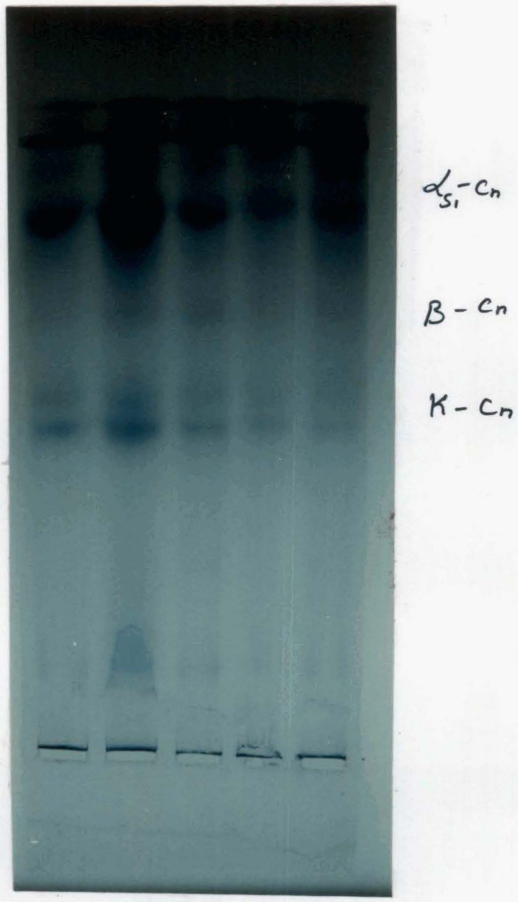

Fig.2 Phenotypes of αS_1, β and K-casein

	1	2	3	4	5
α_{s1} - casein	CC	BC	CC	BB	CC
β - casein	AB	BB	AB	AB	AB
K - casein	AA	AB	AB	AB	AB

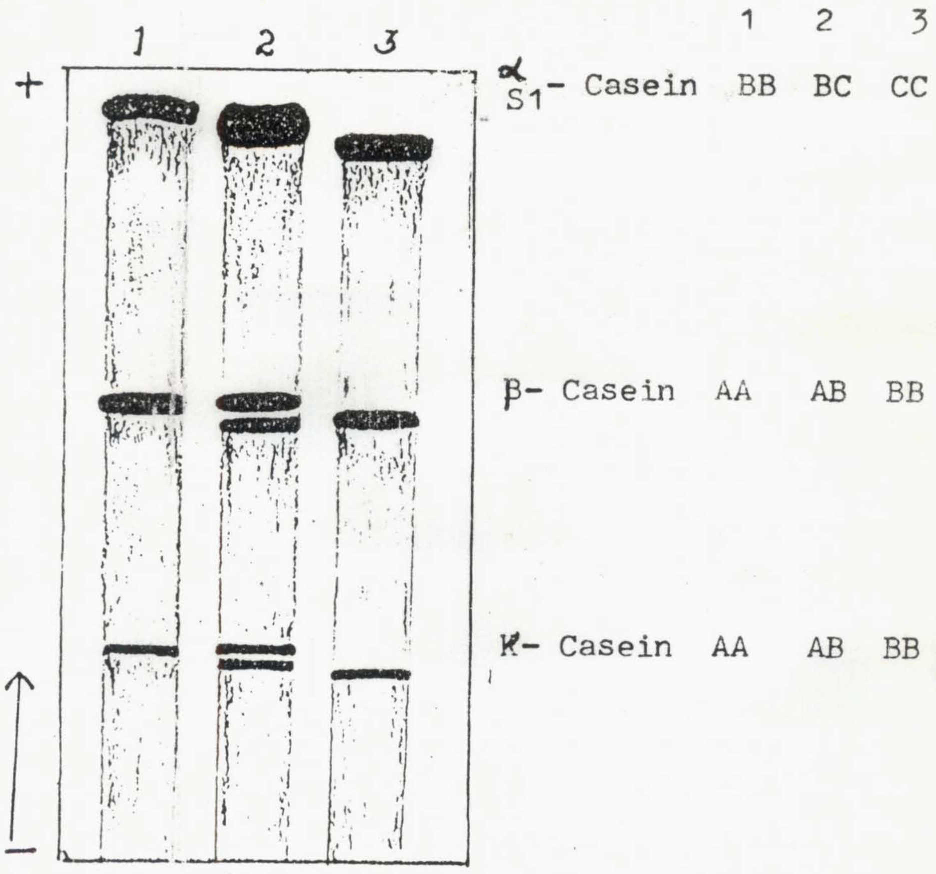

Fig.3 Diagramatic representation of αS_1, β and K-casein phenotypes

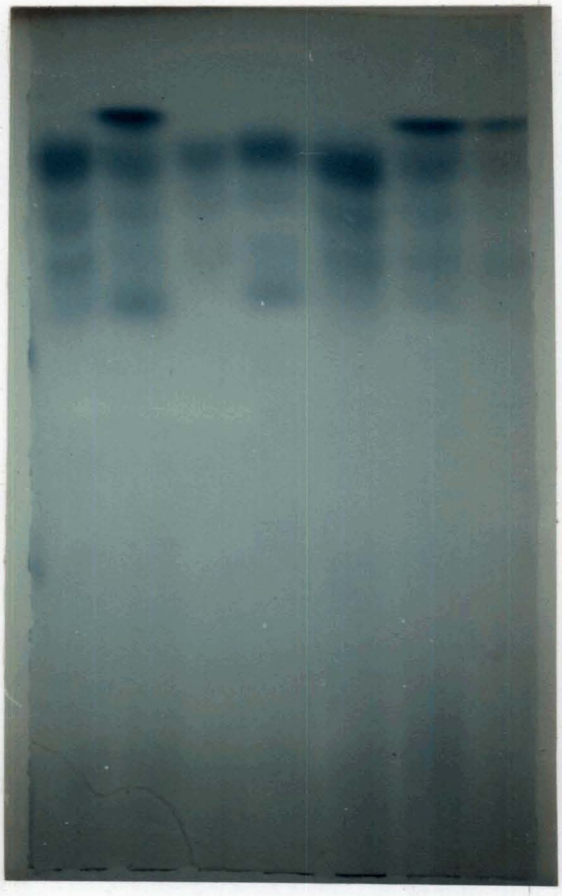

Fig.4 Phenotypes of α-lactalbumin and β-lactoglobulin

	1	2	3	4	5	6	7
α -lactalbumin	AB	AB	AA	AA	AB	AA	AA
β -lactoglobulin	AB	AB	AA	AA	BB	AA	AA

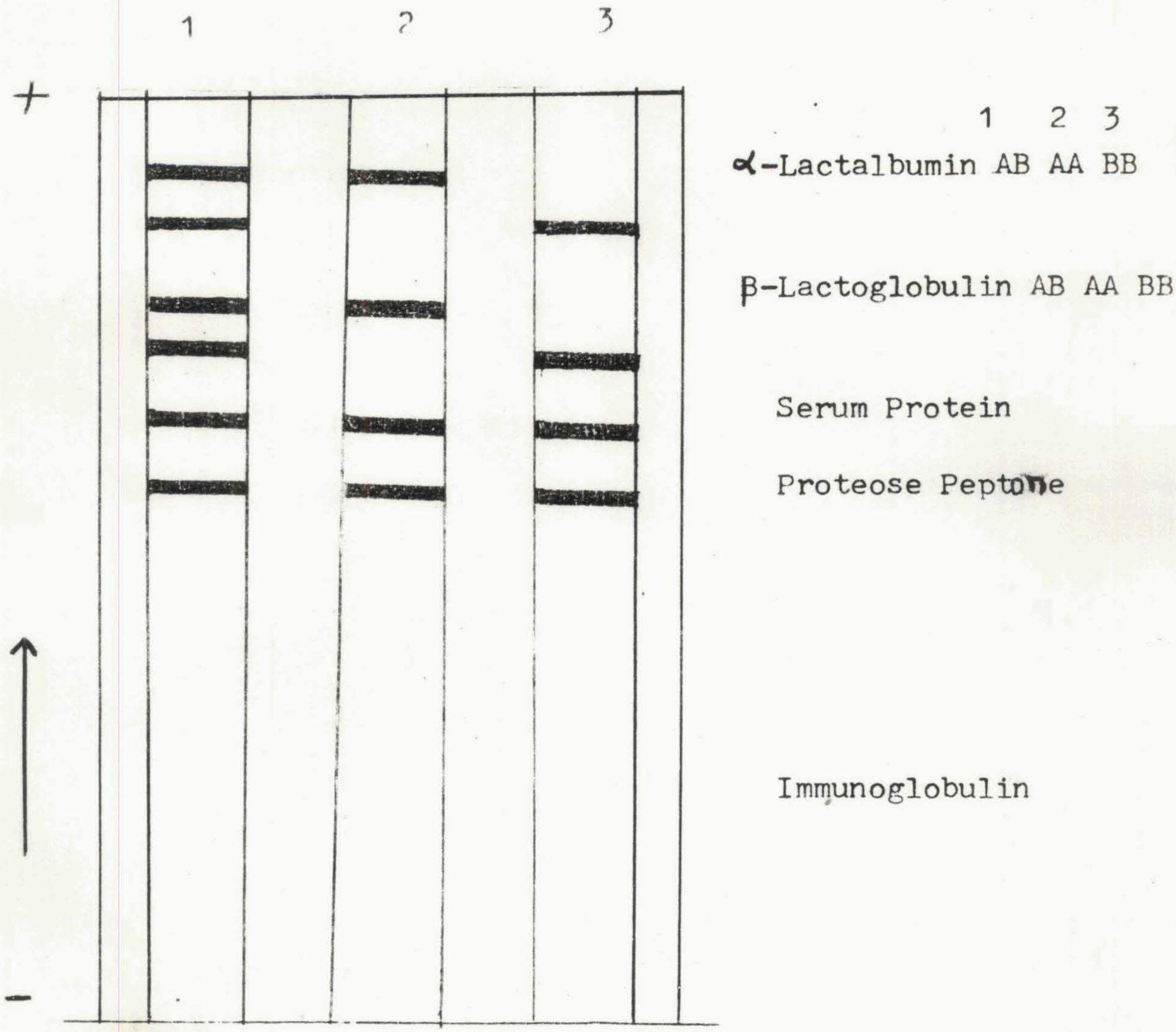

Fig.5 Diagrammatic representation of α-lactalbumin and β-lactoglobulin phenotypes

of variation are given in Table 4.2. The average of milk fat percentage from weeks 1 to 44 was 5.95 ± 0.12 and 6.62 ± 0.13 for morning and evening milk. The average of morning and evening fat percentage was 6.23. The mean fat percentage at first week was 4.05 ± 0.19, 4.65 ± 0.17 and by 20^{th} week of lactation 5.92 ± 0.08, 6.55 ± 0.07 in the morning and evening milk respectively. The increasing trend continued thereafter The effect of stage of lactation is given in Fig.6. Least squares analysis of variance showed that differences (Table 4.3) in fat percentage for different weeks from 1 to 44 were highly significant. so also the difference between morning and evening milk. The least square mean for 1 to 44 weeks (Table 4.4) varied from 4.43 ± 0.12 to 7.52 ± 0.20. The overall mean was 6.13 ± 0.04 and for morning and evening 5.99 ± 0.03 and 6.62 ± 0.03 respectively.

4.2.2 Total solids percentage

The total solids in the morning and evening estimated by gravimetric method at weekly intervals. The mean total solids percentage with standard error and coefficient of variation are presented in Table 4.5. The average total solids percentage was 14.79 ± 0.13 in the morning and 15.53 ± 0.12 in the evening for 1 - 44

Table 4.2 Average fat percentage of milk at weekly intervals in Vechur cattle

Weeks	Morning		Evening	
	Mean	CV%	Mean	CV%
1	4.05±0.19 (9)	12.72	4.65±0.17 (9)	12.61
2	4.33±0.19 (9)	11.60	4.82±0.14 (9)	9.55
3	4.59±0.20 (9)	13.39	5.11±0.21 (9)	12.97
4	4.75±0.16 (9)	10.27	5.31±0.16 (9)	9.42
5	4.82±0.23 (9)	12.77	5.34±0.20 (9)	12.42
6	5.10+0.26 (9)	13.87	5.67±0.21 (9)	12.23
7	5.26±0.20 (9)	9.87	5.79±0.18 (9)	9.89
8	5.29±0.18 (9)	9.05	5.86±0.19 (9)	9.94
9	5.34±0.13 (9)	6.33	5.93±0.15 (9)	8.01
10	5.44±0.15 (9)	7.18	5.95±0.16 (9)	8.47
11	5.53±0.16 (9)	7.32	6.03±0.16 (9)	8.27
12	5.56±0.10 (7)	4.37	6.04±0.11 (7)	5.00

Contd....

Table 4.2 contd..

