STUDIES ON THE WHEY PROTEINS OF COWS' MILK IN INDUCED LACTATION

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "STUDIES ON THE WHEY PROTEINS OF COWS' MILK IN INDUCED LACTATION" is a bona fide record of research work done by me during the course of research and that the 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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CERTIFICATE

Certified that this thesis, entitled "STUDIES ON THE WHEY PROTEINS OF COWS' MILK IN INDUCED LACTATION" is a record of research work done independently by Sri Sadananda Talukdar under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to him.

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INTRODUCTION

Milk is considered as nature's most perfect food. It plays a very significant role as a source of nutrients. Cows' milk, as a food of animal origin, contributes greatly to human nutrition. Proteins in the milk are unique from both nutritional and immunological point of view.

The relationship between whey protein and casein is found to be around 1:4 in cows' milk (Hambraeus, 1982). But, the case is reverse with human milk. It is postulated that casein in cows' milk is insufficient to enrich milk with all essential amino acids present; but it is whey protein with the abundance of amino acids that can supplement and enhance the nutritive value.

Cows' milk is widely used for making humanized milk. Special interest has been devoted now-a-days to the utilization of whey proteins in human diet. With the advances in the knowledge on dietetics and growing awareness for balanced food, the value of milk proteins has been overwhelmingly appreciated. The presence of milk protein as low as 10% of total protein intake in our diet is regarded as a significant contribution (Hambraeus, 1982). It can supplement nutritionally inferior proteins available in cereals and vegetables. Hence, it has drawn wide attention all over the world in recent times.

The practice of animal husbandry and dairying has much to contribute in this content. It can provide a wider scope for the qualitative and quantitative improvement of nutrition. There is also ample scope for providing employment opportunities in a predominantly agrarian country like India.

Meanwhile, several encouraging measures have been adopted with the application of latest innovations in the field of science and technology. The cross-breeding policy involving the selected exotic and other superior breeds has been rightly accepted as a worthwhile and suitable approach for the creation of a new generation of high yielding cattle. The liberalized bank loan facilities and other developmental programmes have encouraged the farmers to take up dairying in an organized manner. Thus, the practice of animal husbandry and dairying has emerged as a profitable proposition on par with any other industry.

In spite of all the developmental efforts, the outcome will be rather a disappointing one when the animal is struck with infertility problems. The animal even with apparently normal health may take prolonged time to attain breedable stage; it may not conceive in time or abort before the term. Even after calving, there are chances of cow going dry before completing the normal length of lactation. Various factors like hormonal, hereditary, managemental, anatomical and physiological defects may be attributed to such eventualities.

The varied and complex situations may stand on the way to our success. The animal with a good genetic potentiality for higher yield, under such circumstances, becomes useless. The owner is ultimately bound to incur a great loss. Consequently, the enthusiastically adopted development programmes may have to suffer a setback or may fail to get the desired impetus.

In order to overcome such an adverse situation and wipe out the very uncertainty, hormonal induction of lactation is considered to be an alternative measure. By this, the higher potentiality of the animal can be exploited. Besides, the animal is later found to get the ability to conceive and she no longer remains as a "problem breeder".

Various aspects like physiological and histological changes, synthesis of milk constituents by the mammary tissue, and the role of circulatory hormones can be studied by bringing the animal into lactation. Investigations on mammogenesis, lactogenesis and related problems can be carried out by the application of such technique.

On the other hand, it is advocated that selection goals for all cows should be towards milk with higher protein content. Among the milk proteins, the biological value of whey proteins is appreciably high. In milk intended for fluid consumption, there has been a preference to have increased whey protein content from nutritional point of view.

These economical, biological and technological importances have prompted today for extensive research in milk proteins.

The whey powder now-a-days has received attention as stock feeds and confectionary ingredients. It can replace the costly skim milk powder. In infant food formulae, the consideration of whey powder is important in view of the occurrence of milk allergy. The whey powder concentrates are being produced commercially. Manufacture of protein ingredients from cheese or casein whey is now an established part of dairy industry. Since the disposal of untreated liquid whey causes heavy water pollution for its high BOD, strict regulation has been enforced. This accounts for the shift towards product manufacture from the disposable whey. As the milk products of different choices are coming up, relevant informations are highly essential.

The milk production and processing statistics (1983) of International Dairy Federation indicated that the total amount of cheese made in the world approximated 10 million metric tons. World liquid whey production reportedly was about 85 million metric tons which has been described by Zall (1984) as an "ocean" of liquid whey that could yield a sufficient quantity of whey proteins.

Informations on the response of hormonal treatment in

infertile cattle stand inadequate. The synthesis of various components of milk in the mammary tissue is yet to be understood fully. Moreover, studies on the composition of milk in induced lactation are still limited. Reports on protein content in this aspect are very few; the same on its different components, particularly the whey proteins, are still wanted.

In the present study, therefore, an attempt has been made to determine the levels of different components of protein in general and whey protein in particular in the milk of cows induced to lactate. Those levels have been compared with those of milk from normal lactation.

Review of Literature

REVIEW OF LITERATURE

Development of mammary gland

The pioneering work of Bouin and Ancel (1909) on the influence of hormones on mammary growth revealed the vital role of corpus luteum in the preparation of the mammary gland of rabbit for lactation. The authors concluded that the corpus luteum exerted a stimulatory activity on the lobulo-alveolar growth and development.

Following one and a half decade, an observation was made by Huxley (1924) on the active phase of mammary growth and proliferation depending upon nature of sex cycle. He developed a relative growth analysis in which the growth rate of an organ could be related to that of the body as a whole.

Application of aforesaid Huxley's technique in the work of Folley et al. (1939) in rhesus monkey evolved valuable informations about mammary growth process. They reported that in the non-pregnant female monkey, there was a period of accelerated mammary growth when the total mammary area increased 2.7 times faster than the body surface.

A new dimension on the role of hormones in mammary gland development was opened by Nixmer and Turner (1943). They observed several histological abnormalities like cystic

alveoli, papillomatous outgrowths into alveolar lumen, immature ducts and alveoli without lumen.

Later, Sykes and Wrenn (1951) treated calves with the daily injection of diethylstilboestrol and progesterone at 6 mg and 250 mg respectively for 5 months. This treatment resulted in the growth of the udder tissue without marked histological abnormality.

The use of estrogen and progesterone together by Cowie et al. (1952) and Benson et al. (1955) in an attempt to study the mammary gland development in goats showed no abnormality as before. Henceforth, it was accepted that for the normal mammary gland development, estrogen and progesterone together played undoubtedly a leading role.

Cowie et al. (1965) after conducting another experiment on goats confirmed the above findings. Findings relating to the hormonally developed udder with special reference to the triggering dose of estrogen were conclusive and confirmatory. However, one additional information was available later when Cowie et al. (1966) could notice that estrogen plus progesterone exerted no mammogenic effect in the absence of the pituitary gland.

Cowie (1970) have elaborately reviewed the outstanding achievements of several decades on the influence of hormones on mammary growth and milk secretion. He also explained the

hormonal mechanisms involved in the change over from the functionally quiescent to functionally active mammary gland.

Induction of mammary gland growth in normal and hypophysectomised heifers were done by Sud et al. (1968, 1971). Injections of 400 µg of estradiol-17 beta and 100 mg of progesterone, three times weekly for 20 weeks were given. Udder tissues were found histologically normal. Sud (1972) used this method for the development of mammary gland followed by the use of 9-Fluoroprednesolone to initiate lactation.

In another study conducted by Sud (1973a), administration of 50 µg estradiol plus one or four mg progesterone for 20 days evoked optimal growth of mammary tissue in the castrated guinea-pig. The results indicated that for the development of mammary tissue in the guinea-pig, both estrogen and progesterone were required.

The histological and metabolic changes in the mammary gland during induced lactation was studied by Fleming et al. (1977). They took 23 cows, injected oestradiol-17 beta and progesterone for 21 days and dexamethasone on day 31-34. On day 35, milking was initiated. The mammary biopsy revealed that epithelial area increased linearly with the course of injections.

The role of progesterone and insulin in the growth and differentiation of mouse mammary epithelium was investigated

by Freeman and Topper (1977). Their conclusion was that ductal cell proliferation in the mouse mammary gland *in vivo* required estrogen and progesterone; alveolar formation required estrogen-progesterone, and either progesterone or placental lactogen. Insulin was not required for growth of either ducts or alveoli *in vivo*.

The report of McFadden *et al.* (1985) was related to hormone induced synthesis and secretion of milk proteins by mammary explants from prepuberal Angus and Holstein heifers. The study was concerned with the effect of breed on protein synthesis and secretary responses of prepuberal mammary tissue to lactogenic stimuli. Injections with oestradiol-17 beta plus progesterone (0.1 and 0.25 mg/kg/day respectively) were given to Angus and Holstein heifers for seven days. On the 15th day, they were slaughtered. The mammary tissue was explanted and cultured for 96 hours in basal medium or stimulatory medium. Prolactin was added to the medium. There was synthesis and secretion of protein in prepuberal mammary tissue after hormone treatment. The Holsteins were more productive than the Angus.

Kazmer *et al.* (1985) collected mammary tissue from nine Holstein cows in three different periods - within one week of parturation, at 60 and again at 180 days post-partum. The aim was to study the lactogenic hormone receptors. It was concluded that lactogenic receptor concentrations in

bovine mammary tissue increased with onset of lactation, following a pattern similar to that observed in nonruminants.

Induction of lactation

Giving special importance on the composition of milk in induced lactation, a study was conducted by Perin (1955). The author brought identical twins into lactation using progesterone and synthetic estrogen. It was found that mammary secretions were initially colostral in nature. As milk flow became established, the secretion gradually turned into a milk of normal composition.

Turner et al. (1956) took a group of infertile heifers and injected estrogen and progesterone daily for 180 days. As a consequence, hyperemia of skin of the udder was found. Another experiment with estrogen alone produced oestrus only. Combination of both the hormones resulted in copious secretion of milk in the Jersey and the Holstein whereas the Guernsey freemartin did not respond. Maximum yield was reached after fourteen weeks from the beginning of treatment. It was also observed that the maximum yield was earlier than in case they would have calved normally.

In a report on hormonal control of mammary growth and lactation in the goat, Meller and Reinkote (1958) stated that sexually mature goats were distinctly more responsive to the treatment. The combination of stilboestrol and

progesterone brought into rapid onset of lactation; but they could not find out a clearcut indication for an optimum ratio between the two hormones for the mammary growth and lactation.

The hormonal set up during pregnancy was studied by Erb et al. (1968) from urinary excretion in bovine. These findings helped later to establish similar condition artificially in order to initiate udder development and milk secretion.

There was a successful result on the use of diethylstilboestrol alone. Naito et al. (1968) found it in 30 Holstein heifers which were given 5 mg every third day subcutaneously for a period of 30 days. The author concluded that the procedure would be useful to predict future production of the heifers.

Contrary to the above report, there was another one where sterile cattle were used for induction of lactation. The study was conducted by Garg and Nangia (1972). A total of four Haryana cows between 6-10 years of age with a history of sterility were taken for the experiment. Injections of (i) estrogen or (ii) progesterone in two different doses and for different periods of time were given by intramuscular route. Estrogen injected was 370 mg in total for 18 weeks and likewise, progesterone was 1750 mg in total for

14 weeks. The response was better from these animals having received higher dose for shorter period. Average total milk was 95.5 litres over a period of 376 days in cows under group I and 98.4 litres in 446 days in group II. The composition was normal after a week.

In another work, Eeb et al. (1973) injected 17-beta estradiol and progesterone subcutaneously to five heifers and three cows. They divided daily doses into two and injected on an interval of 12 hours. From 10th to 21st day of commencement of injection the animals started giving milk and gradually reached the peak 60 days after the last injection.

Sud (1973b) observed gonodal hormone eliciting adequate development of mammary glands in heifers but he expressed doubts over the secretory activity in full. He claimed that melengestrol acetate (MGA) had a similar effect on both development and secretion of the mammary gland.

Piper and Williams (1974) reported that milking in nonpregnant, nonlactating cows was initiated within eight to ten days after the last injection consisting of oestradiol 17-beta plus progesterone at the rate of 0.1 mg and 0.25 mg per kg body weight respectively for seven days. One animal with broken hip was made useful which yielded 15000 pounds of milk till the completion of the study.

Smith and Schanbacher (1973, 1974) administered subcutaneously oestradiol-17-beta and progesterone at the rate of 0.1 mg and 0.25 mg/kg body weight/day respectively, for seven days into nine cows and one heifer. Daily dose was divided into two and given at 12 hour interval. The animals received injection for 7 days. Seven out of ten animals started lactation from 11-21 days after first injection. Peak yield was attained within 30-50 days after lactation began. Animal that received treatment from fifth to seventh day post estrus responded better.

Studies on milk production and reproductive performance of the cows hormonally induced to lactation were undertaken by Collier et al. (1975a,b). They took 11 cows and 5 heifers and administered oestraiol-17-beta with progesterone for seven days followed by dexamethasone on days 19 and 20. Among the animals, only two failed to lactate. The peak milk yield was not reached until an average of 8.8 weeks following commencement of milking.

In another work, eight non-pregnant dairy cows (one dry and seven producing milk) were injected with a mixture of progesterone at 0.125 mg/kg body weight and oestradiol-17-beta at 0.05 mg/kg body weight dissolving in absolute ethanol. As a secondary treatment, dexamethasone or their combination followed the primary treatment with no significant effect in lactation. Sequential changes in milk

composition were reported to be indicative of mammary gland regression followed by lactogenesis. Only 0.007% of injected oestradiol-17 beta was recovered and hence inference was drawn that milk was a minor excretory pathway for that hormone (Mollett et al. 1975).

Erb et al. (1976) induced lactation in 3 cows and 5 heifers with 0.05 mg oestradiol-17 beta and 0.125 mg progesterone per kg body weight twice daily for 7 days, and blood, urine and milk samples were studied by radioimmunoassay for hormones. Total free estrogen in milk decreased significantly by a maximum of 18 days after the last injection. Milk and plasma progesterone never exceeded concentration than what was reported for untreated cows during pregnancy. Milk estrogen was similar after the second day of lactation. Milk yield reached 2.5 kg/day from 9 to 13 days after first injection.

The investigation of Chakriyarat et al. (1978) concerned with regards to the physiological and hormonal response in induced lactation in bovine helped in the exposition of useful information. They used oestradiol-17 beta (0.1 mg/kg body weight) and progesterone (0.25 mg/kg body weight) daily by subcutaneous route for 7 days. Dexamethasone was injected (0.028 mg/kg body weight) on days 18 to 20. Milking initiated on the 21st day. All the animals showed

pre-oestrous activity within two days after the first steroid injection. After 16-20 days, these activities reappeared again in many of them. Seven induced cows averaged 73% of their previous yield of natural lactations. Concentration of prolactin increased in the week following steroid injections (8th to 15th day). There was a higher concentration of prolactin in the cows producing more than five kg/day but this was seen in other cows also during the third week.

Fulkerson (1978) also reported his work on artificial induction of lactation. He took four groups of normal heifers, and estrogen combined with progesterone was injected. Additional treatment with glucocorticoid was given to groups two to four. Milk yield was found similar in all the four groups and it was 65-70% of that in control heifers during normal lactation. The colostral type secretion gradually changed to normal milk after two weeks of milking in group two to four and after two to four weeks in group one.

In another experiment conducted by Hormess et al. (1978), estradiol benzoate was injected to a group of six heifers and six cows. To another set of animals consisting of six heifers and six cows, both oestradiol benzoate and progesterone were injected. The first group commenced milking on the 11th day and the other group on the 20th day.

All the heifers and only three cows belonging to the first group came to the lactation. In the second group, lactation was induced in five heifers and two cows only. The yield was higher in the group injected with both the drugs. The transition to normal composition of milk was slower for single than double treatment.

Skrzeczkowski et al. (1979) induced 15 cows into lactation by subcutaneous injection of oestradiol-17 beta (@ 0.1 mg/kg body weight) and progesterone (@ 0.25 mg/kg body weight) daily at 12 hour intervals for seven days. Treatment was started four to five days after estrus keeping in view the low blood progesterone concentration. Initiation of lactation began 10 days after completion of injections in all the animals. Milk yield was 73% of equivalent average yield in previous normal lactations. Protein and fat yields were 80% and 83%, respectively of corresponding previous yields.

An investigation was carried out by Meninger and Hauman (1979) on nonpregnant, nonlactating dairy cows using oestradiol-17 beta and progesterone in the same dose rate as Skrzeczkowski et al. (1979). It revealed that there were seasonal differences on the yield in induced lactation; the spring resulted in the larger yield. It was also concluded that those differences demonstrated a critical role of prolactin in the treatment to induce lactation.

In another investigation, six heifers having reproductive abnormalities (failure to conceive to numerous natural matings or breeding mummified foetus) were selected by Field et al. (1979). Other six nulliparous heifers (normal) were taken in another group. All the animals were induced into lactation by treatment with estrogen plus progesterone triethylacetate. They found that the length of the treatment period had no effect on milk yield. The secretion in the induced lactation had the appearance of colesterum for first six to seven days. It resembled normal milk in both appearance and composition except for a high fat content; in the normal induced heifers it was 5% and in the heifers with reproductive abnormalities with 5.6% against 4.1% in heifers lactating after calving.

The report of Alifakiotis et al. (1980) indicated the possibility for induction of lactation in ewes by the injections consisting of 25 mg oestradiol-17 beta and 70 mg progesterone together for seven consecutive days. Other additional treatments given were dexamethasone, prednisolone and prostaglandin F_2 alpha in different groups. Milking was initiated on the 16th day. There was colostral secretion for two days after which it resembled normal milk. The authors further observed greater lactogenic response and yield of milk by injecting corticoides in their induction

schemes with estrogen and progesterone but insulin and prostaglandin diminished their responses.

Chakravarty et al. (1981) took 5 cows and 10 heifers with established history of infertility. The animals were treated with estradiol-17 beta and progesterone at the rate of 0.1 mg and 0.25 mg per kg body weight respectively daily by subcutaneous route for 7 days. Five heifers and three cows out of the 15 animals initiated lactation. Only two heifers required additional treatment with intramuscular injection of dexamethasone on day 18, 19 and 20 of treatment and responded well.

Luktuke and Purbey (1982) reported on a preliminary study on induction of lactation in the crossbred cow. They took one 11-year old sterile crossbred cow and oestrogenic hormone was given intramuscularly at the rate of 10 mg every alternate day for 15 days. This was accompanied by application of 2% stilboestrol ointment and udder massage twice daily. Five more injections were given after a gap of one week. Within a week of initiation of treatment, udder enlargement and onset of lactation were observed. Milk yield was 6 litres/day by completion of 15 injections and rose to 8.5 litres/day after the end of the course of second injection. At 8 months, the yield was 2.5 to 3 litres/day. At three week, chemical analysis of milk revealed normal

values for the constituents except for higher fat per cent at initial stage.

The yield and composition of lacteal secretion of heifers during hormonal induction of lactation was studied by Skarda et al. (1983). They administered 56 mg of estradiol dipropionate plus 100 mg progesterone daily for seven days. Milking started on the 18th day after the first injection. There was no significant difference in milk composition between induced and non-induced secretions.

Berchtold et al. (1983) studied on the utility of inducing lactation in dry cows with mummified foetus. After abortion, the cows were induced to lactate. The authors injected oestradiol benzoate (0.05 mg/kg body weight) and progesterone (0.125 mg/kg body weight) twice daily for a week. This was followed by two injections of 100 i.u. carticotropin on the 19th and 20th days of treatment. Milking commenced on the 21st day of treatment. Daily yield in 18 out of the 20 cows was 10-24 kg within 2 months. There was no influence of hormonally induced abortion on milk yield.

Sharma and Rao (1984) treated sterile cattle with dexamethasone for three days and estrogen plus progesterone for seven days. Udder was massaged twice daily. From the 20th day of estrogen plus progesterone treatment, chloropromazine hydrochloride was injected for a period of three days.

After this, milking was commenced. The authors noted that the milk was fit for human consumption after 3-5 days of milking. This technique of artificial induction of lactation was found successful in 70-80% cows.

The proteins in milk

Barry (1961) stated that in the cow, colostron contained about 11% globulin plus albumin; but globulin came down to about 0.2% and albumin to 0.4% on an average in normal milk.

Carroll (1961) observed that in drying off secretion, mastitis secretion and colostrol whey, the relative concentrations of individual proteins were similar. But, there was a rise in all constituents when milk production dropped.

According to the report of Dellamonica et al. (1965) distributions of non-casein nitrogen in milks were 119.15, 114.6, 102.76 and 107.47 mg/100 g milk during summer, fall, winter and spring respectively.

Morr and Lin (1970) in an investigation on alcohol precipitated whey protein concentrate observed that beta-lactoglobulin, alpha-lactalbumin and immunoglobulin was in the order of decreasing electrophoretic mobility.

Khan and Saraswat (1972) have studied the composition of milk during lactation. They found that during colostral

period, percentages of total protein and casein were 5.87 and 3.24 respectively. During the first 25 days, a tendency for the decline of major constituents was noted. After that there was a general increase, though not regular, with the advancement of lactation.

Gone (1973) observed a whey protein/casein ratio in a feeding trial with silage. The author observed the ratio as 18:82 in normal and 17:83 in experimental animals.

Total nitrogen content in cows' milk was studied by Mariani (1974). From 45 farms, two samples of herd milk each were collected for analysis. The values for nitrogen in mg/100 ml milk were: Friesian herds, 549.0; Italian Brown, 554.6; Reggio, 574.0; and Modena, 586.4.

Another attempt was made by Mariani (1975) to investigate the protein distribution in milk of Friesian, Brown Swiss, Reggio and Modena cows. Four samples, two during May/June and two during September/October, were collected from 45 farms. There were 369 Friesian, 273 Brown Swiss, 156 Reggio and 118 Modena cows. There were significant differences between breeds on average contents and per cent distribution of nitrogen fractions in respect of casein, total protein, total albumin and globulin by electrophoretic analysis. Again, in respect of per cent distribution of casein fractions, there were no significant differences between breeds.

Nishikawa et al. (1976) recorded the distribution of nitrogen in the milk of both human and bovine. In cows' milk, casein nitrogen accounted for 78% of total nitrogen. The values for whey protein nitrogen and non-protein nitrogen were 16.9% and 5.1% respectively in cows' milk on 10-15 days post partum. The authors have stressed on accurate measurement of true protein content in milk and other foods with high levels of non-protein nitrogen so that true assessment of nutritive value could be made.

Thibier et al. (1976) reported that milk protein content was 2.96% (measured at monthly intervals). They concluded that contribution of milk protein to variations in progesterone was much higher than that of milk fat content and milk protein was the most important character in predicting progesterone concentration in milk.