13	5.57±0.11 (7)	4.40	6.09±0.12 (7)	5.65
14	5.66±0.12 (8)	5.40	6.11±0.16 (8)	6.61
15	5.68±0.12 (7)	5.35	6.20±0.09 (8)	4.56
16	5.67±0.09 (8)	4.78	6.26±0.12 (8)	6.60
17	5.68±0.11 (8)	4.66	6.33±0.08 (8)	4.20
18	5.73±0.09 (8)	4.30	6.46±0.06 (8)	2.86
19	5.76±0.11 (8)	4.53	6.53±0.11 (8)	4.75
20	5.92±0.08 (6)	2.89	6.55±0.07 (6)	2.99
21	5.93±0.07 (6)	2.54	6.57±0.08 (6)	3.27
22	5.93±0.18 (8)	8.08	6.60±0.14 (8)	6.50
23	5.94±0.19 (10)	8.80	6.62±0.15 (10)	7.71
24	5.94±0.18 (10)	8.62	6.64±0.14 (10)	7.62
25	6.02±0.19 (10)	8.85	6.73±0.13 (10)	6.91

Contd....

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Table 4.2 contd. ..

26	6.10±0.15 (8)	6.42	6.80±0.12 (8)	5.41
27	6.15±0.16 (8)	6.39	6.84±0.12 (8)	5.34
28	6.20±0.17 (8)	6.21	6.93±0.10 (8)	3.84
29	6.30±0.22 (5)	7.02	7.10±0.10 (5)	3.95
30	6.42±0.25 (5)	7.24	7.22±0.07 (5)	2.32
31	6.50±0.25 (4)	7.18	7.28±0.09 (4)	2.80
32	6.56±0.20 (4)	5.80	7.32±0.15 (4)	4.74
33	6.68±0.19 (4)	5.36	7.34±0.15 (4)	4.42
34	6.68±0.23 (4)	6.20	7.35±0.14 (4)	4.29
35	6.73±0.28 (4)	7.40	7.53±0.10 (4)	3.04
36	6.83±0.27 (4)	7.42	7.55±0.13 (4)	3.78
37	6.84±0.24 (5)	7.18	7.68±0.24 (5)	7.93
38	6.88±0.23 (5)	6.42	7.78±0.25 (5)	8.13

Contd....

Table 4.2 contd...

39	6.80±0.14 (3)	3.27	7.84±0.15 (3)	3.80
40	6.83±0.14 (3)	3.29	7.80±0.15 (3)	3.79
41	6.83±0.12 (3)	2.69	7.63±0.15 (3)	3.79
42	6.93±0.07 (3)	1.63	7.66±0.09 (3)	2.45
43	6.97±0.12 (3)	2.80	7.69±0.09 (3)	2.44
44	7.03±0.11 (3)	3.85	7.87±0.10 (3)	2.10

* Numbers in parenthesis denotes number of observation

Fig.6 **EFFECT OF STAGE OF LACTATION ON FAT, TOTAL SOLIDS AND SOLIDS NOT FAT PERCENTAGE OF MILK**

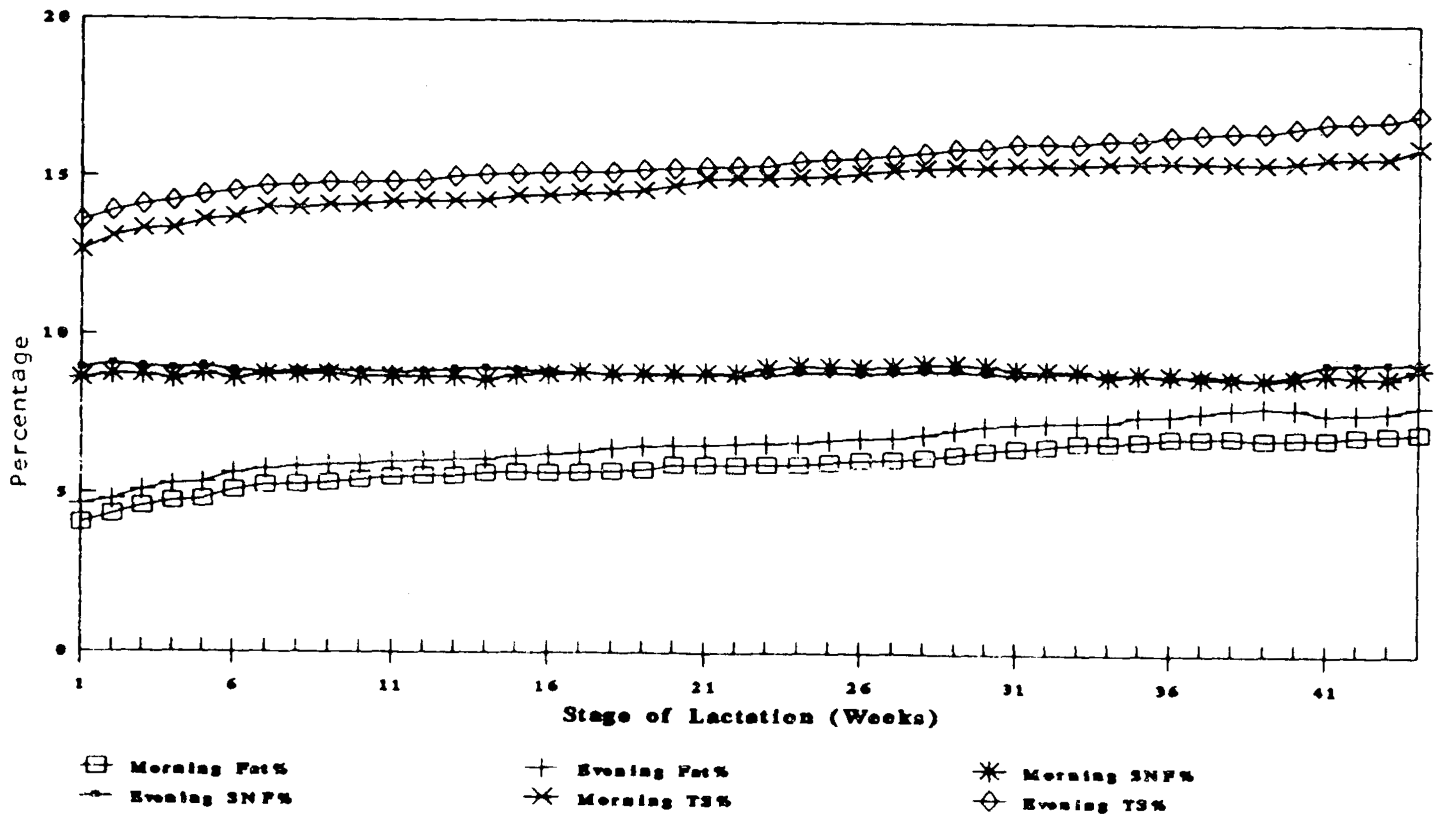

Table 4.3 Least-squares analysis of variance of milk fat percentage in Vechur cattle

Source	D.F.	Sum of squares	Mean squares	F value
Total	550	449.56		
Total reduction	45	327.89	7.29	30.25
MU-YM	1	15.15	15.15	62.90
Stage	43	274.04	6.37	26.45**
Time	1	53.85	53.85	223.53**
Remainder	505	121.66	0.24	

Mean = 6.13 Error standard deviation = 0.49
CV(%) = 8.01 R squared = 0.729 R = 0.854

** Significant at 2 per cent level

Table 4.4 Least squares means and standard errors for fat and total solids percentage of milk at weekly intervals in Vechur cattle

Weeks	Least squares means (%)	
	Fat	Total solids
1	4.43±0.12	13.07±0.19
2	4.84±0.12	13.75±0.19
3	5.02±0.12	13.80±0.19
4	5.14±0.12	13.77±0.19
5	5.33±0.12	14.17±0.19
6	5.58±0.12	14.31±0.19
7	5.56±0.13	14.53±0.19
8	5.62±0.13	14.55±0.20
9	5.27±0.13	14.67±0.20
10	5.72±0.13	14.62±0.20
11	5.81±0.13	14.67±0.20
12	5.82±0.13	14.74±0.20
13	5.77±0.13	14.78±0.19
14	5.91±0.12	14.79±0.19
15	5.99±0.12	14.89±0.19
16	6.08±0.12	14.97±0.18
17	6.00±0.12	14.96±0.18
18	6.07±0.12	14.95±0.19
19	6.13±0.14	14.94±0.22
20	6.23±0.14	15.03±0.22
21	6.25±0.14	15.15±0.22
22	6.39±0.13	15.26±0.20
23	6.33±0.12	15.45±0.18
24	6.36±0.12	15.32±0.18
25	6.43±0.12	15.43±0.18
26	6.44±0.12	15.43±0.19

Contd....

Table 4.4 contd....

27	6.41±0.13	15.45±0.20
28	6.58±0.14	15.61±0.22
29	6.81±0.16	15.70±0.23
30	6.86±0.16	15.74±0.24
31	6.86±0.16	15.78±0.24
32	6.90±0.17	15.49±0.26
33	6.98±0.17	15.83±0.26
34	7.01±0.17	15.86±0.26
35	7.11±0.17	15.87±0.26
36	7.18±0.17	16.01±0.26
37	7.23±0.16	15.97±0.24
38	7.46±0.16	15.98±0.24
39	7.16±0.20	15.85±0.31
40	7.08±0.20	16.11±0.31
41	7.23±0.20	16.27±0.31
42	7.30±0.20	16.32±0.31
43	7.30±0.20	16.49±0.31
44	7.52±0.20	16.61±0.31
Morning	5.99±0.03	14.85±0.05
Evening	6.62±0.03	15.56±0.05
Overall	6.13±0.04	15.02±0.05

Table 4.5 Average total solids percentage in milk at weekly intervals in Vechur cattle

Week	Morning		Evening	
	Mean	CV%	Mean	CV%
1	12.64±0.17 (9)	3.94	13.57±0.19 (9)	4.39
2	13.25±0.10 (9)	2.40	14.03±0.17 (9)	3.82
3	13.34±0.14 (9)	3.25	14.11±0.27 (9)	5.88
4	13.35±0.19 (9)	4.30	14.24±0.28 (9)	5.99
5	13.63±0.19 (9)	4.23	14.44±0.25 (9)	5.40
6	13.74±0.20 (9)	4.48	14.57±0.27 (9)	5.75
7	14.03±0.19 (8)	4.01	14.72±0.24 (8)	4.72
8	14.04±0.19 (8)	3.98	14.74±0.27 (8)	5.26
9	14.11±0.24 (8)	4.76	14.82±0.24 (8)	4.59
10	14.14±0.25 (8)	4.94	14.83±0.35 (8)	6.52
11	14.23±0.21 (8)	4.18	14.86±0.37 (8)	7.08
12	14.26±0.26 (7)	4.90	14.91±0.33 (7)	5.88

Contd....

Table 4.5 contd....

13	14.26±0.26 (7)	4.48	15.02±0.20 (7)	3.51
14	14.27±0.12 (8)	2.40	15.10±0.13 (8)	2.51
15	14.43±0.18 (8)	3.57	15.12±0.19 (8)	3.53
16	14.44±0.22 (8)	4.49	15.14±0.19 (8)	3.96
17	14.51±0.19 (8)	3.70	15.18±0.23 (8)	4.24
18	14.53±0.29 (7)	5.26	15.19±0.28 (7)	4.99
19	14.60±0.31 (6)	5.10	15.27±0.28 (6)	4.51
20	14.75±0.13 (6)	2.12	15.31±0.18 (6)	2.85
21	14.94±0.18 (6)	2.99	15.34±0.22 (6)	3.53
22	15.00±0.16 (8)	3.07	15.39±0.19 (8)	3.61
23	15.01±0.10 (10)	2.13	15.40±0.15 (10)	3.18
24	15.04±0.11 (9)	2.23	15.56±0.15 (9)	2.88
25	15.10±0.16 (9)	3.22	15.64±0.19 (9)	3.86

Contd.....

Table 4.5 contd....

26	15.21±0.17 (8)	3.28	15.70±0.15 (8)	2.78
27	15.31±0.21 (7)	3.67	15.78±0.18 (7)	3.10
28	15.36±0.17 (6)	2.87	15.87±0.11 (6)	2.18
29	15.40±0.19 (5)	2.84	15.98±0.20 (5)	2.86
30	15.40±0.24 (5)	3.09	16.03±0.33 (5)	4.31
31	15.47±0.27 (4)	3.62	16.17±0.22 (4)	2.76
32	15.46±0.22 (4)	2.95	16.16±0.21 (4)	2.70
33	15.46±0.23 (4)	2.98	16.17±0.34 (4)	4.21
34	15.54±0.20 (4)	2.63	16.27±0.25 (4)	3.14
35	15.56±0.16 (4)	2.12	16.28±0.17 (4)	2.16
36	15.59±0.23 (4)	2.95	16.41±0.19 (4)	2.38
37	15.56±0.26 (5)	3.85	16.48±0.26 (5)	3.54
38	15.56±0.31 (5)	4.58	16.56±0.25 (5)	3.46

Contd....

Table 4.5 contd....

39	15.54±0.35 (3)	3.90	16.55±0.12 (3)	1.36
40	15.59±0.52 (3)	5.78	16.70±0.15 (3)	1.50
41	15.72±0.26 (3)	2.93	16.86±0.37 (3)	3.85
42	15.75±0.27 (3)	2.99	16.89±0.31 (3)	3.26
43	15.75±0.25 (3)	2.77	16.94±0.15 (3)	1.58
44	16.09±0.14 (3)	1.49	17.14±0.34 (3)	3.50

* Numbers in parenthesis denotes number of observation

weeks of lactation. The average of morning and evening was 15.16 percent. The mean total solids percentage at first week was 12.64 ± 0.17 and 13.57 ± 0.19 in the morning and evening milk and it steadily increased to 14.75 ± 0.13 and 15.31 ± 0.18 in the morning and evening milk by 20^{th} week of lactation. The effect of stage of lactation on total solids percentage is given in Fig.6. Least squares analysis of variance (Table 4.6) showed that differences in total solids percentage for different weeks from 1 to 44 were highly significant. So also the differences between morning and evening milk. The least squares means for 1 to 44 weeks (Table 4.4) varied from 13.07 ± 0.19 to 16.61 ± 0.31. The overall mean was 15.02 ± 0.05 per cent and for morning and evening was 14.85 ± 0.05 and 15.55 ± 0.05 per cent respectively.

4.2.3 Solids not fat percentage

The average solids not fat percentage for 1 to 44 weeks of lactation in the morning and evening milk is presented in Table 4.7. The average solids not fat percentage was 8.84 ± 0.12 in the morning and 8.92 ± 0.14 in the evening from 1 to 44 weeks. The average of morning and evening was 8.88 ± 0.13 and least squares mean was 8.90 ± 0.12 per cent. The mean solids not fat

Table 4.6 Least-squares analysis of variance of total solids percentage of milk in Vechur cattle

Source	D.F.	Sum of squares	Mean squares	F value
Total	550	657.72		
Total reduction	45	375.29	8.34	14.91
MU-YM	1	15.91	15.91	28.45
Stage	43	307.53	7.15	12.79**
Time	1	67.75	67.75	121.15**
Remainder	505	282.44	0.56	

Mean = 15.02 Error standard deviation = 0.75
CV(%) = 4.98 R squared = 0.571 R = 0.755

** Significant at 2 per cent level

Table 4.7 Average solids not fat percentage of milk at weekly intervals in Vechur cattle

Weeks	Morning		Evening	
	Mean	CV%	Mean	CV%
1	8.60±0.24 (9)	8.39	8.93±0.22 (9)	7.45
2	8.75±0.20 (9)	6.98	9.08±0.16 (9)	5.60
3	8.75±0.23 (9)	7.92	9.01±0.27 (9)	9.24
4	8.62±0.22 (9)	7.65	8.94±0.25 (9)	8.51
5	8.81±0.21 (9)	7.13	9.03±0.24 (9)	8.42
6	8.65±0.20 (9)	7.01	8.91±0.18 (9)	6.28
7	8.77±0.24 (8)	8.50	8.82±0.23 (8)	7.51
8	8.76±0.20 (8)	6.38	8.88±0.25 (8)	8.01
9	8.78±0.23 (8)	7.43	8.90±0.24 (8)	7.36
10	8.71±0.24 (8)	7.79	8.88±0.27 (8)	8.39
11	8.70±0.26 (8)	8.44	8.84±0.26 (8)	7.80
12	8.71±0.31 (7)	9.48	8.87±0.25 (7)	8.99

Contd....

Table 4.7 contd....

13	8.70±0.24 (7)	8.36	8.94±0.23 (7)	6.90
14	8.61±0.12 (8)	3.98	8.99±0.23 (8)	7.24
15	8.76±0.19 (8)	6.42	8.92±0.22 (8)	7.03
16	8.78±0.24 (8)	8.20	8.89±0.12 (8)	4.14
17	8.84±0.14 (8)	4.56	8.86±0.15 (8)	4.81
18	8.83±0.25 (7)	7.62	8.78±0.22 (7)	6.72
19	8.82±0.23 (6)	6.31	8.81±0.19 (6)	5.75
20	8.83±0.11 (6)	3.12	8.76±0.15 (6)	4.24
21	8.82±0.18 (6)	4.88	8.81±0.18 (6)	5.07
22	8.83±0.17 (8)	5.30	8.76±0.18 (8)	6.00
23	9.01±0.13 (10)	4.75	8.78±0.16 (10)	6.09
24	9.08±0.09 (9)	3.29	8.87±0.17 (9)	5.88
25	9.07±0.09 (9)	2.90	8.89±0.16 (9)	5.74

Contd....

Table 4.7 contd....

26	9.05±0.12 (8)	3.67	8.84±0.15 (8)	5.19
27	9.11±0.17 (7)	4.98	8.91±0.11 (7)	4.09
28	9.17±0.15 (6)	4.05	8.94±0.18 (6)	5.05
29	9.16±0.12 (5)	3.14	8.95±0.16 (5)	3.70
30	9.12±0.21 (5)	4.77	8.88±0.15 (5)	3.31
31	8.98±0.29 (4)	6.60	8.82±0.15 (4)	3.40
32	8.97±0.25 (4)	5.57	8.90±0.24 (4)	5.15
33	8.96±0.10 (4)	2.29	8.91±0.34 (4)	7.52
34	8.79±0.13 (4)	2.93	8.85±0.17 (4)	3.89
35	8.87±0.09 (4)	2.13	8.89±0.33 (4)	7.32
36	8.84±0.15 (4)	3.32	8.76±0.23 (4)	5.19
37	8.77±0.21 (5)	5.42	8.86±0.24 (5)	6.03
38	8.72±0.22 (5)	5.82	8.80±0.27 (5)	5.31

Contd.....

Table 4.7 contd....

39	8.71±0.32 (3)	6.36	8.71±0.20 (3)	3.97
40	8.76±0.18 (3)	5.60	8.90±0.15 (3)	2.92
41	8.79±0.21 (3)	4.09	9.23±0.31 (3)	5.88
42	8.82±0.17 (3)	3.42	9.23±0.25 (3)	4.83
43	8.78±0.15 (3)	3.04	9.25±0.18 (3)	3.44
44	9.06±0.13 (3)	2.48	9.27±0.33 (3)	6.23

* Numbers in parenthesis denotes number of observation

percentage at first week was 8.60 ± 0.24 and 8.93 ± 0.22 in the morning and evening milk. It was 8.83 ± 0.11 and 8.76 ± 0.15 at 20th week of lactation in the morning and evening milk respectively. The total solids percentage was not having any trend during the stage of lactation as seen Fig.6.

Least square analysis of variance (Table 4.8) showed that stage of lactation as well as time of milking did not exhibit significant effect on the character.

4.2.4 Inter-relationship among constituents

The correlation coefficient values between fat percentage, total solids percentage and solids not fat percentage at third, 13th and 23rd weeks of lactation are presented in Table 4.9. Positive correlation was observed between fat and total solids content, as well as total solids and solids not fat content but mostly non-significant. Negative correlation values were observed between fat and solids not fat percentage at third, 13th and 23rd week of lactation but mostly non-significant both in the morning and evening milk. Significant correlation values were observed between fat

Table 4.8 Least-squares analysis of variance of solids not fat percentage of milk in Vechur cattle

Source	D.F.	Sum of squares	Mean squares	F value
Total	550	294.54		
Total reduction	45	8.96	0.20	0.35
MU-YM	1	0.04	0.04	0.06
Stage	43	7.70	0.18	0.32^{NS}
Time	1	1.23	1.26	2.23^{NS}
Remainder	505	285.58	0.57	

Mean = 8.90 Error standard deviation = 0.75

CV(%) = 8.45 R squared = 0.030 R = 0.174

NS - Not significant

Table 4.9 Correlation coeffecient values between fat, total solids and solids not fat percentage at different stages of lactation in Vechur cattle

Stages of lactation	Fat and TS		Fat and SNF		TS and SNF	
	Morning	Evening	Morning	Evening	Morning	Evening
3rd week	0.148	0.397	- 0.791*	-0.376	0.482	0.693
13th week	0.587	0.540	-0.456	-0.412	0.902*	0.890*
23rd week	0.435	0.531	-0.792*	-0.623	0.205	0.332

* Significant at 5 per cent level

and solids not fat percentage in the morning milk at third and 23rd week of lactation. The total solids and solids not fat percentage had significant correlation coefficient values in the morning milk at 13th week and in the evening milk at third and 23rd week of lactation.

4.3.4 Fat globule size

The per cent distribution of number of fat globules of different size groups in the morning and evening milk is given in Table 4.10 and Fig.7, 8. The fat globules size varied from 1 to 12 μ. The mean diameter of fat globules is 3.02 μ in the morning and 3.40 μ in the evening and the average of morning and evening is 3.21 μ. The average size of fat globules in the morning and evening milk are presented in Table 4.11 and Fig.9. The average fat globules size ranged from 2.54 to 3.73 μ in the morning and 2.75 to 4.07 μ in the evening milk. A comparison of the fat globule size of Vechur cow with the crossbred cow of Kerala, goats and Murrah buffaloes was done. In the case of crossbred, goats and buffaloes pooled samples were taken and the results are based on four pooled samples. The pooled samples were from 60 crossbred cows, 10 goats and 12 buffaloes. The results are given in Table 4.12.

Table 4.10 Per cent distribution of fat globules in the morning and evening milk at weekly intervals in Vechur cattle

Week	Morning				Evening			
	0-3μ	3-6μ	6-9μ	9-12μ	0-3μ	3-6μ	6-9μ	9-12μ
1	50.08	36.49	13.44		37.92	46.20	12.33	4.33
2	50.42	35.57	10.91	3.12	37.29	45.71	12.06	5.85
3	52.07	33.54	10.62	3.79	39.88	47.50	12.60	
4	52.41	33.69	10.24	3.89	39.94	47.55	9.72	3.55
5	54.56	34.43	11.06		40.78	46.96	9.40	3.26
6	55.09	33.05	9.91	2.81	40.11	47.88	11.99	
7	55.11	33.46	10.90		40.23	48.00	11.76	
8	54.84	33.01	8.69		42.23	47.37	10.56	
9	55.40	33.19	11.29		42.23	44.90	9.45	3.90
10	55.63	34.10	10.50		42.96	45.05	9.41	3.16
11	55.45	35.39	9.15		42.93	46.63	10.43	
12	55.68	34.96	8.05	1.82	43.96	46.62	9.40	
13	56.06	34.15	8.15	2.06	44.57	46.84	9.36	
14	56.44	35.42	6.18	2.83	43.57	45.31	8.56	2.92
15	56.52	34.26	5.32	4.29	44.00	44.57	8.84	3.36
16	56.55	36.15	5.92	1.85	44.27	45.14	10.58	
17	56.91	35.20	8.88		44.88	45.14	9.99	
18	57.43	35.70	6.85		44.48	43.42	9.51	3.41
19	57.18	35.07	6.02	2.03	44.09	43.42	10.40	3.06
20	57.60	35.73	7.25		44.92	43.58	9.65	2.62
21	57.37	33.83	6.83	2.02	44.41	43.64	9.75	2.22
22	59.03	33.30	6.43	1.86	45.69	42.54	8.29	3.65
23	59.04	31.19	6.18	4.47	45.04	43.09	8.63	3.81
24	59.61	29.41	8.63	2.38	45.57	42.16	8.92	3.08

Contd.....

Table 4.10 contd....