Disc electrophoresis patterns of whey proteins in cows' milk, human milk and Vitalkt humanized milk were analysed taking six samples of each type by Kalashnikova (1977). Polyacrylamide gel was used for this analysis. The gels were subjected to densitometer examination. Cows' milk contained 0.76% whey proteins with 11 peaks. Two peaks corresponded to immunoglobulins, three to protease peptones, one to serum albumin, one to alpha lactalbumin, one to an intermediate fraction, two to beta lactoglobulins A and B, and one to glucopeptides. Human milk had whey protein content

of 0.45% and there were seven peaks visible. Out of them not a single one was corresponding to beta lactoglobulin and no intermediate fraction. There were only two proteose peptone peaks. In the humanized milk, on the other hand, whey protein content was 0.94% with nine peaks. Two of the proteose peptone peaks observed in cows' milk were absent. Humanized milk which had been heated for 10 minutes at 105°C contained only 0.37% whey proteins with marked changes in B-lactoglobulin and immunoglobulin; and there was absence of intermediate fraction also.

In the observation of Sumonic-Bijeliac (1977) on Black Pied cows, the mean value for total protein was 3.36±0.09 per cent.

The blood derived whey proteins were shown to be increased with decrease in potassium and lactose content in the milk from the mammary gland affected by latent mastitis (Grigoryan et al. 1978).

Sing and Sing (1980) observed that whey proteins were 0.7, 0.7, 0.8 and 1.1 g per 100 ml of milk in the Jamunapari, Beetal, Barbari and Black Bengal goats respectively. Breed differences in respect of total whey protein and colloidal proteins were statistically significant. There was a trend of increasing whey protein with the advancement of lactation.

Chakravarty et al. (1981) observed different levels of protein in a study on the composition of milk in a group of

crossbred cows. The average percentage values were 12.44 ± 0.75, 3.85 ± 0.07, 3.43 ± 0.06 and 4.02 ± 0.05 during 1st day, early, mid and late lactations respectively. During the corresponding periods, the values for casein were 5.59 ± 0.48, 3.05 ± 0.06, 2.71 ± 0.05 and 3.20 ± 0.05. There was also a comparative study between the milks of normal and induced lactation. The authors reported that the average percentage values of protein were 3.93 ± 0.02 and 3.76 ± 0.11 in normal and induced milk respectively. Corresponding values for casein were 2.99 ± 0.03 and 2.99 ± 0.09 in the two conditions respectively.

While narrating the distribution of proteins in bovine milk, Swaisgood (1982) mentioned that casein content was 30-35 g/litre and the whey protein was 5-7 g/litre.

In a report of Yamauchi et al. (1983) it was stated that electrophoretic and immunological properties of whey proteins from two Indian buffalo breeds were similar.

Beta-lactoglobulin

Rolleri et al. (1956) made a comparative study on this fraction of whey protein in the Holstein, Ayrshire, Brown Swiss, Guernsey and Jersey breeds. They reported that the Holstein had significantly less beta-lactoglobulin than in the other four breeds. Correlation co-efficient between beta-lactoglobulin and alpha-lactalbumin was found to be

0.327. The percentage values for beta-lactoglobulin were 0.31, 0.31, 0.35, 0.30 and 0.39 for the Ayrshire, Brown Swiss, Guernsey, Holstein and Jersey cows respectively.

Aschaffenburg and Drewry (1957) isolated beta-lactoglobulin as a distinct fraction in the whey by adjusting pH and crystallising later. They have suggested some form of quantitative relationship between beta-lactoglobulin and casein synthesis. It was concluded that for consumption of fluid milk, cow homozygous for beta-lactoglobulin would be suitable for higher whey protein content.

According to the report of Brunner et al. (1960) beta-lactoglobulin in cows' milk was 0.30 g/100 ml of milk. It was 7-12% of skim milk protein.

Beta-lactoglobulin was reported to exhibit C and D variants also. This was in addition to A and B variants reported earlier. It was observed by Wake and Baldwin (1961) using starch gel electrophoresis.

Paper electrophoresis was done with whey protein of the cows' milk as well as that of the buffalo. Samples of milk from 138 zebu cattle of different breeds and from 15 crosses between Jersey and Haryana dams were examined. Cow beta-lactoglobulin B and buffalo beta-lactoglobulin showed close similarity in their properties indicating approximately same molecular weights (Bhattacharya et al. 1963).

Jokhe et al. (1964) reported on the immunological relationships of beta-lactoglobulin with alpha-lactalbumin in various species. They observed that antiserum to cow beta-lactoglobulin had reacted more strongly with sheep and goat beta-lactoglobulin than cow beta-lactoglobulin itself. The results indicated that it would not be possible to use the antiserum to cow beta-lactoglobulin or alpha-lactalbumin to distinguish between these milks and adulteration of camel, horse, pig or some other species milk with that of goat, buffalo, sheep or cow could be readily detected.

Bellamonica et al. (1965) recorded beta-lactoglobulin in terms of mg nitrogen/100 ml milk. Their observations revealed highest concentration of this component (39.91 mg/100 ml) during winter. The values were 30.49, 38.50 and 33.04 mg/100 ml in summer, fall and spring respectively.

Studies relating to the postpartum changes in milk protein fractions by Porter and Conrad (1967) revealed that in ten days a fairly stable relationship had established between relative concentration of the four protein fractions of milk serum with beta-lactoglobulin being the majority. This fraction was recorded to be the highest (2%) on the first day of calving while on the 21st day next, the same settled down at 0.33%. The decrease in gramme percent of beta-lactoglobulin in 60 hours was approximately five-fold.

Kraeling and Gerrits (1969) studied the polymorphism of sow's whey. The beta-lactoglobulin was found with two variants A and B. They were fast moving with greater electrophoretic mobility. The sow polymorphic protein (A, B and AB) migrated faster than cow's beta-lactoglobulin when subjected to electrophoresis under similar condition in the same gel.

Marr and Lin (1970) demonstrated the properties of an alcohol-precipitated whey protein concentrate. They have also displayed the electrophoretic pattern of beta-lactoglobulin being fractionated further into two, beta-lactoglobulin A and B.

Sing and Ganguli (1975b) reported their findings on changes in whey protein fractions in mastitis, milk, colostrum and drying off secretions in Tharparkar cows. There was significant lower proportion of beta-lactoglobulin in all the abnormal milk samples compared with that in normal ones.

The investigation of Cerebulis and Farrell (1975) revealed that beta-lactoglobulin nitrogen in whey was 26.6, 33.6, 26.9, 22.0, 24.4 and 19.9% in the Holstein, Jersey, Guernsey, Ayrshire, Brown Swiss and Shorthorn breeds, respectively. In terms of protein percentage, the corresponding values were given as: 0.134 ± 0.051, 0.281 ± 0.074, 0.222 ± 0.047, 0.163 ± 0.051, 0.222 ± 0.062 and 0.768 ± 0.034 respectively.

In a quantitative estimation by immunochemical method, Hodate and Jokte (1976) studied the beta-lactoglobulin nitrogen concentration in bovine milk. The mean values were 332 ± 18.4 and 292.6 ± 16.7 mg/100 ml at 6 and 13 days post-partum respectively.

In another investigation, Imbert-Pondeven (1977) found that beta-lactoglobulin was higher in the acid whey than in the sweet whey. The conclusion was that it was possible to store whey at low temperature for up to 5 days without any type of denaturation.

Elfagam and Wheelock (1977) reported that denaturation of beta-lactoglobulin in whey was much greater than in milk. At a temperature more than 74°C there was a greater degree of denaturation of beta-lactoglobulin in heated milk than in heated whey.

The interesting feature in respect of beta-lactoglobulin was that while in cows' milk whey proteins it played a dominant role, in human milk whey protein, it was reported to be absent. Of late, the report of Liberatari et al. (1979) however, showed that beta-lactoglobulin occurred in minor quantity in human milk.

A decrease of beta-lactoglobulin in quantity was observed by Ishikawa et al. (1982) in a study conducted during a period of subclinical mastitis.

Weiss et al. (1982) detected one "x" variant electrophoretically in the milk of two Murnau-Werdenfels cows, daughter of one bull. The variant occurred between beta-lactoglobulin A and B. It was established by amino acid analysis that the new variant was not beta-lactoglobulin C.

Hambraeus (1982)gave the value for beta-lactoglobulin as 3.0 mg/ml of cows' milk.

The above value falls within the range given by Swaisgood (1982). According to the author, it was 2-4 g/litre of bovine milk.

On the third day of calving, beta-lactoglobulin was reported to attain normal level (Sing et al. 1982). In the buffalo milk, on the contrary, it was 55% of the total whey protein.

By isoelectric precipitation of casein, whey was obtained from 36 individual and composite skim milk samples of buffaloes and Friesian cows during the 4th, 5th and 6th months of lactation. The whey proteins were separated by paper electrophoresis at pH 8.6. Four distinct bands were identified, and beta-lactoglobulin was 57.2 and 52.3% of total whey protein from individual cows and buffaloes respectively (Aboul-Khier et al. 1983).

Alpha-lactalbumin

This is known to play an important role as B subunit of

the enzyme lactose synthetase complex, the other subunit A being galactosyl transferase.

Electrophoretic study of Slatter and Winkle (1952) in skim milk revealed that the mobilities of the protein in unfractionated skim milk were apparently lower than the mobilities of the corresponding isolated proteins; this was true for lactalbumin also. Best electrophoretic resolution was observed at pH 6.6.

Rolleri et al. (1956) investigated the protein constituents of milk and specific proteins present in the serum proteins isolated from various breeds. The authors found that alphalactalbumin fractions were 21.7, 21.3, 19.7, 23.3 and 22.2% of serum protein for the Ayrshire, Brown Swiss, Guernsey, Holstein and Jersey cows respectively. In the skim milk of these breeds mentioned, the average amounts of alpha-lactalbumin were 0.11, 0.11, 0.11, 0.13 and 0.15 g/100 ml as against total serum proteins in respective breeds being 0.51, 0.53, 0.59, 0.56 and 0.66 g/100 ml skim milk. The alpha-lactalbumin, though not statistically significant, had a higher average content as in the case with immune globulins. Within the breed, the authors observed, the correlation coefficient of alpha-lactalbumin and beta-lactoglobulin was found to be 0.845, and between alpha-lactalbumin and serum albumin 0.310.

The report of Blumberg and Tambs (1958) draw much attention since they could show for the first time the polymorphism of alpha-lactalbumin. They showed two variants of alpha-lactalbumin in the Zebu cattle.

There are also reports on seasonal variations of different whey protein fractions (Ghosh and Anantakrishnan, 1963). Albumin was reported to be 0.23% in the months of April-May and 0.30% in September which were the lowest and highest average values respectively in cows' milk. The same component was found lowest (0.26%) during April and highest (0.38%) during September in the buffalo milk. There was a tendency in general, to lower the values in dry and hot months from February to June. Again, in August to November, it attained a higher value, as the authors observed. Seasonal effect on albumin only in cows' milk was found to be highly significant. This was not observed in buffalo milk.

In another investigation on the inherited alpha-lactalbumin polymorphism in the Indian Zebu cattle and that in the buffalo by Bhattacharya et al. (1963), it was found to have close similarity in their properties in the same manner as in case of cow beta-lactoglobulin and the single buffalo beta-lactoglobulin. The alpha-lactalbumin A migrated approximately the same distance as buffalo alpha-lactalbumin did. That there was a combination of alpha-lactalbumin A and beta-lactoglobulin B in zebu milk was revealed in the study.

Effect of season on the whey protein nitrogen was investigated by Bellamonica et al. (1965) by Kjeldahl nitrogen method. Analysis of their data indicated that total albumin fraction did not vary with season; and concentration of all non-casein fractions (except beta-lactoglobulin) was highest during summer.

Milk serum protein fractions were studied by Porter and Conrad (1967) for the first 21 days post-partum. Their finding was that alpha-lactalbumin was highest only on the first day. The decrease in gram % of alpha-lactalbumin in 60 hours was approximately two fold. On the first day, it was 0.26%, and on the second day the percentage came down to 0.13. It was 0.12% and 0.10% on the 7th and 21st day respectively.