25	60.19	30.84	6.03	3.09	46.27	43.91	9.83	
26	60.64	31.76	7.59		47.16	42.25	7.80	3.36
27	60.92	30.69	6.44	2.01	48.36	41.36	7.54	2.87
28	61.24	30.60	6.01	2.29	48.77	41.39	7.47	2.80
29	61.21	29.31	8.40	1.03	49.17	42.05	8.52	
30	62.22	28.94	8.83		49.81	40.88	6.52	2.83
31	62.53	29.13	8.33		49.51	40.89	6.25	4.05
32	62.73	28.33	8.93		49.53	40.44	8.55	1.87
33	63.52	30.60	5.83		50.77	38.78	7.58	3.21
34	63.38	32.00	5.23		51.45	39.97	8.11	
35	63.89	29.22	6.88		51.47	34.25	8.73	3.93
36	64.62	27.71	7.86		52.53	35.98	11.29	
37	65.90	27.59	6.51		52.06	35.06	8.98	4.36
38	66.40	27.31	7.08		53.06	35.65	8.22	3.68
39	68.40	26.70	5.67		55.16	34.59	8.24	
40	69.66	25.41	5.88		58.79	30.29	8.88	2.09

Fig.7 **PERCENT DISTRIBUTION OF FAT GLOBULES AT DIFFERENT STAGES OF LACTATION IN THE MORNING MILK**

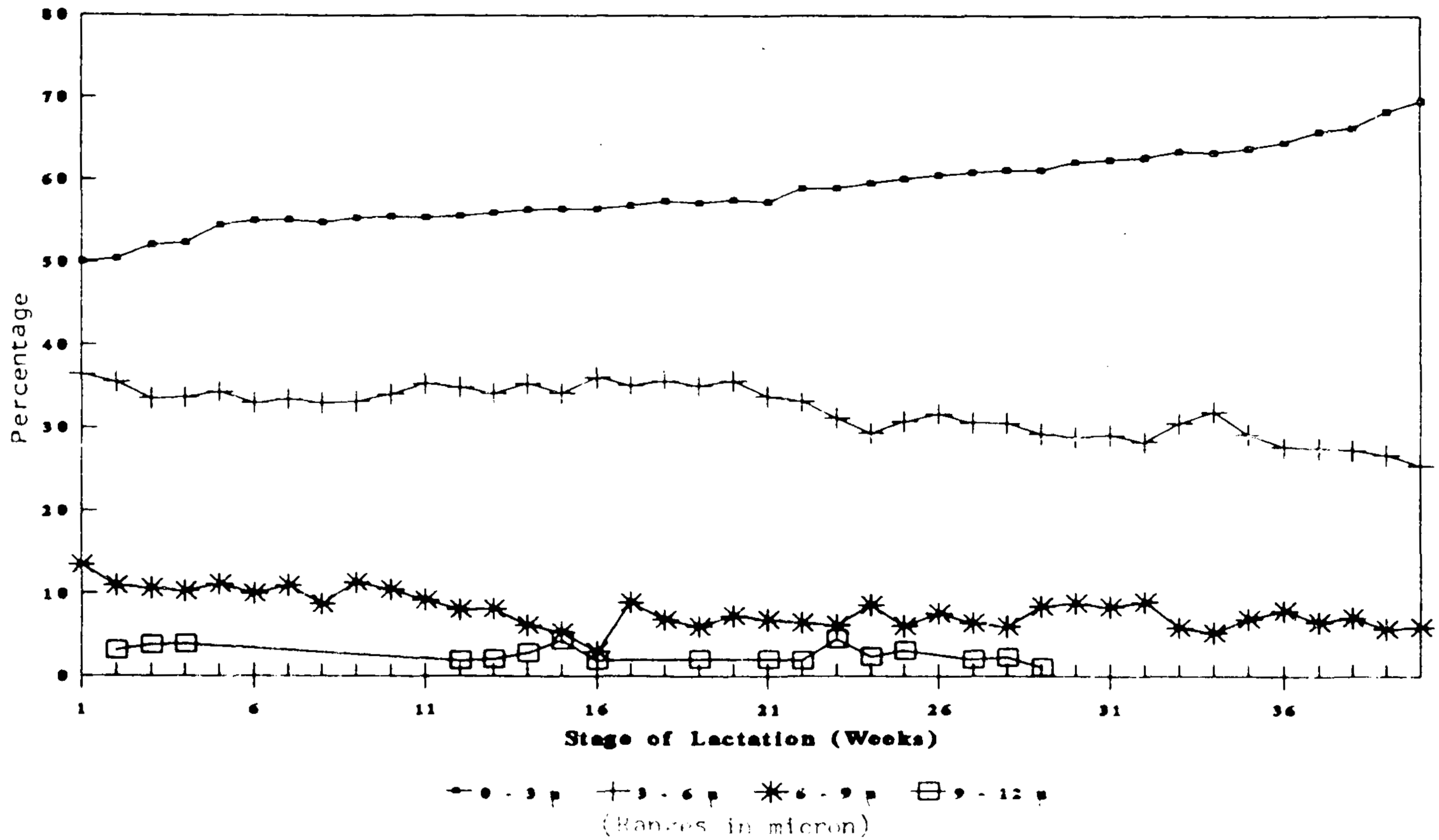

Fig.8 **PERCENT DISTRIBUTION OF FAT GLOBULES AT DIFFERENT STAGES OF LACTATION IN THE EVENING MILK**

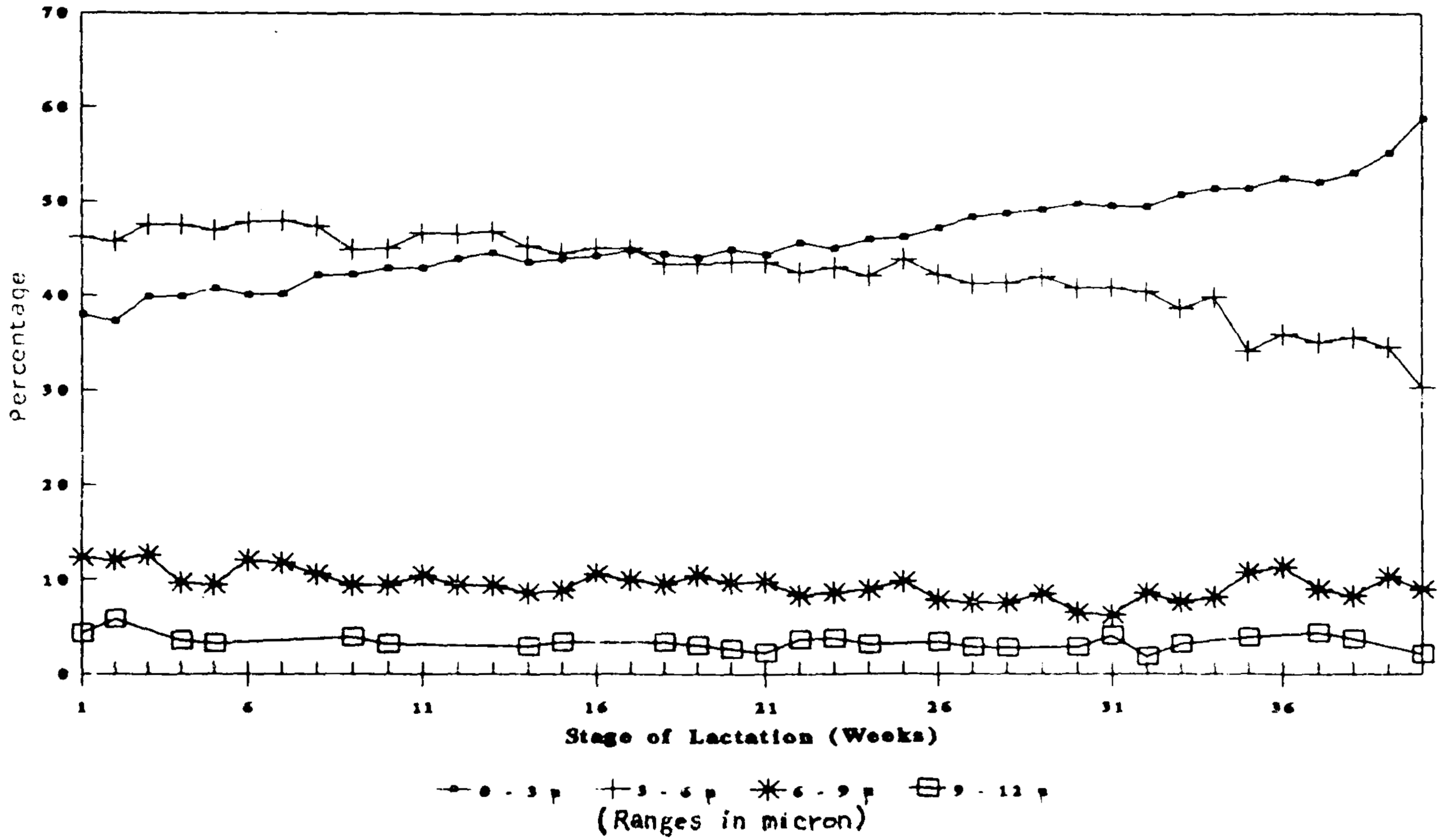

Table 4.11 Mean size of fat globule in the morning and evening milk at weekly intervals in Vechur cattle

Week	Number of observation	Morning	Evening
1	9	3.73	4.07
2	9	3.67	3.85
3	9	3.49	3.79
4	9	3.45	3.74
5	9	3.36	3.65
6	9	3.38	3.64
7	9	3.27	3.63
8	9	3.19	3.57
9	9	3.12	3.73
10	9	3.10	3.71
11	8	3.07	3.55
12	8	3.09	3.56
13	8	3.17	3.65
14	8	3.16	3.66
15	8	3.06	3.52
16	8	3.00	3.52
17	8	3.00	3.62
18	8	3.01	3.56
19	8	2.93	3.60
20	8	2.88	3.55
21	8	3.02	3.52
22	11	2.91	3.42
23	11	3.06	3.42
24	11	2.89	3.33
25	11	3.05	3.29
26	11	3.07	3.25

Contd.......

Table 4.11 contd....

27	11	3.15	3.17
28	11	3.13	3.15
29	7	2.98	3.09
30	7	2.80	3.10
31	7	2.82	3.13
32	7	2.25	3.07
33	6	2.70	3.06
34	6	2.67	3.05
35	6	2.70	3.02
36	6	2.66	3.10
37	6	2.54	2.99
38	5	2.55	2.92
39	5	2.54	2.90
40	5	2.55	2.81
Overall		3.05	3.40

Fig.9 **EFFECT OF STAGE OF LACTATION ON SIZE OF FAT GLOBULES IN MILK**

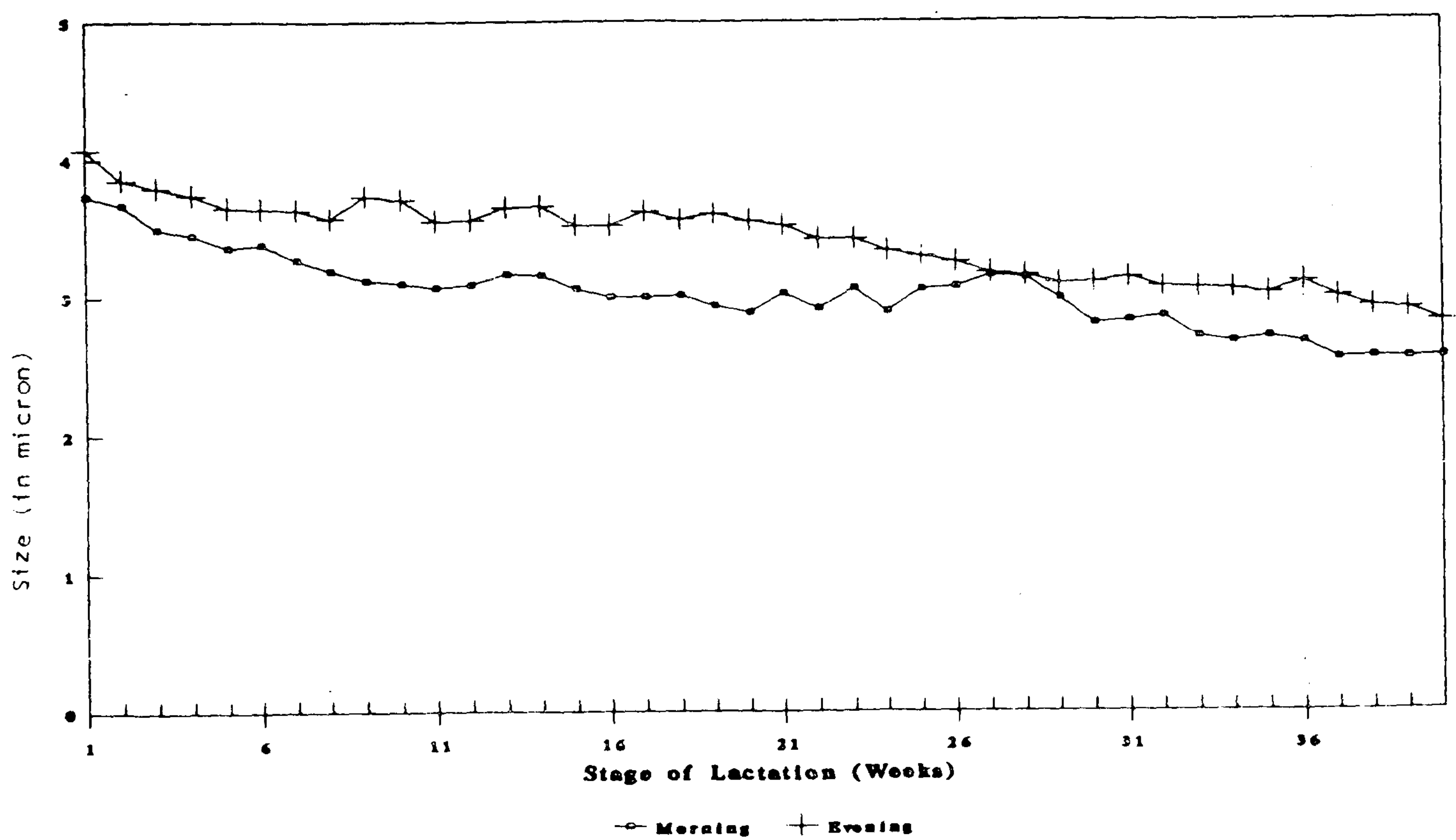

Table 4.12 The average size of milk fat globules in goat, Vechur cattle, crossbred cattle and buffalo

Species	0-3 (μ)	3-6 (μ)	6-9 (μ)	9-12 (μ)	12-15 (μ)	Total	Size (μ)
Goat (Malabari and their crosses)	68	29	4	-	-	101	2.60
Vechur cattle	57	37	8	3	-	105	3.21
Crossbred cattle	30	39	18	9	2	98	4.87
Murrah buffalo	20	28	33	10	3	94	5.85

4.3.1 Correlation coefficient between fat globule size and milk fat percentage at 3rd, 13th and 23rd week of lactation

A significant positive correlation obtained between fat globule size and fat percentage in the morning milk at 3rd week of lactation. The inter-relationship between fat globules size and fat percentage at various stages of lactation are given in Table 4.13.

4.4 Iodine value

Iodine number was estimated from milk of 15 Vechur cows. The mean iodine value was 29.60 ± 2.20. It ranged from 28.61 to 30.26. The average iodine number for nine fortnights are presented in the Table 4.14.

Table 4.13 Correlation coefficient values between milk fat percentage and fat glouble size at different stages of lactation in Vechur cattle

Stage of lactation	Morning	Evening
Third week	0.706*	-0.231
Thirteenth week	0.063	-0.532
Twentythird week	0.004	-0.120

* Significant at 5 per cent level

Table 4.14 Mean iodine value at fortnightly intervals in Vechur cattle

Fortnights	Mean	CV (%)
1	25.59±0.19 (15)	2.46
2	30.29±0.21 (15)	2.67
3	29.57±0.22 (15)	2.86
4	30.14±0.20 (14)	2.42
5	30.26±0.20 (13)	2.35
6	29.16±0.19 (12)	2.24
7	29.82±0.06 (11)	0.64
8	28.93±0.10 (11)	1.23
9	28.61±0.12 (11)	1.40

* Numbers in the parenthesis denotes number of observation

Discussion

DISCUSSION

5.1 Milk Protein polymorphism

5.1.1 Casein

5.1.1.1 α_{s1}-casein

Three phenotypes viz., BB, BC and CC (Table 4.1 and Fig. 2) were observed at α_{s1}-casein locus, with frequency of 0.14, 0.22 and 0.64 respectively. The gene frequency was 0.25 and 0.75 for B and C allele. Several authors (Kiddy *et al.*, 1964; Juneja and Chaudhary, 1973; Jairam and Nair, 1983 b; Mclean et al, 1984; Madhavan, 1985) reported only B and C allele at α_{s1}-casein locus. But Aschaffenburg (1968); Ng-Kwai-Hang *et al.* (1984) reported three alleles A, B and C at α_{s1}-casein locus in Holstein population. Kiddy *et al.* (1964) Aschaffenburg (1968); Hussain (1974); Mclean *et al.* (1984); Lin *et al.* (1986); Eenemaan (1991) reported very high gene frequency of α_{s1}-casein B variant in western breeds (Ayrshire, Holstein Friesian Brown Swiss, Guernsey). Aschaffenburg (1968); Juneja and Chaudhary (1973); Jairam and Nair (1983b) reported the predominance of C variant (0.90) in Indian breeds like Hariana, Sahiwal, Tharparkar, Red Sindhi and Desi cows. Frequency of B allele reported to be 0.59

in Jersey X Sahiwal (Juneja and Choudhary 1973) and 0.51 in Brown Swiss X Sahiwal crosses (Jairam and Nair, 1983b). Madhavan (1995) reported that the frequency of C allele as 0.63, 0.61 and 0.63 in Jersey, Brown Swiss and Holstein crossbred cattle of Kerala. In the present study the frequency of C allele was 0.75 which probably indicates that the local cattle of Kerala had separate line of inheritance.

5.1.1.2 β-casein

Two variants A and B with phenotypic combinations AA AB and BB were observed (Table 4.1 and Fig. 2). The frequency of AB phenotype was the highest (0.57) and the frequency of A and B allele were 0.58 and 0.42 respectively. Though several β-casein variants viz., A1, A2, A3, B, C, D and E were reported in cattle (Eigel *et al.*, 1984), only A and B could be observed in the present study. The frequency of B allele was reported to be very low in Indian breeds. Aschaffenburg (1968) reported that the frequency of B allele in Sahiwal, Tharparkar and Red Sindhi as 0.05, 0.07 and 0.10. Juneja and Chaudhary (1970); Jairam and Nair (1983b) also reported a very low frequency of B allele for Indian breeds at β-casein locus. Ng-Kwai-Hang *et al.* (1984)

reported a high frequency of A allele in Holstein population. Madhavan (1995) reported a high frequency of A allele as 0.81, 0.75 and 0.71 in Brown Swiss, Jersey and Holstein Friesians crossbreds. This again support the probability that the Vechur cattle has a probably different B allele frequency at β-casein locus compared to the other Indian cattle. Crossbreds formed as a result of the introduction of exotic bulls show a halving of B allele and a proportionate increase of A allele.

5.1.1.3 k-casein

Two phenotypes AA (0.17) and AB (0.83) controlled by two alleles A and B were observed at k-casein locus (Table 4.1 and Fig.2). The frequency of A was 0.59 and B 0.41. Several authors Aschaffenburg (1968); Juneja and Choudhary (1973); Jairam and Nair (1983b); Ng-Kwai-Hang *et al.* (1984) have reported only two allele in the k-casein locus. Aschaffenburg (1968) observed low frequency of B allele in Sahiwal (0.30), Tharparkar (0.26) and Red Sindhi (0.30) breeds. Majumdar and Ganguly (1970); Juneja and Choudhary (1970); Jairam and Nair (1983b) also reported very low frequency of B allele in Indian breeds. Jairam and Nair (1983b);

Madhavan (1995) reported a significantly higher frequency of B allele in crossbred cows of India. It appears that in Vechur cattle of Kerala, the indication is that A allele has predominance but a conclusion can not be drawn due to the low number of observations.

5.1.2. Whey proteins

5.1.2.1 α-lactalbumin

Two Phenotypes AA and AB were observed at α-lactalbumin locus with the frequency of 0.57 and 0.43 (Table 4.1 and Fig. 4). The frequency of A allele was 0.78 and B allele 0.22. Bhattacharya *et al.* (1963) observed a frequency of A allele as 0.20 in crossbreds and 0.37 in Sahiwal cows. Mawal (1967) reported frequency of A allele as 0.38 in Gir and 0.40 in Gavathi cows. Aschaffenburg (1968) reported the frequency of A allele as 0.40 in Desi cows and 0.05 in African breeds like Ankole and Boran. Jairam and Nair (1983b) reported a low frequency of A allele as 0.16 in Red Sindhi, 0.17 in Tharparkar, 0.18 in Sahiwal and 0.06 in Brown Swiss X Sahiwal crossbred. The frequency of A allele in Vechur cattle was quite higher compared to the reports on other zebu breeds probably indicative of separate line of inheritance.

5.1.2.2 β-Lactoglobulin

Three phenotypes of β-lactoglobulin viz., AA, AB and BB with the frequency of 0.57, 0.29 and 0.14 were observed (Table 4.1 and Fig.4). The frequency of A allele was 0.71 and B allele 0.29. Aschaffenburg and Drewry (1955) observed two alleles A and B at β-lactoglobulin locus. Singh and Khanna (1972) observed three alleles A, B and C in Hariana cattle. Several authors Bhattacharya *et al.* (1963); Aschaffenburg (1968); Juneja and Choudhary (1970) reported the low frequency of B allele (0.05 - 0.21) in Indian breeds like Sahiwal, Gir and Desi cows. Singh and Bhat (1980) reported that the β-lactoglobulin B was the most common allele among indigenous breeds. β-lactoglobulin 'A' had highest frequency in crossbred Holstein Friesian cattle. Jairam and Nair (1983b) reported very low (0.23) frequency of A allele in Sahiwal, Tharparkar and Red Sindhi breeds. Madhavan (1995) reported the presence of A and B allele and the frequency of A allele as 0.46 in Holstein, 0.43 in Jersey and 0.40 in Brown Swiss crossbreds, which comes to about half of that of Vechur cattle. The results are indicative of a separate line of inheritance from other breeds of cattle. Any inheritance from foreign breeds would have probably reduced the frequency of A allele.

The study was based on samples collected from 14 animals only. The number of observations are small but these can be considered indicative of the trend of Vechur breed.

5.2 Milk Composition

5.2.1 Milk fat percentage

The milk fat percentage from weeks 1 - 44 ranged from 4.05 to 7.03 and 4.65 to 7.87 in the morning and evening milk respectively and the range was higher for evening milk. The mean milk fat percentage at mid lactation ie 20th week of lactation was 5.92 ± 0.08 in the morning and 6.55 ± 0.07 in the evening. The average for milk fat percent from weeks 1 - 44 was 5.95 ± 0.12 and 6.62 ± 0.13 for morning and evening which was quite comparable to the mid-lactation fat percentage. The average morning and evening together is 6.18 percent. The milk fat percentage is was found to be higher than exotic breeds like Ayrshire, Brown Swiss, Guernsey, Holstein Friesian and Jersey as reported by Byron *et al.* (1972) and Indian breeds like Gir, Ongole, Sahiwal, Sindhi, Tharparkar and their crossbreds (Ghosh and Anantakrishnan, 1963).

Fat content of the milk increased uniformly as the lactation advanced as can be seen from the results given in Table 4.2 and Fig.6. These findings concur with the results of Bayoumi (1959); Singh *et al.* (1961); Gosh and Anantakrishnan (1964); Arora and Gupta (1969); Sadana *et al.* (1978); Iype *et al.* (1994). The evening milk fat percentage was uniformly higher during all stages of lactation than morning milk fat percentage. This finding was in close agreement with the reports made by Iype *et al.* (1994) in crossbred cattle of Kerala. This higher milk fat in evening milk is probably due to lesser milking interval ie. only nine and half hours between morning and evening milking. Least squares analysis of variance showed highly significant differences between morning and evening milk (Table 4.3). Least squares mean was 5.99 ± 0.03 and 6.62 ± 0.03 for morning and evening respectively with a difference of 0.63. and the overall mean was 6.13 ± 0.04.

Increase in fat percentage between weeks was more in initial stages of lactation. But there was a gradual increase at a lesser rate between subsequent weeks upto 44 weeks of lactation. The increase in fat percentage was 1.39% in the morning and 1.3% in the evening from first to 10th week of lactation. Then the rate of increase in fat percentage was found to be almost consistent both in the morning (0.39%, 0.49% & 0.33%) and evening (0.52%, 0.65% & 0.52%) milk from 11 to 20 weeks, 21 to 30 weeks and 31 to 40 weeks of lactation. The increase in fat percentage from one to seven month of lactation was more than 2% as against 1.49% percentage reported by Arora and Gupta (1969). The coefficient of variation (Table 4.2) of weekly milk fat percentage was around 8 percent. Least squares analysis of variance (Table 4.3) revealed highly significant differences between fat percentage at weekly intervals. The least squares means (Table 4.4) which ranged from 4.43 ± 0.12 to 7.52 + 0.20 showed a gradual increase in percentage from the beginning till the end of the lactation. These findings were in agreement with the reports of Ghosh and Anantakrishnan (1964); Arora and Gupta (1969) and Sadana *et al.* (1978).

5.2.2 Total solids percentage

The total solids percentage for weeks 1 - 44 ranged from 12.64 to 16.09 and 13.57 to 17.14 in the morning and evening milk respectively and the range was higher for evening milk. The mean total solids percentage at mid lactation ie 20th week of lactation was 14.75 ± 0.13 in the morning and 15.31 ± 0.12 in the evening. The average for total solids per cent from weeks 1 - 44 was 14.79 ± 0.13 and 15.53 ± 0.12 for morning and evening which is quite comparable to the mid-lactation total solids percentage. The average morning and evening total solids was 15.16 ± 0.05 per cent. The least squares means for 1 - 44 (Table 4.4) weeks varied from 13.07 ± 0.19 to 16.61 ± 0.31 and the overall mean was 15.02 ± 0.05 per cent. Least squares means for morning (14.85 ±0.05) and evening (15.56 ± 0.05) showed a difference of 0.70 which was similar to that of fat percentage. The total solids content was found to be higher than the other Indian breeds (Gir, Ongole, Tharparkar, Sahiwal, Sindhi and their crossbreds) reported in Annual Report National Dairy Research Institute (1948), by Balwant Rai Puri *et al.* (1963); Tilakaratnae *et al.* (1975); Babu Rao and Jayaramakrishna (1983) and exotic breeds (Jersey, Guernsey, Brown Swiss, Ayrshire, Short horn and Holstein Friesian) of cattle reported by Byron *et al.* (1972).