Elmer and Ure (1969) in their account on the biological role of alpha-lactalbumin have noted that this component had a remarkable importance. In the bovine skim milk, this fraction contributed as one of the major components. There was 70-150 mg of alpha-lactalbumin per 100 ml of skim milk.

Lactalbumin as a whole contributed to 0.44% of protein in milk (Jenness and Patton, 1969). The authors separated the casein by filteration after precipitating with acid at pH 4.6. The filtrate thus obtained was subjected to full saturation with magnesium sulphate or half saturation with

ammonium sulphate. After filtering off, the albumin was isolated from the filtrate. Estimating 81 samples, the authors have concluded that albumin + globulin nitrogen together had 13.4% of total nitrogen in milk. Out of that, albumin nitrogen was 8%.

Thompson (1970) has made a detailed review on the phenotyping of milk proteins. He has summarised a variety of methods for phenotyping whey proteins. He observed that polyacrylamide-gel electrophoresis would be far superior to paper electrophoresis. Alpha-lactalbumin was stated to have two variants, A and B of which A occurred only in Bos indicus cattle. Occurrence of B variant was found in the milk of Bos taurus.

In a brief interpretive review on the prolactin, golgi apparatus and milk secretion, Keenan et al. (1970) have elaborated that prolactin played the role to increase synthesis of both A and B proteins of lactose synthetase in organ culture of mouse mammary glands pretreated with insulin and hydrocortisone.

Sing and Ganguli (1975b) in a report expressed that alpha-lactalbumin in Tharparkar cows had significantly lower proportion in the milk samples during the stage of colostrum, drying off and mastitis.

Quantitative estimations of major whey protein

fractions were done by Hodate and Jokte (1976) using immunochemical method. At 6 and 13 days postpartum, mean concentrations of alpha-lactalbumin in bovine milk were 131.6 ± 3.9 and 121.3 ± 2.7 mg/100 ml respectively.

Albumin and globulin values together was 0.64 ± 0.04 per cent in Black Pied cows in an investigation on whey proteins and their distribution in relation to total milk protein by Sumenic-Bijeliac (1977).

By the use of polyacrylamide gel electrophoresis, Hillier et al. (1979) measured residual native whey proteins remaining after heat treatment of skim milk and cheese whey. The denaturation of alpha-lactalbumin appeared to be first order followed by beta-lactoglobulin A and B.

In the case of buffalo milk, it was observed that alpha-lactalbumin got stabilized on 6th or 7th day following parturition. This process of stabilization was rapid than in case of beta-lactoglobulin. Changes in alpha-lactalbumin component of buffalo milk at different periods of post calving were also studied. These were 2.38 ± 0.21, 26.88 ± 1.14 and 30.49 ± 1.56% of total whey protein on the 1st, 5th and 10th day respectively (Sing et al., 1982).

Alpha-lactalbumin was found to be decreased by the effect of subclinical mastitis in a study conducted by Ishikawa et al. (1982). Conversely, enhanced alpha-lactalbumin

production and simultaneous reduction on the total whey protein was observed as mastitis score decreased.

Cows' milk was reported to contain 0.9 mg of this protein per ml (Hambraeus, 1982).

Swaisgood (1982) stated that this component was 1-1.5 g/litre of bovine milk.

While undertaking a qualitative and quantitative study on the whey proteins, Aboul-Khier (1983) identified four distinct bands in paper electrophoresis. The alpha-lactalbumin was 24.1 and 23.8% of total whey protein in cows' milk and buffalo milk respectively.

Another report stated that alpha-lactalbumin was found to be most heat-resistant whey protein (deWit and Klarenbeek, 1984). The authors also described it as most important protein in the whey.

Immunoglobulin

Bovine immunoglobulins are of tremendous importance from the immunological point of view because, the calf gets the passive immunity from the dam through the colostrum.

Rowland (1938) undertook an investigation on the precipitation of proteins in milk. He observed the following levels of globulin at different values of pH.

pH of the solution	Globulin nitrogen mg/100 ml milk
4.70	45.8
5.63	34.7
6.26	32.3
6.81	31.8
7.78	33.6

His methods were claimed to be superior ones and was accepted largely later on.

More than a four decades ago, Kothavalla (1939-40) reported on the globulin nitrogen present in milk samples from Murrah buffalo. It was 3.90-8.26% of total nitrogen in the milk. On the other hand, the same in the Gir, Sindhi and British breeds of cow were 4.9, 2.9 and 5.5% respectively.

Two fractions of globulin, euglobulin and pseudoglobulin were reportedly estimated as 1.2 and 0.8% of total protein in cows' milk respectively by Mukherjee (1934). The author determined fractions of milk protein electrophoretically.

Reinart and Hechtt (1936) reported that globulin nitrogen as per cent of total nitrogen was distributed as 4.0, 3.6, 3.4, 3.7 and 3.7 of total nitrogen in the Ayrshire, Brown Swiss, Guernsey, Holstein and Jersey breeds respectively.

In the report of Rollori et al. (1956), it was noted that immunoglobulin was 0.06, 0.07, 0.08, 0.09 and 0.08% in the milk samples of the Ayrshire, Brown Swiss, Guernsey, Holstein and Jersey breeds respectively.

The study conducted by Brunner et al. (1960) revealed that the whey protein contained only 0.03 g of immunoglobulin per 100 ml of milk in the cow.

But the report of Barry (1961) stated that cows' milk contained on average about 0.2% globulin.

The immunoglobulin fraction in milk was 0.92%. It was found to increase markedly in mastitis and drying off phases of lactation. During this period, the quantity recorded was similar to that of the colostrum (Carroll, 1961). The author speculated that when milk production dropped, there was local production of globulin.

Ghosh and Anantakrishnan (1969) conducted an investigation on the composition of milk in the cow and the buffalo with special reference to season, breed and species. In cows' milk, globulin content was found to be the lowest (0.15%) during the month of October and highest (0.24%) during March. In the buffalo, the values were 0.15% in October and 0.19% in September. Globulin content lowered down in monsoon season. They could draw a conclusion that seasonal effect on globulin was highly significant in cow milk whereas significant only in buffalo milk. They noticed

a reciprocal relationship between albumin and globulin with a tendency to maintain a constant sum of the two.

According to the report of Dellamonica et al. (1965), globulin nitrogens were 10.37, 7.09, 2.90 and 7.54 mg/100 g milk in summer, fall, winter and spring.

Porter and Conrad (1967) reported that there was a twenty fold decline of immunoglobulin within 60 hours post-partum. The authors observed that on the first day of post-partum, the immunoglobulin was 3.56% and 2.06% of milk serum in two different samplings. On the second day the level came down to 0.52% and 0.31%, on the 3rd day, this was again reduced to 0.16 and 0.14% and on the 5th day it was 0.08% in a single sampling. For the study on those changes in milk serum protein, polyacrylamide-gel disc electrophoresis was carried out. The investigation was, however, confined to a period of 21 days post-partum.

A detailed review on bovine immunoglobulin was given by Butler (1969). IgG as principal immunoglobulin was found to have the highest concentration in bovine serum. IgM, the other immunoglobulin was found to be associated with parasitic infestation of Anaplasma, primary immune response, complement fixation and as agglutinating antibody of the serum. The IgG immunoglobulins were divided into two sub units, IgG_1 and IgG_2. The former subunit was selectively

transported; that was the principal immunoglobulin for the passive immunity to the calf. This was also responsible for sensitising bovine skin. IgG_2 appeared to be more homogenous than IgG_1. It was in high concentration in bovine serum. Bovine IgM was found to occur in serum, colostrum and milk; this was important in primary immune response, complement fixation and as an agglutinating antibody of the serum. IgM was also assumed to have an association with parasitic infestation of Anaplasma. Bovine IgA has been described to occur as "secretory IgA" in milk and colostrum.

Jamuna and Patton (1969) expressed the quantity of lactoglobulin as 20 mg in the form of nitrogen per 100 ml milk. This value is equivalent to 0.13% of protein in milk. This fraction could be estimated in two steps. During the first step, the milk was precipitated acidifying it to pH 4.6. It was filtered. In the next step, the filtrate was fully saturated using Magnesium sulphate and filtered. Nitrogen content in the precipitate was determined which ultimately (% Nitrogen x 6.38) indicated the quantity of lactoglobulin fraction.

Two ratios, fast IgG and slow Ig^G has been described by Smith et al. (1971). Conclusion was drawn that the mechanism for selective transport of fast IgG from blood

stream to lacteal fluid might be operative in the nonlactating gland itself.

While undertaking a study on blood plasma protein in the milk of cows with mastitis, 90 samples were subjected to starch gel electrophoresis. Comparison was made with another 100 samples from normal cows. The authors could, however, demonstrate increase in "some globulin" in the samples from mastitis milk (Garza et al. 1974).

Senft et al. (1974) studied on the variation of immunoglobulin during lactation in 63 Black Pied cows. Highest concentration of IgG (110 mg/ml milk whey) was found in colostrum particularly the first milking one. Lowest level (1.1 mg/ml whey) was by about the 20th day of lactation. There was a little increase towards the end of lactation. Again, the concentration increased with the age of the cows.

It was reported that rapid decrease in normal immunoglobulin levels within the first two days after parturition paralleled the transition from colostrum collected 0-5 hours parturition to normal milk. Only a small decline in immunoglobulin level was usually found to continue after one week following parturition. The magnitude and characters of the decline varied with the species. In cows, the total immunoglobulin level was found to drop greater than fortyfold over the first 2-3 weeks. IgG_1 in cattle comprised 60% of total

IgG but often more than 95% of the colostral IgG (Butler, 1974).

Sing and Ganguli (1975a) have investigated whey protein fractions and relative proportion of different components in it. Immune globulin was found to be 15.32 ± 1.89, 44.36 ± 4.86 and 84.11 ± 2.42 per cent of total whey protein in the milk samples during normal, clinical mastitis and colostrum period respectively. These values were higher than in normal cases.

Considering the predominance of IgG_1 in the milk of all breeds during lactation, Bhatia and Ganguli (1977) took up the work to investigate elaborately. The authors once again confirmed the existing evidence that bulk of IgG_1 was selectively transported from serum to lacteal secretion in the mammary gland. They noted that IgG_2 in blood was in higher concentration but it was not predominant in milk in comparison to IgG_1. On the other hand, IgGM had been a minimum in quantity with 0.03 to 0.06 mg/ml in milk and IgG_1 and IgG_2 together were in much higher concentration. They further noted that there was no significant difference of these immunoglobulins between different breeds either pure or crossbred. In the report, IgG_1 was described as a predominant immunoglobulin in colostrum of both zebu and crossbred cows consisting of Brown Swiss x Sahiwal. It comprised of more than 90% of colostral IgG. IgG_2 that represented

approximately 1/10th the level of IgG_1 on the first day was found decreasing in a rapid manner as it was found in the milk samples of 7th and 8th day post-partum. IgM also followed the similar pattern. This investigation also revealed the selectivity of mammary gland to transport IgG_1 from serum to colostrum. The authors concluded that there was a presence of serum derived IgG_1 in Bovidae.

There was a progressive decrease in the protein, especially immunoglobulin, in a study on the changes in the composition of lacteal secretion from 1st to 5th day post-partum milking in 6 Italian Friesian cows (Cauvin et al., 1982).

In the buffalo milk, immunoglobulin was found to be 82.29 ± 1.34, 13.69 ± 1.37 and 8.01 ± 1.36 per cent of whey protein on the 1st, 5th and 10th day respectively (Sing et al., 1982)

In cows' milk, the values for different components of immunoglobulin have been given by Hambraeus (1982). IgA, IgG and IgM were noted as 0.03, 0.6 and 0.03 mg/ml respectively.

According to Swaisgood (1982), immunoglobulin in bovine milk was 0.6-1 g/litre.

In an investigation on previous udder disease and composition of whey proteins of cows' colostrum, Balbiers et al. (1982) observed certain alterations in the picture

of different components. Acute clinical mastitis, and streptococcal or staphylococcal infections resulted in significant lowering in immunoglobulin content along with total protein. But aseptic inflammation of quarters, secretion disturbances and mild clinical involvement stimulated defence mechanisms and caused significant increase in contents of immunoglobulins and total protein compared with healthy quarters.

The subclinical mastitis in cows was the cause for increased concentration of immunoglobulin along with serum albumin in the investigation of Ishikawa et al. (1982). Streptococcus aureus infection caused a significant increase in these two components.

By adopting a technique of paper electrophoresis, Abou-Khier et al. (1983) observed that immunoglobulin was 13.5 and 17.7% of total whey protein from cows' and buffalo milk respectively.

DeWet and Klaronbeek (1984) have studied certain characteristics of whey protein in the context of physiological and biochemical importance. After coagulation of casein, the whey was observed as major nitrogen compound.

Nonprotein nitrogen

While adopting a procedure to estimate proteins in milk or any other food by way of nitrogen estimation in it, the

relevance of non-protein nitrogen (NPN) is to be considered. Again, the NPN gets much attention in the diagnosis of disturbed secretion. Because, it was found that the blood of cow had the same residual nitrogenous substance (Rowland, 1938). The author observed different values for nonprotein nitrogen and total protein by employing different chemicals like tannic acid, phosphotungstic acid, uracyl acetate and trichloroacetic acid. The attempt was to separate out the non-protein nitrogen from the protein. After a thorough investigation, the author recommended the use of 12% trichloroacetic acid at a room temperature for complete precipitation of protein. This was claimed to be a rapid method where NPN could be accurately determined.

Nitrogen distribution in the milk of native as well as exotic cattle were elaborately investigated by Kothavalla (1939-40). He found that percentage values for NPN were 4.62-11.20 of total nitrogen in Murrah buffalo; 5.2, 7.1 and 5.47 of total nitrogen in the Gir, Sindhi and British breeds respectively. The value in the goat milk was 0.5-0.6% of total nitrogen.

The range of non-protein nitrogen given by Harland et al. (1955) was between 23-42 mg/100 ml milk with a mean value of 31 mg/100 ml. It was 5.5% of total nitrogen in milk.

Non-protein nitrogen has been found to have different values for different breeds. In an investigation, distributions of the same were 5.7, 4.4, 5.5, 5.3, 5.2 and 6.0% of total nitrogen in the Holstein, Jersey, Guernsey, Ayrshire, Red Poll and Brown Swiss breeds respectively (Reinart and Nesbitt, 1956).

Ghosh and Anantakrishnan (1963) found different levels of non-protein nitrogen and influence of season, breeds and species on them. They observed a lowest monthly average value of non-protein nitrogen. Expressed as equivalent to protein, the lowest values were in the month of September, 0.14% for the cow and 0.17% for the buffalo; the highest values were 0.22% for the cow in June and 0.25% for the buffalo in the month of May. The seasonal effect in respect of non-protein nitrogen was highly significant in cows' milk but it was not so in buffalo milk.

In summer, fall, winter and spring, non-protein nitrogen were found to be 32.06, 29.61, 22.17 and 26.60 mg/100 g milk (Dellamonica et al., 1965).

In another report, it was stated that an increase in the uptake of dietary protein increased the non-protein nitrogen of cows' milk. High protein ration to Holstein cows was the reason for increased concentration of serum non-protein nitrogen. Lower level in serum in cows were

considered to be due to either inadequate protein in the ration or lactational drain (Menon et al., 1969).

Khan and Saraswat (1972) noted that there was a decrease in non-protein nitrogen content of buffalo milk as the lactation advanced beyond 57th day of lactation. This tendency was not observed in the course of advancing lactation in cows. The authors took Gir cows and Murrah buffaloes for this study.

Contents of urea and non-protein nitrogen in cows' milk have been recorded by Mariani (1974). Two samples of herd milk from each of 45 farms were analysed and average values for urea nitrogen and non-protein nitrogen (in mg/100 ml) were found to be: for 15 Friesian herds, 16.2 and 29.5 for 10 Italian Brown, 17.9 and 31.5 for 10 Reggio, 15.9 and 29.5 and for 10 Modena, 15.7 and 29.6 respectively. Another notable feature in the study was that herds fed with lucerne had a tendency for higher urea and non-protein nitrogen.

Proteose-peptone

Rowland (1938) evolved an improved procedure and separated out proteose peptone along with non-protein nitrogen. There was proteose-peptone in the non-casein nitrogen fraction. Earlier, the soluble protein other than globulin was considered usually to consist entirely of albumin. The

author determined albumin plus proteose-peptone as the difference between the soluble protein and the globulin. Then the separation of albumin from proteose peptone was possible by precipitating the true albumin and the globulin as well. In the casein-free filtrate, pH was adjusted to 4.75 and denaturation and coagulation accomplished by boiling. The other method was by heating the milk at 95°C for 10-20 minutes and coprecipitating in the denatured form along with casein at pH 4.7 thereby leaving the proteose peptone in solution. From this solution, proteose-peptone could be precipitated later by adding trichloroacetic acid to give final concentration of 8%.

According to Dellamonica et al. (1965), proteose-peptone nitrogen distributions were different in different seasons. In summer, fall, winter and spring, the values were 15.61, 14.03, 12.85 and 11.84 mg/100 g milk respectively.

Post-partum changes in proteose-peptone was investigated by Joshi et al. (1970) using turbidometric method, sialic acid estimation, sephadex gel filtration and starch gel electrophoresis. The colostrum and milk samples were taken from the breeds like Tharparkar, Red Sindhi, Crossbred cows and the Murrah buffalo. In respect of proteose-peptone level, there was a significant difference between colostrum and milk as well as their electrophoretic pattern. There

was an indication of a gradual change of protease-peptone level with the progress of lactation and starch gel electrophoresis was useful to detect this. Finally, the authors concluded that appearance of protease-peptone from blood was most probable in the case of colostrum. But in the case of milk it was likely to originate from mammary function.

Ganguli (1974) reported that concentration of protease-peptone varied between species. Cows' milk showed higher values (220 mg/100 ml), buffalo milk came next (172 mg/100 ml) and goat milk least (56 mg/100 ml). Colostrum samples had a greater concentration being 306 mg/100 ml for cow and 236 mg/100 ml for buffalo. During first three months of lactation, there was a decrease in protease-peptone followed by little elevation during 4th month till 7th month. During 8th and 9th month of lactation, protease-peptone showed a sharp increase followed by a sharp drop corresponding to the onset of dry period. Extreme climates decreased its concentration both in cow and buffalo milk. In the buffalo, it was found maximum (226 mg/100 ml) during the fourth month of lactation period. Different time of milking in a day also showed different values.

Sing and Ganguli (1975b) observed that ratio of protease to peptone decreased with mastitis while it increased in cases of colostrum and drying off secretions. They

conducted this experiment on the Tharparkar cows by paper electrophoresis and gel filtration.

In bovine milk, normally, proteose peptone content has been found to be 0.6-1.8 g/litre as per report of Swaisgood (1982).

In another report by deWit and Klarenbeek (1984), the proteose peptone has been described as a polypeptide coming from degradation product of casein. One fraction of proteose peptone, PP-3 has been identified as one of the components of milk fat globule membrane. The author has cited the names of four different components - PP8f, PP8s, PP5 and PP3. Quantitatively, they found that proteose-peptone content was 0.2 g/litre of milk.

Bovine serum albumin

Carroll (1961) induced mastitis in cows; and by application of ion-exchange cellulose, he observed a marked rise in this component of whey at 8 hour. Another observation made from this experiment was that there was a selective resorption of the serum albumin when milk production dropped. On an absolute basis, the component was found to be 0.24% in cows' milk.

In an acrylamide gel electrophoresis of milk whey, Melachouris (1968) observed that bovine serum albumin fraction was next to alpha-lactalbumin. The author noted that

mobility of beta-lactoglobulin was highest, the next was alpha-lactalbumin and then bovine serum albumin while immunoglobulin was nearer to the origin. The same pattern of electrophoretic mobility was also observed by Morr and Lin (1970) while they undertook an investigation on preparation and properties of alcohol precipitated whey protein concentrate.

Garza et al. (1974) demonstrated the increase in the serum albumin in 90 samples of mastitis milk compared with 100 samples of normal milk by the use of starch gel electrophoresis technique.

Sing and Ganguli (1975b) noted that bovine serum albumin was evident on paper electrophoregram of whey protein from clinical mastitis milk, colostrum and drying off secretions, but not from subclinical mastitis milk.

Different techniques like immunodiffusion, polyacrylamide gel electrophoresis and paper electrophoresis have consistently shown the rise of bovine serum albumin mastitis (Alison and John, 1979).

The percentage values of the serum albumin in total whey proteins were 3.21 ± 0.89, 5.27 ± 0.56 and 6.70 ± 0.62 on day one, five and ten respectively in an investigation conducted by Sing et al. (1982).

Hambraeus (1982) has stated that the serum albumin was 0.3 mg per ml of cows' milk.

Aboul-Khier et al. (1983) identified the fraction of bovine serum albumin in an investigation on whey by paper electrophoresis. They ascertained it to contribute 5.2 and 6.2% to total whey proteins of cows' milk and buffalo milk respectively.

According to the report of deWit and Klarenbeek (1984) bovine serum albumin represented the highest single polypeptide chain of all the whey proteins. This was precipitated at 40-45°C. In the blood circulatory system, it has been known as transport protein. Acidification to pH 4.0 brought about denaturation of its molecules.

Iron binding protein and other whey proteins

In the bovine whey protein fraction, Sorensen and Sorensen (1939) noted the red and green proteins for the first time.

Groves (1966) isolated Lactollin from bovine milk. This protein was found to be associated with lactoferrin and that too in bovine milk only.

Hanson et al. (1967) reported another copper-binding protein, ceruloplasmin. It was detected both in colostrum, mature milk and blood serum of cattle.

McKenzie (1967) gave the following description:

Characteristic of the protein	Name
Red coloured, iron-containing protein specific to milk	Lactoferrin
Iron-free, from lactoferrin	Apo-lactoferrin
Red coloured, iron-containing protein of serum	Serum transferrin
Serum transferrin when isolated from milk	Serum transferrin (milk)
To indicate species from which red protein is isolated, a prefix is used	Bovine lactoferrin

The author also reported that lactollin was not likely to be synthesised in the udder and most likely to be related to immune protein.

Jenness (1974) reported the values for lactoferrin and lactollin as > 0.018 and 0.002 g per litre of milk respectively.

Variations in the lactoferrin level during the lactation period of Black pied cows were observed by Senft et al. (1974). They took 63 cows and found that this protein had highest concentration (1275 µg/ml whey) in colostrum particularly in the 1st milking. Lowest level (98 µg/ml whey)

was observed on around 20th day of lactation. IgG showed little increase towards the end of lactation. Lactoferrin, contrary to IgG, rose to 900 µg/ml. There was a tendency for increase with advancing age of the cows. However, no correlation was found between lactoferrin and IgG.

By electroimmuno diffusion of whey proteins from 80 quarters of 20 normally lactating Holstein-Friesian and Jersey cows, mean lactoferrin concentration was found to be 0.38 mg/ml. In mastitis milk, the increase of lactoferrin was marked and it was significantly related to cell count (Harmon et al., 1975).