The total solids percentage showed an increasing trend (Table 4.5 and Fig. 6) with the progress of lactation. Therefore the findings in the present study are in close agreement with reports made earlier by Bayoumi (1959); Gosh and Anantakrishnan (1963); Parekh and Gangwar (1968); Darshanlal and Narayanan (1991); Mohran and Fahmy (1992). But there was no decline in total solids content at fourth week of lactation as reported by Ghosh and Anantakrishnan (1963); Parekh and Gangwar (1968) in Sindhi, Gir and crossbred. The total solids percentage was uniformly higher in the evening than morning milk, which may be due to lesser milking interval. The maximum increase was noticed from first to second week of lactation (0.44% in morning and 0.33% in evening). Then there was a constant increase of about 0.1% or lesser between subsequent weeks of lactation. Least squares analysis of variance (Table 4.6) revealed highly significant differences between morning and evening milk.

The rate of increase was high in the morning (1.5%, 0.52%) than in the evening milk (1.26% & 0.45%) from 1 to 10 and 10 to 20 weeks of lactation. From 20 to 30 weeks and 30 to 40 weeks the rate of increase was high in the evening milk (0.69% & 0.53%) as compared to morning milk (0.46% & 0.12%). The coefficient of variation was around 5 per cent. These findings were in agreement with the reports of Bayoumi (1959); Ghosh and Anantakrishnan (1964) and Parekh and Gangwar (1968).

From this study it can be concluded that in Vechur cattle the increasing trend with advancing stage of lactation in the total solids percentage followed that of the milk fat percentage. Least squares analysis of variance showed highly significant differences between weekly total solids percentage (Table 4.6).

5.2.3 Solids not fat percentage

The solids not fat percentage from weeks 1 - 44 ranged from 8.60 to 9.17 and 8.71 to 9.27 in the morning and evening milk respectively and the range was higher for morning milk. The mean solids not fat percentage at mid lactation ie. 20th week of lactation was 8.83 ± 0.11 in the morning and 8.76 + 0.15 in the evening. The average for solids not fat percentage from weeks 1 - 44 was 8.84 + 0.03 and 8.92 + 0.04 for morning and evening milk respectively. The average of morning and evening is 8.88 per cent and least squares mean was 8.90 ±0.04. This was found to be similar to the other Indian breeds like Sindhi, Gir, Tharparkar, Sahiwal, Ongole and crossbred cattle reported by Ghosh and Anantakrishnan (1963); Babu Rao and Jayaramakrishna (1983) and some breeds like Ayrshire, brown Swiss, Guernsey and Jersey as reported by Byron *et al*. (1972).

From the Table 4.7 and Fig.6, it can be seen that no definite trend was visible in the solids not fat percentage in relation to stage of lactation. The differences in solids not fat percentage was not significant for stage (weekly) as in the case of time of milking. This results was in close agreement with the findings made earlier by Ghosh and Anantakrishnan (1964); Parekh and Gangwar (1968); Arora and Gupta (1969). But it was in disagreement with the reports made by Bayoumi (1959); Singh *et al.* (1961) as they observed an increasing trend in the solids not fat content with the progress of lactation. The coefficient of variation (Table 4.7) was around 9 per cent. The least squares analysis of variance (Table 4.8) showed no significant differences between the morning and evening solids not fat percentage. It was inferred that solids not fat percentage increased or decreased more or less in similar pattern at every stage of lactation as well as in the morning and evening milk.

5.2.4 Inter-relationship among constituents

No significant correlation was observed between (Table 4.9) fat and total solids percentage both in the morning and evening milk at 3rd, 13 th and 23 rd weeks of lactation. Total solids percentage had positive correlation with solids not fat percentage and significant at 13 th week of lactation. Generally negative correlation coefficient was noticed between fat and solids not fat percentage.

5.3 Fat globule size

The fat globules size varied from 1 to 12μ. The observation on the distribution of fat globules in different classes of 0-3μ, 3-6μ, 6-9μ and 9-12μ revealed that 50 - 70 per cent of globules were in the 0-3μ class in the morning milk (Table 4.10) and 38 - 59 per cent in the evening milk at different weekly intervals.

The overall average size irrespective of stage of lactation and time of milking was found to be 3.21 + 0.10μ. The mean diameter of fat globules (Table 4.11) irrespective of stage of lactation was 3.02 + 0.05μ and 3.40+0.05μ in the morning and evening milk respectively. A study made for comparison of Vechur milk fat globules with goat, crossbred cattle and buffalo milk fat globules. The average size of fat globule (Table 4.12) were found to be 2.60μ, 4.87μ and 5.85μ in goat (Malabari and their crosses), Crossbred cow and buffalo (Murrah) respectively. The average size for Vechur cows was found to be higher than that of goat but much lower than crossbred cow and buffalo. The fat globule size in this study tended towards that of the size of fat globules of goat. The average of 3.21ú for Vechur cow in this study was lower than the average size of buffalo, cow, goat and sheep fat globules reported by Fahmi *et al.* (1956) and 0.29ú and 1.39ú lower than the report made by Kuchroo and Narayanan (1977b) in cow and buffalos milk. The size was found to be higher than the fat globules in goat reported by Balwant Rai Puri *et al.* (1960); George (1981).

The average size was uniformly higher in the evening milk from 1 to 40 weeks of lactation than in the morning. It may be due to lesser milking interval as reported by Byron *et al.* (1972). A general decreasing trend was observed in fat globule size (Table 4.11 and Fig. 9) as lactation advanced. This finding was in close agreement with the earlier reports made by Upadhyaya *et al.* (1973) in buffalos; Katiyar *et al.* (1973) in Sahiwal cows; Kuchroo and Narayanan (1977b) in cows and buffalos and George (1981) in goats.

The average fat globule size ranged from 2.54 to 3.73ú in the morning and 2.75 to 4.07ú in the evening milk for different weeks. The evening milk had higher range (1.26ú) than morning (1.19ú) milk. The average size was attained by mid lactation in morning and evening milk. From Table 4.10 and Fig. 7,8 it was clear that the fat globules below 3ú in diameter showed an increasing trend and decreased trend was observed in the proportion of larger size fat globules. The morning milk uniformly had higher proportion of smaller size fat globules compared to the evening milk. The proportion of smaller size fat globules increased at accelerated rate during 30 to 40 weeks of lactation. The finding of this study was in close agreement with the reports made earlier by Upadhyaya *et al.* (1973) in buffaloes; Katiyar *et al.* (1973) in Sahiwal cows; Kuchroo and Narayanan (1977b) in cows and buffalos and George (1981) in goats. The proportion of small size fat globules was found to be higher than the exotic breeds like Jersey, Guernsey, Ayrshire and Holstein as reported by Jenness and Patton (1959).

The lower size of fat globules in the milk is associated with greater surface area and higher phospholipid content (Kuchroo and Narayanan, 1977b). The smaller size fat globules are easily digested due to greater surface area exposed to lipases action (Jenness, 1980). Phospholipids are an important factor in the development of brain and nervous tissues and play a vital role in the fat absorption and digestion (Norman, 1974). In general it was seen that the fat globule size in Vechur cow was much lower than other Indian breeds and exotic breeds of cattle and buffaloes, but higher than the goats. The general trend of increase in percentage of smaller size fat globules and decrease in percentage of larger size of fat globules in Vechur cow milk was similar to other breeds of cattle, buffaloes and goats. The lower size of fat globules in milk probably indicates a better digestibility and higher phospholipid content.

5.3.1 Correlation coefficient between fat globule size and fat percentage at different stages of lactation

A significant positive correlation (0.702) was noticed between the fat globule size and fat percentage in the morning milk at 3 rd week of lactation. Correlation at 13 th and 23 rd week were not significant. Milk fat percentage and fat globule size did not show a positive association in general (Table 4.13). This findings was in agreement with the reports made earlier by Kuchroo and Narayanan (1977a&b).

5.4 Iodine value

The average iodine value was 29.60 + 2.20 and it varied from 28.61 to 30.29 (Table 4.14). The findings of the present study though falls in range reported by Ramamurthy *et al.* (1978) in cow milk fat, it was lower than 32.8 to 33.9 reported by Basu (1962) and 30 to 34 reported by Henry and Newlander (1987). It was higher than the value of 24.95 and 25.05 reported in the milk of Alpine Malabari and Saanen Malabari crossbred goat by George (1981) and similar to that in buffalo milk (29.40) reported by Basu. As the iodine value indicates the degree of unsaturation, a lower iodine value for milk fat from Vechur cow would be probably indicative of more saturated fatty acids. The saturated fatty acids are digested easily by β-oxidation than unsaturated fatty acids and found to be therapeutically useful in malabsorption syndromes (Vasudevan and Sreekumari, 1995). The iodine number did not show any increasing or decreasing trend in relation to stage of lactation. Similar trend reported by Basu (1962); George (1981); Fahmi and Fahmy (1972).

Conclusion

In the case of casein the frequency of C allele at α_{s1}-casein locus, A allele at β- and k-casein locus were lower than other Indian breeds like Sahiwal, Tharparkar, Red Sindhi and Rathi. But in the case of whey proteins at α-lactalbumin and β-lactoglobulin locus Vechur cattle had much higher frequency for A variant.

Compared to the results reported by Madhavan (1995) in crossbred cattle of Kerala, Vechur cows had higher frequency of C allele at α_{s1}-casein locus and lower frequency of A allele at β- casein and k-casein locus, but higher frequency of α-lactalbumin and β-lactoglobulin A allele.

The fat percentage and total solids percentage were higher than other breeds of cattle, but lower to buffalo. These results scientifically support the belief of the people that the Vechur cow milk is thicker than general cow's milk.

The mean size of fat globules (3.21μ) was higher than the goat and lower to the other breeds of cattle and buffalo. An increase in the number of small size fat globule is always associated with greater surface area and high phospholipid content. So the fat is digested readily because of greater surface exposed to lipases action. Phospholipids are an important factor in the development of brain and nervous tissues in babies and play a vital role in the fat absorption and digestion. The iodine value (29.60) indicates higher proportion of saturated fatty acids in the milk fat. The saturated fatty acids are digested easily by β-oxidation than unsaturated fatty acids. Since the milk fat has smaller size of fat globules and more proportion of saturated fatty acids, it would be therapeutically useful in malabsorption syndromes due to its easy digestibility. This results pinpoint the need for more detailed study into the suitability of Vechur cow milk for patients with cardiac problems. The high fat content of milk can act as a regulator of infants appetite because of its satiating effect. Thus Vechur cow milk appears to be more suitable for infants due to its high fat content and low fat globule size. It is to be thought that it is more advantageous for adult also due to lower unsaturated fatty acids content.

The scientific studies on milk composition and looking into the size of fat globule and level of saturated fatty acids revealed that the popular beliefs has been with adequate basis though the scientific secrets behind were not fully known earlier.

It can be concluded that the Vechur cattle of Kerala has unique characteristics of its own and have separate identity from other breeds of cattle not only by its diminutive size, but also due to its milk protein variants, composition of milk, size of fat globules and level of saturated fatty acids in milk fat.

Summary

SUMMARY

1. Milk samples from individual Vechur cows were collected to study biochemical polymorphism of milk proteins, milk components such as fat, total solids and solids not fat, fat globule size and iodine value as a part of characterisation.

2. Fourteen Vechur cows available at ICAR Scheme on "Conservation of germplasm of Vechur cattle" were used for this study.

3. At α_{s1}-casein locus three phenotypes viz. BB, BC and CC determined by two allele B and C were observed. Frequency of CC phenotype (0.64) was highest a followed by BC (0.22) and BB (0.14). Frequency of B and C allele were 0.25 and 0.75.

4. At β-casein locus three phenotypes viz. AA, AB and BB with A and B allele were observed. The frequency of AB phenotype was highest (0.57) followed by AA (0.29) and BB (0.14). Frequency of A allele was 0.57. C allele which was specific for Brown Swiss was not identified.

5. At k-casein locus two phenotypes AA and AB with A and B allele were identified. The frequency of AB was (0.83). The frequency of A allele was 0.59.

6. Two phenotypes AA and AB with two allele A and B were identified at α-lactalbumin locus. The frequency of AA and AB phenotypes were 0.57 and 0.43. The frequency of A and B allele were 0.78 and 0.22 respectively.

7. At β-lactoglobulin locus consisted of three phenotypes viz. AA, AB and BB with A and B allele with frequency of 0.57, 0.29 and 0.14 respectively were identified. The frequency of A allele was 0.71.