Several valuable informations are available on lactoferrin in the report of Welty et al. (1976). They investigated the concentration of lactoferrin during involution of bovine mammary gland. In this work, electroimmuno diffusion assay was used to quantitate the changes in its concentration in mammary secretions. After 2-4 days of cessation of regular milking, lactoferrin concentration began to increase. It went on increasing in a linear fashion, the rate being 1.5 mg/ml/day. After 2-4 weeks of involution, it was 20 mg/ml which was a maximum concentration. The level was 100-fold more than that in normal milk. Marked decrease of this concentration was noticed prior to parturition and onset of lactation.

A study was undertaken by Gaunt et al. (1980) on the variation of lactoferrin concentration in different situations of Holstein cows. There were 830 cows and the observation was made for a period of one year. Concentration of lactoferrin in colostrum was 10-15 times more than that of normal milk (1-5 mg/ml). In dry secretions, its increase was 100 times that of normal milk (20-30 mg). There was a linear increase starting from 2-4 days of cessation of regular milking. On the other hand, mastitis increased the lactoferrin concentration. It increased with the increase in the amount of infection, advancing stage of lactation, and the growing age of the cow. For the higher heritability (0.44 ± 0.33) for lactoferrin that can fight infection, it was suggested to consider this protein as a selection criterion from genetic point of view.

There was 0.012 mg lactoferrin/ml of cows' milk as against 1.7 mg/ml of human milk. The Lysozyme is another whey protein (an enzyme) known for its distinct physiological role. It destroys the bacterial cell wall. It was found to be 0.0001 mg/ml in cow milk (Hambraeus, 1982).

Materials and Methods

MATERIALS AND METHODS

Crossbred cows from the University Livestock Farm, Mannuthy were used for the experiments. The experimental group consisted of 3 heifers between 3½-4½ years of age which were listed for culling due to infertility. The control animals of first lactation were 3 cows of approximately same age group selected randomly.

All the animals were maintained under similar farm conditions.

The heifers were observed both in the morning and the evening daily for the symptoms of oestrus.

I. Induction of lactation

The experimental animals were induced to lactate by injection of steroid hormones in the following doses recommended by Smith and Schanbacher (1973):

Estrogen (Vetoestrol, M&B) at the rate of 0.1 mg/kg body weight and progesterone (Lutocyclin, Hindustan Ciba-Geigy) at the rate of 0.25 mg/kg body weight daily.

The necessary doses of the two hormones were mixed together and given as a single intramuscular injection in the gluteal muscle alternately on the right and left sides at 10 AM, every day for 7 days consecutively.

Massaging of the udder was done daily once for 5-10 minutes to each animal during the course of treatment.

In the experimental group, hand milking was done twice daily starting from the 21st day of the first injection and collection of samples was started from the 16th day of first milking. The control group was in the 3rd month of lactation at the time of sample collection. Afternoon milk on every 7th day in the case of both the groups were collected for analysis and 6 samples were thus collected from each animal. All the chemical analysis were conducted from each fresh sample.

II. Chemical analysis of milk

The samples were processed immediately for the estimation of-

a) Albumin
b) Globulin
c) Total whey nitrogen
d) Non-protein nitrogen
e) Total whey protein
f) Other whey protein (Total whey protein minus albumin and globulin)
g) Total protein
h) Casein
i) Crude protein

a) Albumin

The method described by ISI (1981) was followed for the estimation. About 5 ml of milk sample was weighed.

It was mixed with 50 ml of water at 40°C. Then, 0.5 ml of acetic acid (10% V/V) was added and mixed well. After 10 minutes, 0.5 ml sodium acetate solution (1N) was added and mixed again. It was allowed to cool down to about 20°C, then filtered using Whatman filter paper No.40. The filtrate was exactly neutralised with sodium hydroxide (1% W/V). Again 0.3 ml of dilute acetic acid (1:9 by volume) was mixed with the filtrate and heated in a steam bath till the albumin was completely precipitated. Lastly, filtration was done using Whatman filter paper No.40 and the precipitate was collected.

The precipitate was transferred to a 300 ml Kjeldahl digestion flask, 25 ml of concentrated sulphuric acid and 0.2 g copper sulphate were added to it. After thorough mixing, it was put for digestion. In the beginning it was put in a low heat till the frothing subsided. Afterwards, it was cooled, added about 2 g of anhydrous sodium sulphate, washed the neck of the flask pouring some distilled water and put for digestion again till the solution became clear and greenish blue in colour. After cooling, the material was transferred to a 250 ml volumetric flask and the volume made up with distilled water. From this, 10 ml was transferred into the Micro-Kjeldahl distillation apparatus (Oser, 1965) followed by addition of 10 ml sodium hydroxide solution (50% W/V) and finally some amount of distilled water.

This was subjected to steam distillation. Resulted ammonia was collected in a conical flask by allowing to pass into standard sulphuric acid (0.1 N) where 2 drops of methyl red was added as indicator. This was titrated against 0.1 N standard sodium hydroxide solution and titre value recorded. This distillation was repeated thrice for each sample. The average value was taken for calculation.

Calculation-

Weight of milk taken	= W g
Volume of digest	= V ml
Amount of digest taken for distillation	= V_1 ml
Volume of standard acid (0.1 N) taken	= V_2 ml
Titre value	= V_3 ml
Volume of 0.1 N acid neutralized by ammonia	= V_2-V_3

$$\% \text{ Nitrogen} = \frac{V_2-V_3 \times 1.4 \times V \times 100}{W \times V_1 \times 1000}$$

% Protein = (% Nitrogen) x 6.38

b) Globulin

Ten ml of milk was weighed. Casein was precipitated out adjusting the pH to 4.6 by using Hydrochloric acid (1 N). It was filtered through Whatman filter paper No.40. The filtrate was neutralised by sodium hydroxide solution

(0.1 N) and then it was fully saturated with magnesium sulphate (Jenness and Patton, 1969). The precipitate was collected by filtering through Whatman filter paper No.40. This was subjected to similar treatments for the determination of nitrogen and the calculation for protein value as under (a) above.

c) Total whey nitrogen

Ten ml of milk was weighed, casein was precipitated out and it was filtered as under (b) mentioned earlier. The filtrate was treated as under (a) above for getting total whey nitrogen and corresponding value for protein.

d) Non-protein nitrogen

This was estimated as per procedure described by ISI (1981). Ten ml of milk was weighed and 40 ml of trichloroacetic acid (15%) was added to it. After settling down the precipitate, this was filtered using Whatman filter paper No.40. The filtrate was digested, distillated, titrated and calculated as under (a) mentioned earlier for getting the nitrogen value and also its equivalent value for protein.

e) Total whey protein

This was calculated by subtracting the value of non-protein nitrogen as under (d) above from that of total whey nitrogen as under (c) mentioned earlier in a milk sample.

Corresponding value for protein was obtained by calculating as under (a) mentioned earlier.

f) Other whey proteins

This was comprising of proteose peptone, iron binding proteins, ceruloplasmin, lysozyme, etc. The value as a whole was calculated by subtracting the value for albumin + globulin from that of the total whey protein.

g) Total protein

The precipitate of (d) mentioned earlier was subjected for digestion, distillation, titration and calculation as described under (a) earlier to get the value for total protein.

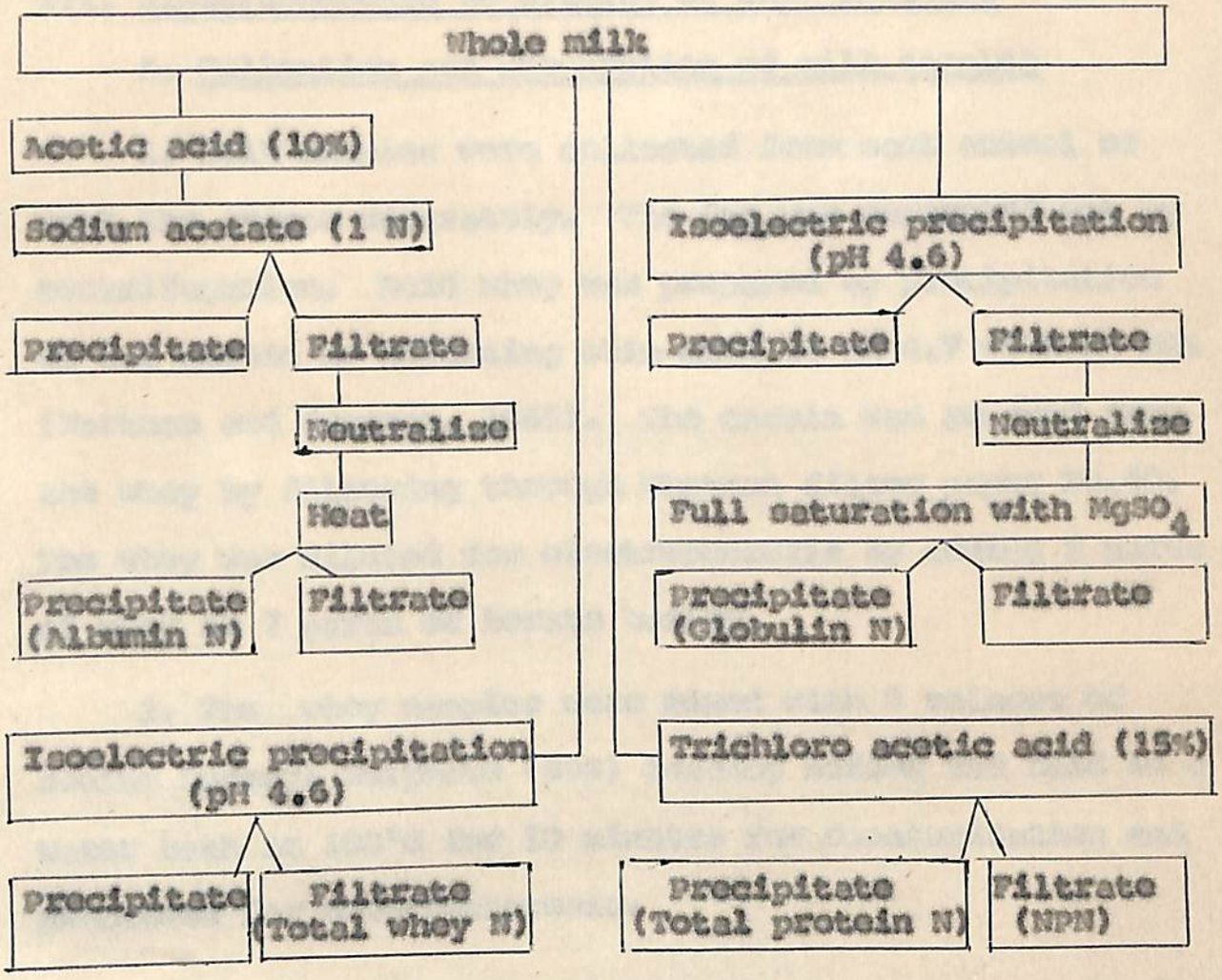
h) Casein

The value for total whey protein was subtracted from that of the total protein to get the value for casein.

i) Crude protein

The nitrogen value for total protein and non-protein nitrogen together gave the nitrogen value for crude protein from which equivalent value for protein was calculated as under (a) above.

Fig.1. Flow-sheet diagram for the chemical fractionation of milk proteins:



Total whey protein N = (Total whey N)-(NPN)

Other whey protein N = (Total whey protein N)-(Albumin N + Globulin N)

Casein N = (Total protein N)-(Total whey protein N)

Crude protein N = (Total protein N) + (NPN)

Protein % = N% x 6.38 N = Nitrogen

NPN = Non-protein nitrogen

III. Electrophoretic separation of whey proteins

A. Collection and preparation of milk samples

1. Milk samples were collected from each animal of both the groups separately. The fat was separated out by centrifugation. Acid whey was prepared by precipitation of the casein by adjusting skim milk to pH 4.7 with 1M HCl (Hartman and Swanson, 1965). The casein was removed from the whey by filtering through Whatman filter paper No.40. The whey was diluted for electrophoresis by adding 3 parts of whey to 7 parts of borate buffer.

2. The whey samples were mixed with 9 volumes of sodium Dodecyl Sulphate (SDS) loading buffer and held in a water bath at 100°C for 10 minutes for denaturisation and proceeded for electrophoresis.

SDS Loading buffer (Garvey et al., 1977)

2% Sodium dodecyl sulphate (SDS)	..	1 g
2% Marcaptoethanol	..	1 ml
0.1 M Borate buffer	..	49.0 ml
0.1% Pyronin Y	..	50 mg
Glycerine	..	10 ml

The treated samples were cooled prior to loading the gel tubes.

B. Electrophoresis

A disc gel electrophoresis apparatus as described by Darling and Dickson (1979) with a capacity to hold 12 sample tubes vertically were used. The glass tubes for accommodating gels used were 7.0 cm long with 0.5 cm diameter (internal). The gel tubes were prepared as follows:

Tubes were immersed in chromic acid for overnight and cleaned with detergent. They were then washed with tap water, finally rinsed with distilled water and allowed to dry in hot air oven .

A modified Neurath's (Neurath and Hill, 1975) 10% acrylamide gel system in Borate buffer (Borax 38.1 g/l plus Boric acid to adjust the pH) of pH 8.3 was prepared as under.

a) The following were mixed in a 50 cc beaker.

0.2 M Borate buffer (pH 8.3) containing 1 g SDS per litre	..	22.0 ml
TEMED (Tetramethyl ethyl diamine)	..	0.04 ml
Ammonium persulphate	..	20.0 mg
Riboflavin (0.5 mg/ml)	..	1 ml

After the air bubbles (due to addition of ammonium persulphate) have completely subsided, stock acrylamide solution was carefully added to the contents and thoroughly mixed by swirling.

Stock acrylamide solution:

Acrylamide	..	44.4 g	37:1
N,N-Methylenebisacrylamide		1.2 g	
Water upto	..	100 ml	

A quantity of 6.8 ml from this stock acrylamide solution was added to the Borate buffer prepared earlier.

b) After closing the bottom and placing vertically the tubes were filled up to 2 cm below the top by using a clean pasteure pipette.

c) A vew drops of isobutyl alcohol was added to each tube so as to obtain an evenly flat surface of the gel.

d) The tubes were then exposed to diffuse natural light by placing them near a lighted window. In about 15-20 minutes, cross linking (polymerisation) was found to be over. Such tube could be easily recognised by a sharply demarkated flat minuses below the isobutyle alcohol layer and slightly more opelescence of the gel as compared to the alcohol layers.

e) The alcohol on the top was then discarded by inverting and shaking the tubes. The top samples was rinsed with running buffer and inserted into gromets of the apparatus.

f) Running buffer (0.1 M Borate buffer pH 8.3) was added to top and bottom compartment taking care not to trap any air bubble at the ends of the tubes.

g) The apparatus was connected to electrophoresis power supply and run for 15 minutes to allow excess ammonium persulphate to migrate out.

h) The tubes were loaded with 0.1 ml of samples treated as outlined under 2. Glycerine was mixed with the prepared sample before putting into the tubes to increase the density of the sample so that it may settle over the gel while charging.

i) The sample thus loaded were subjected to electrophoresis for 3 hours at a constant power supply of 5 mA/tube at 150 V using a separate power pack to get DC.

j) At the end of electrophoresis, the gels were removed from the tubes by water pressure and put in Coomassie Blue stain for several hours.

Coomassie Blue stain-

Coomassie Blue	..	1.25 g
Methanol	..	227 ml
Glacial acetic acid	..	46 ml
Water	..	500 ml

k) Destaining of the background in the gels were done by putting them in a 10% acetic acid solution.

l) The stained gels were preserved in 5% acetic acid solution.

Results

RESULTS

I. Induction of lactation

In response to the hormonal treatment, there were gradual development of the mammary glands in all the three heifers. The enlargement was appreciable from external appearance towards the end of the course of treatment. After the termination of the treatment till the initiation of milking, the udders were engorged and turgid. Colostrum-like mammary secretion was obtained on the 18th day after first injection. The colostral secretion resembled normal milk within 5 days of regular milking. The yield, in a day, varied from 200 ml (in the heifer No.032) to a maximum of 4,500 ml (in the heifer No.670) till the end of the experiment.

II. Chemical analysis of milk

a) Albumin

The average percentage value recorded for albumin in the milk of cows in normal lactation was 0.383 ± 0.0097. The same in the cows induced to lactate was found to be 0.603 ± 0.066 (Table 2). On statistical analysis, the difference between the two groups in respect of albumin was not significant. However, differences between individuals within the group was found to be highly significant

($P < 0.01$, Table 3, Fig.2). Out of total nitrogen in milk, albumin nitrogen accounted for 12.09 per cent in normal and 15.04 per cent in induced milk (Table 20); out of total protein, albumin accounted for 12.9 and 15.9 per cent in normal and induced lactation respectively (Table 22, Fig.2).

b) Globulin

The average percentage of globulin in milk in the control group was estimated to be 0.178 ± 0.010, but the value was 0.284 ± 0.051 in the experimental group (Table 4). The difference in globulin content between the two groups as well as between individuals was not significant on statistical analysis (Table 5).

Out of total nitrogen in milk, globulin nitrogen, on an average, accounted for 5.64 per cent in normal and 7.09 per cent in the induced milk (Table 20); corresponding values for globulin were 6.02 per cent and 7.50 per cent out of total protein in normal and induced milk respectively (Table 22).

c) Total whey nitrogen (equivalent to protein)

The total whey nitrogen expressed as equivalent to protein was 0.841 ± 0.010 per cent on an average in the milk of the control group. In the experimental group, the value was 1.263 ± 0.078 per cent (Table 6). Between groups, the difference was significant ($P < 0.05$) whereas individual

differences were highly significant ($P < 0.01$) on statistical analysis (Table 7, Fig.2). Total whey nitrogen as the fraction of total nitrogen in milk was 26.53 per cent in normal and 31.52 per cent in induced milk on an average (Table 20).

d) Non-protein nitrogen (NPN)

The NPN was 31.68 mg/100 g milk on average in the control group. The value was 34.11 mg/100 g milk in the experimental group (Table 8). As equivalent to protein, the NPN was 0.202 ± 0.009 per cent and 0.2175 ± 0.007 per cent in the said groups respectively (Table 8). In respect of this component, neither group difference nor individual difference was significant on statistical analysis (Table 9). The average NPN value was 6.38 per cent in normal and 5.43 per cent in induced milk out of total nitrogen in milk (Table 20).

e) Total whey protein

This was true whey protein obtained after eliminating the non-protein nitrogen from the total whey nitrogen before converting into protein value. Average percentage values of total whey protein in the control and the experimental groups were 0.640 ± 0.014 and 1.045 ± 0.079 per cent respectively (Table 10). On statistical analysis, it was revealed that the difference between the two groups was

significant ($P < 0.05$) whereas the difference was highly significant ($P < 0.01$) between the individuals in a group (Table 11, Fig.2). Out of the total nitrogen in milk, the total whey protein nitrogen, on an average, accounted for 20.368 and 26.093 per cent in the normal and the induced lactation respectively (Table 20); correspondingly, total whey proteins were 22.22 and 27.59 per cent of total protein in milk in normal and induced lactations (Table 22).

f) Other whey proteins (comprising of proteose-peptone, iron binding proteins, ceruloplasmin, lysozyme, etc.)

These proteins altogether were found to be very less in quantity in both the groups. Percentage values, on an average, in the control and the experimental group were 0.10 ± 0.0178 and 0.158 ± 0.0184 respectively (Table 12). On statistical analysis, the differences were not found significant (Table 13).

Other whey proteins constituted only 3.15 and 3.95 per cent each out of total nitrogen in normal and induced milk respectively (Table 20). Out of total protein in milk, the other whey protein accounted for 3.30 and 4.18 per cent in normal and induced milk respectively (Table 22).

g) Total protein in milk

In the control group, the average percentage of total protein was 2.97 ± 0.048. The same in the experimental

group was found to be 3.79 ± 0.155 (Table 14). Between the two groups, the values were having significant difference (P 0.05) statistically; differences between individuals within a group were highly significant (P < 0.01, Table 15, Fig.2).

The total protein nitrogen accounted for 93.624 and 94.567 per cent of the total nitrogen content of milk (Table 20).

h) Casein

The average percentage values of casein in the control and the experimental group were 2.330 ± 0.053 and 2.773 ± 0.126 respectively (Table 16). The difference between the two groups was not significant on statistical analysis; only individual differences were found to be significant (P 0.05, Table 17, Fig. 2).

Casein nitrogen contributed 73.47 and 68.21 per cent to the total nitrogen content of milk (Table 20). The whey protein:casein ratio was found to be 22:78 in the normal milk and 27:73 in the induced milk. These ratio would be 1:3.64 and 1:2.65 respectively.

i) Crude protein

Average values of crude protein for the control and the experimental groups were 3.17 ± 0.052 and 4.007 ± 0.159 per cent respectively (Table 18). Statistical analysis

showed that both group difference and individual differences were highly significant (Table 19).

III. Electrophoretic separation of whey proteins

Fractions of different whey proteins were observed in the form of bands in the polyacrylamide gel electrophoresis (PAGE) which has been displayed in the Plate No.I to VI.

Separation of the whey samples from the control group has been shown in plate IV to VI. With varying prominence in the electrophorograms, 6 bands were observed in each case (Plate IV-V). Each plate represents individual animal. In the plate VI, however, variations in respect of the quantity of sample and time for the run during electrophoresis have been shown. Mode of separation was different depending upon the quantity as well as time. Gel 1 and 2 (Plate VI) were the results of comparatively lesser time with lesser quantity of sample. For the gel 2 in the same plate, time was same as the previous two but quantity of sample was comparatively higher. For the gel 4, longer time was allowed with a quantity same as in the case of gel 1 and 3.

In case of induced lactation, there were 6 bands for each sample with varying prominence. This has been displayed in plate No.I to III. The electrophoretic mobility for a

particular sample was same but the mode of fractionation was different from individual to individual.