8. The frequency of C allele at α_{s1}-casein locus was lower than the other Indian breeds like Sahiwal, Tharparkar, Red Sindhi and Rathi, but higher than exotic breeds like Jersey and Holstein and crossbred cattle of Kerala. The A variant at β and k-casein locus had frequency lower to other Indian breeds, exotic breeds of cattle and crossbred cattle of Kerala.

9. The A variant at α lactalbumin and β- lactoglobulin locus had much higher frequency than other Indian, exotic breeds of cattle and crossbred cattle of Kerala. B variant in Vechur cattle was lower in frequency compared to other Indian breeds.

10. The average milk fat percentage was 6.23 ± 0.19. Average for the morning was 5.95 ± 0.12 percent and for the evening 6.62 ± 0.13 percent.

11. The mean milk fat percentage for the morning and evening at first week was 4.05 ± 0.19, 4.65 ± 0.17 and by 20th week of lactation was 5.92 ± 0.08, 6.55 ± 0.07

12. The evening milk had uniformly higher fat percentage than morning milk The fat percentage showed an increasing trend with advancing stage of lactation. Increase in fat percentage was more in the initial stages of lactation, by 10 th week there was an increase of 1.39 per cent in the morning and 1.30 per cent in the evening. Least squares analysis of variance for effects of weeks and time of milking showed highly significant differences for both. The least squares means for

morning and evening milk were 5.99 ± 0.03 and 6.62 ± 0.03 respectively. The least squares means for 44 weeks ranged from 4.43 ± 0.12 at weeek to 7.52 ± 0.20 at 44 weeks and overall mean was 6.13 ± 0.04.

13. The morning and evening milk averages of 14.79 ± 0.13 and 15.53 ± 0.13 respectively. The average total solids percentage in milk was 15.16 ± 0.11 per cent.

14 . The mean total solids percentage of milk at first week was 12.64 ± 0.17 and 13.57 ± 0.19 in the morning and evening milk and it steadily increased to 14.75 ± 0.13 and 15.31 ± 0.18 in the morning and evening milk by 20th week of lactation .

15. An increasing trend was noticed in total solids percentage as the lactation advanced. The evening milk showed uniformly higher total solids percentage compared to morning. Least squares analysis of variance showed a significant effect on time of milking and stage of lactation. The overall squares mean was 15.02 ± 0.05 and the adjusted mean for morning and evening was 14.85 ± 0.05 and 15.55 ± 0.05 per cent.

16. The average solids not fat percentage was 8.88 ± 0.13 and for morning 8.84 ± 0.12 percent and for evening 8.92 ± 0.14 per cent respectively.

17. The mean solids not fat percentage of milk at first week was 8.60 ± 0.24 and 8.93 ± 0.22. It was 8.83 ± 0.11 and 8.76 ± 0.15 per cent at 20th week of lactation in the morning and evening milk respectively.

18. No trend of increase was noticed in the solids not fat percentage in relation to the stage of lactation as in the case of fat and total solids percentage. Least square analysis of variance showed non-significant effect of time of milking and stage of lactation on solids not fat percentage.

19. Total solids and solids not fat did not have strong association in general. The positive correlation between total solids and fat percentage were not significant. The negative correlation between fat and solids not fat percentage were also not significant.

20. The milk fat and total solids percentage were higher than those of other Indian breeds of cattle, but lower to buffalo. The solids not fat percentage of milk was similar to other Indian breeds of cattle.

21. The mean size of fat globule was 3.21 μ and the range was 2.54 to 4.07 μ. The mean diameter of fat globules was found to be 3.02 ± 0.05 μ in the morning and 3.40 ± 0.05 μ in the evening milk. The mean size of fat globules estimated in goats (Malabari and their crosses), crossbred cattle and Murrah buffalo were 2.60 μ, 4.87 μ and 5.81 μ respectively for the comparison.

22. The average fat globule size was found to be decreasing as the lactation advanced. The proportion of small size fat globules were found to be increased and the larger size of fat globules decreased towards the end of lactation.

23. No correlation was noticed between fat globules size and fat percentage.

24. The iodine value ranged from 28.61 to 30.29 and the average iodine value was 29.60 ± 2.20. Based on available reports this appeared to be similar to buffalo, higher than goat and lower than cows. The iodine value indicates the level of unsaturated fatty acids and it appears that Vechur cow milk has less unsaturated fatty acids compared to other cattle breeds.

The larger proportion of small size fat globule is associated with greater surface area and high phospholipid content. So the fat is digested more readily because of greater surface exposed to lipases action. Phospholipids are important in the development of brain and nervous tissues and also play avital role in the fat absorption and digestion. The saturated fatty acids are easily digested by beta oxidation than unsaturated fatty acids. Since the milk fat has higher proportion of smaller size fat globules and saturated fatty acids, it would be therapeutically useful in malabsorption syndromes due to its easy digestability. Thus Vechur cow milk appear to be suitable for infants and even sick. Studies on more number of cows are required for confirmation of results.

It can be concluded that Vechur cattle of Kerala has unique characteristics of its own and have separate identity from other breeds of cattle not only by its diminutive size but also due to its milk protein variants, composition of milk, size of fat globules and level of saturated fatty acids.

References

REFERNCES

Annual Report of National Dairy Research Institute (1948). Average composition of cows and buffalo milk in India. (cited in *Dairy India* 1987 pp: 218)

Arora, S.P and Gupta, B.S. (1969). Variation in the milk componants of Nimari cows. *Indian J. Dairy Sci.,* **22**: 65-72

Aschaffenburg, R. (1961). Inherited casein variants in cow's milk. *Nature* **192**: 431

Aschaffenburg, R. (1968). Reviews of the progress of the dairy Science, Section G. Genetics. Genetic variants of milk proteins : their breed distribution. *J. Dairy Res.* **35** : 447-460

Aschaffenburg, R. and Drewry, J. (1955). Occurrence of different β-lactoglobulin in cow's milk. *Nature* **176** :216

Babu Rao, T. and Jayaramakrishna, V. (1983). Milk constituents of three genetic groups of cows. *Indian J. Dairy Sci.,* **36**(3): 286-293

Balwant Rai puri, Satparkash and Chandan, R.C (1961). Studies in physico-chemical properties of milk. Part IX. Variation in fat globule size distribution curves of cow and buffalo milk, on the removal of fat and additionof goat milk. *Indian J. Dairy Sci.,* **14**: 31-35.

Barbano and Dellavalle, M.E (1985). Seasonal variation in milk solid components in various regions of the US D.M. *J. Dairy Sci.*, 68(1): 71-76

Basu, K.P. (1962). Composition of milk and ghee. ICAR Report Series No.8 Bayoumi, M.S. (1959) Effect of season and stage of lactation on the yield and composition of milk in two herds of University of Wales, Aberystwyth. *Indian J. Dairy Sci.*, 12: 87-89

Bayomi, M.S.(1959). Effect of season and stage of lactation on the yield and composition of milk in two herds of University College of Wales. Aberystwyth. *Indian J. Dairy Sci.*, 12: 87-99

Belyaev, D.K and Kiseleva, ˜.S. (1966). Some genetic parameters of major milk constituents in cattle. *Genetica* 9 : 58-79 (cited in Dairy Sci., Abstr. 29(4): 1260

Bhattacharya, S.D., Roy Chowdhary, A.K., Sinha,N.K. and Sen, A. (1963). Inherited ÿ-lactalbumin and Ö-lactoglobulin polymorphism in Indian zebu cattle. *Nature* 197:797

Byron H.Webb, Arnold H. Johnson, John A. Alford (1972). *Fundamentals of Dairy Chemistry.* CBS Publishers and Ditributors, Delhi pp : 9-11

Darshanlal and Narayanan, K.M. (1991). Effect of lactation number of the animal on milk total solids. *Indian J. Anim. Sci.*,61(3): 311-315

Eennemaan, A.L. and Medrano, J.F (1991). Milk protein polymorphism in California cattle. *J. Dairy Sci.*, **74**(5): 1730-1742

Eigel, W.W., Butler, J.E., Ernstrom, C.A., Farrel, H.M., Harwalkar, V.R., Jenness, R and Whitney, R. (1984). Nomenclature of milk proteins. Fifth revision. *J. Dairy Sci.*, **67**: 1599-1631

Fahmi, A.H and Fahmy, T.K (1972). Studies on some chemical properties of Samn. III. The degree of saturation present in Etyptian Samn. *Agricultural Research Review* **50**(3): 191-196

Fahmi,A.H., Sirry,I and Safwat, A (1956). The size of fat globulesand the creaming power of cow, buffalo, sheep and goat milk. *Indian J. Dairy Sci.*, **9**: 124-130

George, B. (1981). Properties of milk fat of crossbred goats. M.V.Sc. Thesis submitted to the Kerala Agricultural University

Ghosh, S.N. and Anantakrishnan, C.P. (1963). Composition of milk part IV. Influence of season, breed and species. *Indian J. Dairy Sci.*, **17**: 190-202

Ghosh and Anantakrishnan, C.P. (1964). Composition of milk. part V. Effectof stage of lactation. *Indian J. Dairy Sci.*, **17**: 17-28.

Harvey, W.R. (1986). Mixed model Least- Squares and Maximum Likelihood Computer Program. PC version (PC-1) LSMLMW with Parmcard.

Henry, V. Atherton and Newlander J.A. (1987). *Chemistry and Testing of Dairy Products*. CBS Publishers and Distributors, Delhi pp: 9

Hussain, M.K. (1974). Genetic Variants of milk proteins and their importance in dairying. *Kieler Milchwirts Chaffliche forschengsberichte* **26**(1): 65-77

Indian standards : 1479 (1961). Determination of total solids in milk (Gravimetric method) Methods of Tests for Dairy Industry: part-II - *Chemical analysis of milk*. Indian Standards Institution, New Delhi pp :6

Indian standards : 1224 (1977). Determination of fat percentage by Gerber method. Part-I *Milk* (first revision) Indian Standards Institution, New Delhi, pp: 4-8

Indian standards : 3508-1966 (1980). Determination of Iodine value (Wiji's method). *Methods of Sampling and Tests for Ghee (Butter Fat)*. Indian Standards Institution, New Delhi pp. 30-34

Iype, Rahavan, K.C., Girija, C.R., Aravindakshan, T.V., Radakrishnan, J and Mukundan, G. (1994). Milk fat percentage at various stages of lactation of the crossbred cattle in Kerala. *Indian J. Anim. Sci.*,**64**(3): 312-313

Jairam,B.T. and Nair, P.G.(1983a). Genetic polymorphism of milk proteins in different breeds of cattle. *Indian J. Dairy Sci.*, **36**: 5-11.

Jairam, B.T. and Nair, P.G. (1983b). Genetic polymorphism of milk proteins and economic characters in dairy animals. *Indian J. Dairy Sci.*, **53**: 1-8

Jenness, R. (1980). Composition and charateristics of Goat Milk: Review 1968-1979. *J. Dairy Sci.*, **63**(10): 1605-1630

Jenness, R. and Patton, S. (1959). *Principles of Dairy Chemistry*. Wiley Eastern Private Limited, New Delhi. pp: 33

Johnson, G.S. (1995). Preparation of mozzarella cheese using skim milk filled with coconut milk M.V.Sc. Thesis sumitted to Kerala Agricutural University

Juneja, R.K. and Chaudhary, R.P. (1973). Simmultaneous phenotyping of milk proteins in Indian cattle. *Indian J. Dairy Sci.*, **26**(2): 104-106

Katiyar, M.P., Srivastava, R.P and Kushwaha, N.S. (1973). Studies on fat globules in milk. I. Factors affecting size and distribution of fat globules in the milk of Sahiwal cows. *Indian J. Farm Sci.*, **1**(1): 90-95

Kiddy, C.A., Johnson, J.O. and Thompson, M.P. (1964). Genetic polymorphism in caseins of cow's milk. I. Genetic control of α_{s1}-casein variation. *Indian J. Dairy Sci.*, **47**: 149

Kohli, M.L., Suri, K.R., Bhatnagar, V.K. and Lohia, K.L. (1961). Studies of some economic characters in relation to age at first calving in Hariana cattle. *Indian J. Dairy Sci.*, **14:** 154-160.

Kuchroo, T.K. and Narayanan, K.M. (1977a). Effect of stage of lactation on distribution of fat globules and phospholipids content of milk. *Indian J. Dairy Sci.*, **30**(2): 99-104

Kuchroo, T.K. and Narayanan, K.M. (1977b). Effect of sequencing of milking on the distribution of fat globules and phospholipids composition of milk. *Indian J. Dairy Sci.*, **30**(3): 99-104

Lin, C.Y., McAllister, A.J., Ng-Kwai-Hang, H.F. and Hayes, J.F. (1986). Effects of milk protein loci on first lactation performance in dairy cattle *Indian J. Dairy Sci.*,69; 704-712.

Madhavan, K. (1995). Milk protein polymorphism in crossbred cattle of Kerala, M.V.Sc. Thesis submitted to the Kerala Agricultural University

Majumder, G.C. and Ganguly, N.C. (1970). Genetic polymorphism of kappa casein in Indian zebu cattle and buffaloes. *Indian J. Dairy Sci.*, **23:** 201-204

Mawal, R.B. (1967). Evaluation of α - lactalbumin and β-lactoglobulin in cows and buffaloes by electrophoresis and column chromatography. *Acta. Physiol. Pharmac. Neer.* **14:** 317-324

Mclean, D.M., Graham, E.R.B., Ponzoni, R.W. and Mckenzie, H.A. (1984). Effects of milk protein genetic variants on milk yield and composition. *J. Dairy Res.*, **51**: 531- 546

Melton, A.A., Riggs, J.K., Nelson, L.A. and Cartwright, T.C.(1967). Milk production, composition and calf gains of Angus, Charolais and Hereford cows. J. Anim. Sci., **26**(4): 804-809

Mohran, M.A. and Fahmy, M.A. (1992). Physico-chemical characteristic of milk produced in upper Egypt. Effect of animal, breed and lactation period. *Egyptian J. Dairy Sci.*, **55**(7): 307-310

Naikare, B.D., Kale, K.M., Jagtap, D.Z. and Narwade, U.S. (1992). Factors affecting fat percentage in Gir crosses. *Indian J. Anim. Sci.*, **62**(12): 1209-1211

Ng-Kwai-Hang, K.F., Hayes, J.F., Moxeley, J.E. and Monardes, H.G. (1984). Association of genetic variants of casein and milk proteins with milk, fat and protein production by dairy cattle. *Indian J. Dairy Sci.*, **67**: 835-840

Norman, N. Potter. (1978). *Food Science*, CBS Publishing and Distributors, New Delhi, pp: 72

Parekh, H.K.B. and Gangwar, P.C. (1968). Chemical composition of buffalo milk and effect of stage of lactation and pregnancy period on its composition. *Indian J. Dairy Sci.*, **27**: 177-182

Rama Murthy, M.K., Krishana Rao and Venkateswara Rao (1978). Change in the refractive index of milk fat due to its reaction with mercuric acetate and its use for determination of iodine number of milk fat. *Indian J. Dairy Sci.*, **31**(4) : 375-376

Rangappa, K.S. (1964). Studies on fat globules in milk. *Indian J. Dairy Sci.*, **17**: 95-96

Sadana, D.K., Basu, S.B and Bhatia, K.L. (1978). Effects of lactation number and stage of lactation on milk fat percentage of Karen Swiss cattle. *Indian J. Dairy Sci.*, **31**(2): 177-178

Sathian, C.T. (1992). Effect of feeding additives on total solids of cow milk, M.V.Sc. Thesis submitted to Kerala Agricultural University

Sharma,A.K., Wilcox, C.T., Martin, F.G and Thatcher, W.W. (1990). Effects of stage of lactation and pregnancy and their interactions on milk yield and constituents. *Indian J. Dairy Sci.*,**73**(6):1586-1592

Singh, H. and Khanna, N.D. (1972). Milk protein polymorphism in Hariana cattle. *Indian J. Anim. Sci.*, **42** 7) : 468-469

Singh, H and Bhat, P.N. (1980). β-lactoglobulin polymorphism in indegenous cattle. *Indian J. Anim. Sci.*, **50**: 932-937

Singh, R.P., Singh, M., Rao, Y.S. and Singh, S.N. (1961). Quantitative relations among milk constituents I. Effect of breed, stage of lactation and age of animals. *Indian J. Dairy Sci.*, **14** : 119-129

Snedecor, G.W. and Cohran, W.G. (1967). *Statistical Methods*. (Third edn.) Oxford and IBH publishing co. New Delhi.

Sommerfeldt, J.L., Baer, R.J. and Tucker, W.L. (1985). Bi-weekly variability of herd milk components. *Indian J. Dairy Sci.*, **68** (1) : 72-75

Taha, F. and Puhan, Z.(1993). Milk protein polymorphism in Swiss dairy cattle. *Agrl. Sci.*,Finland **2**(5): 423-429

Taparia, A.L. (1969). Rumen fermentation in relation to fat and SNF contents of milk - A review. *Indian J. Dairy Sci.*, **22**:205-210

Thompson, M.P. and Kiddy, C.A. (1964). Genetic polymorphism in caseins of cow's milk IV. Isolation and properties of αs1-casein A, B and C. *J. Dairy Sci.*, **47**: 626

Thompson, M.P. and Pepper, L. (1964). Genetic polymorphism in caseins of cow's milk IV. Isolation and properties of β-casein A, B and C. *J. Dairy Sci.*, **47**: 633

Tilakaratne, N. Ranawana, S.S.E. and Buwanendran, V. (1975). A note on the milk composition of European cattle and their crosses with Sindhi. *Cyelon Vet. J.* **23** (1): 14 - 50

Vasudevan, D.M. and Sreekumari, S. (1995). *Text Book of Biochemistry.* Jaypee Brothers Medical Publishers (p) Ltd., New Delhi pp: 154

Velu pillai, T.K. (1940). *Travancore State Mannual* Vol. III. Published by Government of Travancore pp: 383

Upadhyaya, V.S., Katiyar, M.P., Srivastava, R.P. and Kushwaha, N.S. (1973). Studies on fat globules of milk II. Factors affecting the size and distribution of fat globules in the milk of Murrah buffaloe. *Indian J. Farm Sci.*,**1**(1):96-101

Whittlestone, W.G. (1958). Fat globule size distribution of buffalo milk. *Indian J. Dairy Sci.*,**11** :43-47

MILK PROTEIN POLYMORPHISM AND MILK COMPOSITION IN VECHUR CATTLE

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ABSTRACT OF A THESIS

Submitted in partial fulfilment of the
requirement for the degree

Master of Veterinary Science

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1996

ABSTRACT

The native cattle of Kerala have been evolved through several generations of natural selection against high humidity, heavy rainfall and hot climate. They have been considered as non-descript animals, but there has been variety with characters distinguishing from others and known as Vechur cattle of Kerala. The Vechur cattle had their origin in Vechur near Vaikom of Kottayam district of Kerala. They were very popular four decades back for their relatively higher milk production compared to other local cows. The extremely small size of the cow, good adaptation and high disease resistance are the traits very much favoured by the farmers. Farmers prefered Vechur cattle for ploughing in marshy lands because of the small size and light weight. The emergence of crossbreeding led to the gradual reduction in tradationally reared Vechur cattle. Under this circumstances, the present work was undertaken to characterise the Vechur cattle of Kerala by studying (a) certain genetic markers in milk (b) milk components like fat, total solids and solids not fat percentage (c) fat globule size and distribution and (d) iodine value.

The Vechur cows maintained under the scheme on "Conservation of germplasm of Vechur cattle" formed the material for the study. Milk proteins such as casein and whey proteins were studied by polyacrylamide gel electrophoresis in horizontal dimension (Thompson and Kiddy, 1963). The fat, total solids, solids not fat percentage of milk and iodine value of milk fat were estimated as prescribed by Indian Institution Standards. The fat globule size was measured under microscope by fitting an eye piece micrometer to the eye piece of the microscope. The statistical analysis were done as suggested by Snedecor and Cochran (1967). The influence of stage and time of milking were worked out using least squares analysis of variance as described by Harvey (1986).

Three proteins α, β and k-casein were identified in casein system. At α_{s1}-casein locus three phenotypes viz. BB, BC and CC determined by two allele B and C were observed. Frequency of CC phenotype (0.64) was highest a followed by BC (0.22) and BB (0.14). Frequency of B and C allele were 0.25 and 0.75. The β-casein locus consisted three phenotypes viz. AA, AB and BB with A and B allele. The frequency of AB phenotype was highest (0.57) followed by AA (0.29) and BB (0.14). Frequency of A allele was 0.57. At k-casein locus two phenotypes

AA and AB with A and B allele were identified. The frequency of AB was (0.83) and frequency of A allele was 0.59.

Two whey proteins α-lactalbumin and β-lactoglobulin were identified. At α-lactalbumin locus two phenotypes AA and AB with two allele A and B were observed. The frequency of AA and AB phenotypes were 0.57 and 0.43. The frequency of A and B allele were 0.78 and 0.22 respectively. The β-lactoglobulin locus consisted of three phenotypes viz. AA, AB and BB with A and B allele with frequency of 0.57, 0.29 and 0.14 respectively were identified. The frequency of A allele was 0.71.

The frequency of C allele at α_{s1}-casein locus was lower than the other Indian breeds like Sahiwal, Tharparkar, Red Sindhi and Rathi, but higher than exotic breeds like Jersey and Holstein and crossbred cattle of Kerala. The A variant at β and k-casein locus had frequency lower to other Indian breeds, exotic breeds of cattle and crossbred cattle of Kerala. The A variant at α-lactalbumin and β-lactoglobulin locus had much higher frequency than other Indian, exotic breeds of cattle of cattle and crossbred cattle of Kerala. B variant in Vechur cattle was lower in frequency compared to other Indian breeds.

The average milk fat percentage for 1 - 44 weeks of lactation was 5.95 ±0.12, 6.62 ± 0.13 in the morning and evening respectively and the mean milk fat percentage for morning and evening milk was 6.23 ± 0.19. The mean milk fat percentage at first week was 4.05 ± 0.19, 4.65 ± 0.17 and by 20 th week of lactation was 5.92 ± 0.08, 6.55 ± 0.07 in the morning and evening milk respectively. The evening milk had uniformly higher fat percentage than morning milk. The fat percentage showed an increasing trend with advancing stage of lactation. The least squares means for morning and evening milk were 5.99 ± 0.03 and 6.62 ± 0.03 respectively and overall mean was 6.13 ± 0.04.

The mean total solids percentage of milk at first week was 12.64 ± 0.17 and 13.57 ± 0.19 in the morning and evening milk and it steadily increased to 14.75 ± 0.13 and 15.31 ± 0.18 in the morning and evening milk by 20 th week of lactation. The average total solids percentage in milk from 1 - 44 weeks of lactation was 14.79 ± 0.13 and 15.53 ± 0.13 in the morning and evening respectively and the average for morning and evening together was 15.16 ± 0.11 per cent. An increasing trend was noticed in total solids percentage as the lactation advanced. The evening milk showed uniformly higher total solids percentage compared to morning. The least squares mean was 15.02 and the adjusted mean for morning and evening was 14.85 ± 0.05 and 15.55 ± 0.05 per cent.

Least squares analysis of variance showed a significant effect of time of milking and stage of lactation on milk fat and total solids percentage of milk.

The average solids not fat percentage from 1 - 44 weeks of lactation was 8.84 ± 0.12 and 8.92 ± 0.14 in the morning and evening milk respectively. The average for morning and evening milk was 8.88 ± 0.13 and least squares mean was 8.90. The mean solids not fat percentage of milk at first week was 8.60 ± 0.24 and 8.93 ± 0.22. It was 8.83 ± 0.11 and 8.76 ± 0.15 percent at 20 th week of lactation in the morning and evening milk respectively. No trend of increase was noticed in the solids not fat percentage in relation to the stage of lactation. The least squares analysis of variance showed no significant effect of time of miling and stage of lactation on solids not fat percentage.

Total solids and solids not fat did not have strong association in general. The positive correlation between total solids and fat percentage were not significant. The negative correlation between fat and solids not fat percentage were also not significant.

The milk fat and total solids percentage were higher than other Indian breeds of cattle, but lower to buffalo. The solids not fat percentage of milk was similar to other Indian breeds of cattle.

The mean size of fat globule was 3.21 μ and the range was 2.54 to 4.07 μ. The mean diameter of fat globules was found to be 3.02 + 0.05 μ in the morning and 3.40 + 0.05 μ in the evening milk. The mean size of fat globules estimated in goat (Malabari and their crosses), crossbred cattle and Murrah buffalo were 2.60 μ, 4.87 μ and 5.81 μ respectively. The average fat globule size was found to be decreasing as the lactation advanced. The proportion of small size fat globules were found to be increased and the larger size of fat globules decreased towards the end of lactation. No correlation was noticed between fat globules size and fat percentage.

The iodine value ranged from 28.61 to 30.29 and the average iodine value was 29.60 ± 2.20. Based on available reports this appeared to be similar to buffalo, higher than goat and lower than cows.

The size of fat globule (3.21 μ) was higher than the goat and lower to the other breeds of cattle and buffalo. Since the milk fat has higher proportion of smaller size fat globules and saturated fatty acids, it would be therapeutically useful in malabsorption syndromes due to its easy digestability. The larger proportion of small size fat globule is associated with high phospholipid content because of greater surface area. Phospholipids are important in the development of nervous system in babies. Thus Vechur cow milk appear to be suitable for infants and even sick. Studies on more number of cows are required for confirmation of results.

It can be concluded that Vechur cattle of Kerala has unique characteristics of its own and have separate identity from other breeds of cattle not only by its small size but also due to its milk protein variants, composition of milk, size of fat globules and level of saturated fatty acids.