Tables

Tables

Table 1. Particulars of the experimental heifers

Sl. No.	Tattoo No.	Date of birth	Last date of A.I.	Body wt. (kg)	Date of oestrus	Date of first injection
1	681	9-12-81	10-5-85	341	11-7-85	16-7-85
2	032	6-4-81	19-6-85	303	10-7-85	16-7-85
3	670	1-9-81	26-6-85	256	7-7-85	16-7-85

Table 2. Quantity of albumin in milk from normal and induced lactation

Normal			Induced		
Animal No.	Replications	Albumin %	Animal No.	Replications	Albumin %
280	1	0.374	681	1	0.404
	2	0.383		2	0.386
	3	0.437		3	0.670
	4	0.372		4	0.465
	5	0.369		5	0.453
	6	0.369		6	0.60
	Mean	0.3841		Mean	0.496
040	1	0.383	670	1	0.522
	2	0.357		2	0.453
	3	0.369		3	0.516
	4	0.372		4	0.445
	5	0.442		5	0.445
	6	0.406		6	0.386
	Mean	0.3881		Mean	0.461
267	1	0.404	032	1	0.295
	2	0.479		2	0.893
	3	0.326		3	1.116
	4	0.372		4	1.123
	5	0.296		5	1.212
	6	0.388		6	0.461
	Mean	0.3774		Mean	0.850
Overall mean		0.383±0.0097	Overall mean		0.603±0.066

Table 3. Analysis of variance of albumin in milk

Source	DF	SS	MSS	F
Between treatments	1	0.4335	0.4335	3.123
Between animals within treatment	4	0.5529	0.1383	4.9374**
Error	30	0.8435	0.0281	
Total	35	1.8299		

** Highly significant $P < 0.01$

Table 4. Quantity of globulin in milk from normal and induced lactation

Normal			Induced		
Animal No.	Replications	Globulin %	Animal No.	Replications	Globulin %
280	1	0.188	681	1	0.3942
	2	0.185		2	0.334
	3	0.182		3	0.55
	4	0.149		4	0.224
	5	0.22		5	0.376
	6	0.184		6	0.11
	Mean	0.1847		Mean	0.3314
040	1	0.217	670	1	0.295
	2	0.22		2	0.223
	3	0.277		3	0.144
	4	0.112		4	0.226
	5	0.193		5	0.114
	6	0.223		6	0.112
	Mean	0.2071		Mean	0.186
267	1	0.184	032	1	0.998
	2	0.182		2	0.225
	3	0.145		3	0.341
	4	0.165		4	0.217
	5	0.121		5	0.116
	6	0.121		6	0.115
	Mean	0.1441		Mean	0.335
Overall mean		0.1786±0.01	Overall mean		0.2841±0.051

Table 5. Analysis of variance of globulin in milk

Source	DF	SS	MSS	F
Between treatments	1	0.1002	0.1002	4.0182
Between animals within treatment	4	0.0997	0.0249	1.0329
Error	30	0.7242	0.0241	
Total	35	0.9241		

Table 6. Quantity of total whey nitrogen as equivalent to protein in milk from normal and induced lactation

Normal			Induced		
Animal No.	Replications	Protein %	Animal No.	Replications	Protein %
280	1	0.838	681	1	1.172
	2	0.818		2	1.110
	3	0.823		3	1.544
	4	0.900		4	1.032
	5	0.779		5	1.108
	6	0.836		6	1.108
	Mean	0.833		Mean	1.179
040	1	0.818	670	1	1.116
	2	0.787		2	1.163
	3	0.822		3	1.152
	4	0.939		4	1.145
	5	0.822		5	0.867
	6	0.82		6	0.783
	Mean	0.834		Mean	1.038
267	1	0.861	032	1	1.773
	2	0.845		2	1.563
	3	0.845		3	1.700
	4	0.818		4	1.772
	5	0.938		5	1.676
	6	0.820		6	0.867
	Mean	0.855		Mean	1.572
Overall mean		0.841±0.0103	Overall mean		1.263±0.078

Table 7. Analysis of variance of total whey nitrogen as equivalent to protein in milk

Source	DF	SS	MSS	F
Between treatments	1	1.6065	1.6065	6.9736*
Between animals within treatment	4	0.9215	0.2304	7.1038**
Error	30	0.9729	0.0324	
Total	35	3.5009		

* Significant $P < 0.05$

** Highly significant $P < 0.01$

Table 8. Quantity of non-protein nitrogen as equivalent to protein in milk from normal and induced lactation

Normal				Induced			
Animal No.	Replications	mg nitrogen/ 100 g milk	Equivalent to protein (%)	Animal No.	Replications	mg nitrogen/ 100 g milk	Equivalent to protein (%)
280	1	30.1	0.192	681	1	34.4	0.219
	2	23.7	0.151		2	35.2	0.224
	3	39.8	0.254		3	35.1	0.224
	4	35.0	0.223		4	34.6	0.22
	5	34.6	0.22		5	20.8	0.133
	6	28.8	0.184		6	36.1	0.233
	Mean	32.0	0.2041		Mean	32.7	0.209
040	1	30.2	0.192	670	1	36.8	0.235
	2	36.1	0.23		2	29.6	0.188
	3	35.0	0.223		3	39.1	0.245
	4	23.1	0.147		4	40.9	0.261
	5	23.3	0.149		5	34.5	0.22
	6	28.8	0.184		6	35.4	0.226
	Mean	29.416	0.1876		Mean	36.05	0.23
267	1	35	0.223	032	1	34.6	0.22
	2	32.1	0.205		2	24.3	0.155
	3	46.6	0.297		3	35.9	0.229
	4	23.3	0.149		4	34.9	0.223
	5	34.6	0.22		5	35.2	0.224
	6	30.3	0.193		6	36.6	0.233
	Mean	33.65	0.2146		Mean	33.58	0.214
Overall mean		31.68	0.202±0.0093	Overall mean		34.11	0.2175± 0.0072

Table 9. Analysis of variance of non-protein nitrogen as equivalent to protein in milk

Source	DF	SS	MSS	F
Between treatments	1	0.00219	0.00219	2.4
Between animals within treatment	4	0.00365	0.00091	0.6974
Error	30	0.03920	0.00131	
Total	35	0.0904		

Table 10. Quantity of total whey protein from normal and induced lactation

Normal			Induced		
Animal No.	Replications	Protein %	Animal No.	Replications	Protein %
280	1	0.646	681	1	0.953
	2	0.666		2	0.885
	3	0.57		3	1.32
	4	0.677		4	0.818
	5	0.558		5	0.975
	6	0.658		6	0.878
	Mean	0.63		Mean	0.97
040	1	0.625	670	1	0.882
	2	0.557		2	0.974
	3	0.6		3	0.903
	4	0.792		4	0.884
	5	0.673		5	0.647
	6	0.636		6	0.557
	Mean	0.647		Mean	0.808
267	1	0.638	032	1	1.553
	2	0.64		2	1.408
	3	0.55		3	1.551
	4	0.669		4	1.55
	5	0.718		5	1.451
	6	0.627		6	0.634
	Mean	0.64		Mean	1.358
Overall mean		0.64±0.0142	Overall mean		1.045±0.079

Table 11. Analysis of variance of total whey protein

Source	DF	SS	MSS	F
Between treatments	1	1.4884	1.4886	6.21*
Between animals within treatment	4	0.9588	0.2397	7.1251**
Error	30	1.0093	0.0336	

* Significant $P < 0.05$

** Highly significant $P < 0.01$

Table 12. Quantity of other whey proteins in milk from normal and induced lactation

Normal			Induced		
Animal No.	Replications	Protein %	Animal No.	Replications	Protein %
280	1	0.084	681	1	0.155
	2	0.1		2	0.165
	3	0.065		3	0.099
	4	0.16		4	0.121
	5	0.032		5	0.146
	6	0.104		6	0.166
	Mean	0.091		Mean	0.142
040	1	0.025	670	1	0.064
	2	0.046		2	0.298
	3	0.035		3	0.242
	4	0.308		4	0.214
	5	0.04		5	0.088
	6	0.13		6	0.058
	Mean	0.097		Mean	0.161
267	1	0.05	032	1	0.26
	2	0.033		2	0.29
	3	0.077		3	0.094
	4	0.132		4	0.208
	5	0.24		5	0.124
	6	0.12		6	0.058
	Mean	0.109		Mean	0.172
Overall mean		0.1±0.0178	Overall mean		0.158±0.0184

Table 13. Analysis of variance of other whey proteins

Source	DF	SS	MSS	F
Between treatments	1	0.00107	0.00107	0.0554
Between animals within treatment	4	0.0772	0.0193	0.664
Error	30	0.8719	0.02906	
Total	35	0.9502		

Table 14. Quantity of total protein in milk from normal and induced lactation

Normal			Induced		
Animal No.	Replications	Protein %	Animal No.	Replications	Protein %
280	1	3.15	681	1	4.501
	2	2.663		2	4.06
	3	2.517		3	4.371
	4	2.939		4	4.237
	5	2.871		5	3.618
	6	2.874		6	3.329
	Mean	2.836		Mean	4.019
040	1	3.08	670	1	3.218
	2	3.35		2	3.224
	3	3.014		3	3.118
	4	2.769		4	3.206
	5	3.014		5	3.2
	6	2.849		6	3.189
	Mean	3.013		Mean	3.193
267	1	2.94	032	1	3.905
	2	3.218		2	4.883
	3	3.2		3	3.329
	4	2.865		4	4.662
	5	3.119		5	4.759
	6	2.984		6	3.185
	Mean	3.054		Mean	4.156
Overall mean		2.97±0.0484	Overal mean		3.79±0.155

Table 15. Analysis of variance of total protein in milk

Source	DF	SS	MSS	F
Between treatments	1	6.0642	6.0642	7.1088*
Between animals within treatment	4	3.4122	0.853	5.4659**
Error	30	4.6821	0.1561	
Total	35	14.1585		

* Significant $P < 0.05$

** Highly significant $P < 0.01$

Table 16. Quantity of casein from normal and induced lactation

Normal			Induced		
Animal No.	Replications	Casein %	Animal No.	Replications	Casein %
280	1	2.503	681	1	3.548
	2	1.996		2	3.171
	3	1.950		3	3.051
	4	2.263		4	3.426
	5	2.313		5	2.642
	6	2.216		6	2.451
	Mean	2.206		Mean	3.048
040	1	2.454	670	1	2.145
	2	2.793		2	2.250
	3	2.416		3	2.216
	4	1.980		4	2.322
	5	2.341		5	2.552
	6	2.213		6	2.632
	Mean	2.366		Mean	2.353
267	1	2.302	032	1	2.352
	2	2.577		2	3.475
	3	2.652		3	1.777
	4	2.196		4	3.319
	5	2.401		5	3.307
	6	2.357		6	2.555
	Mean	2.414		Mean	2.798
Overall mean		2.33±0.053	Overall mean		2.773±0.126

Table 17. Analysis of variance of casein

Source	DF	SS	MSS	F
Between treatments	1	1.4680	1.4679	3.5092
Between animals within treatment	4	1.6313	0.4078	2.9597*
Error	30	4.1339	0.1378	
Total	35	7.2332		

* significant $P < 0.05$

Table 18. Quantity of crude protein in milk from normal and induced lactation

Normal			Induced		
Animal No.	Replications	Protein %	Animal No.	Replications	Protein %
280	1	3.42	681	1	4.72
	2	2.814		2	4.282
	3	2.771		3	4.955
	4	3.163		4	4.458
	5	3.092		5	3.750
	6	3.057		6	3.560
	Mean	3.040		Mean	4.227
040	1	3.272	670	1	3.453
	2	3.580		2	3.413
	3	3.238		3	3.368
	4	2.916		4	3.467
	5	3.163		5	3.420
	6	3.033		6	3.415
	Mean	3.200		Mean	3.423
267	1	3.163	032	1	4.126
	2	3.423		2	5.038
	3	3.497		3	3.558
	4	3.014		4	5.09
	5	3.340		5	4.983
	6	3.178		6	3.423
	Mean	3.269		Mean	4.37
Overall mean		3.170±0.052	Overall mean		4.007±0.159

Table 19. Analysis of variance of crude protein in milk

Source	DF	SS	MSS	F
Between treatments	1	6.3037	6.3037	7.648**
Between animals within treatment	4	3.2968	0.824	5.227**
Error	30	4.7304	0.1577	
Total	35	14.3309		

** Highly significant $P < 0.01$

Table 20. Distribution of nitrogen in milk (as percentage of total nitrogen)

Component	% of total nitrogen	
	Normal	Induced
Albumin nitrogen	12.090	15.044
Globulin nitrogen	5.640	7.094
Total whey nitrogen	26.526	31.524
Non-protein nitrogen	6.376	5.432
Total whey protein nitrogen	20.368	26.093
Other whey protein nitrogen	3.150	3.954
Total protein nitrogen	93.624	94.567
Casein nitrogen	73.470	68.210

Table 21. Distribution of nitrogen in whey proteins (as percentage of total whey protein nitrogen)

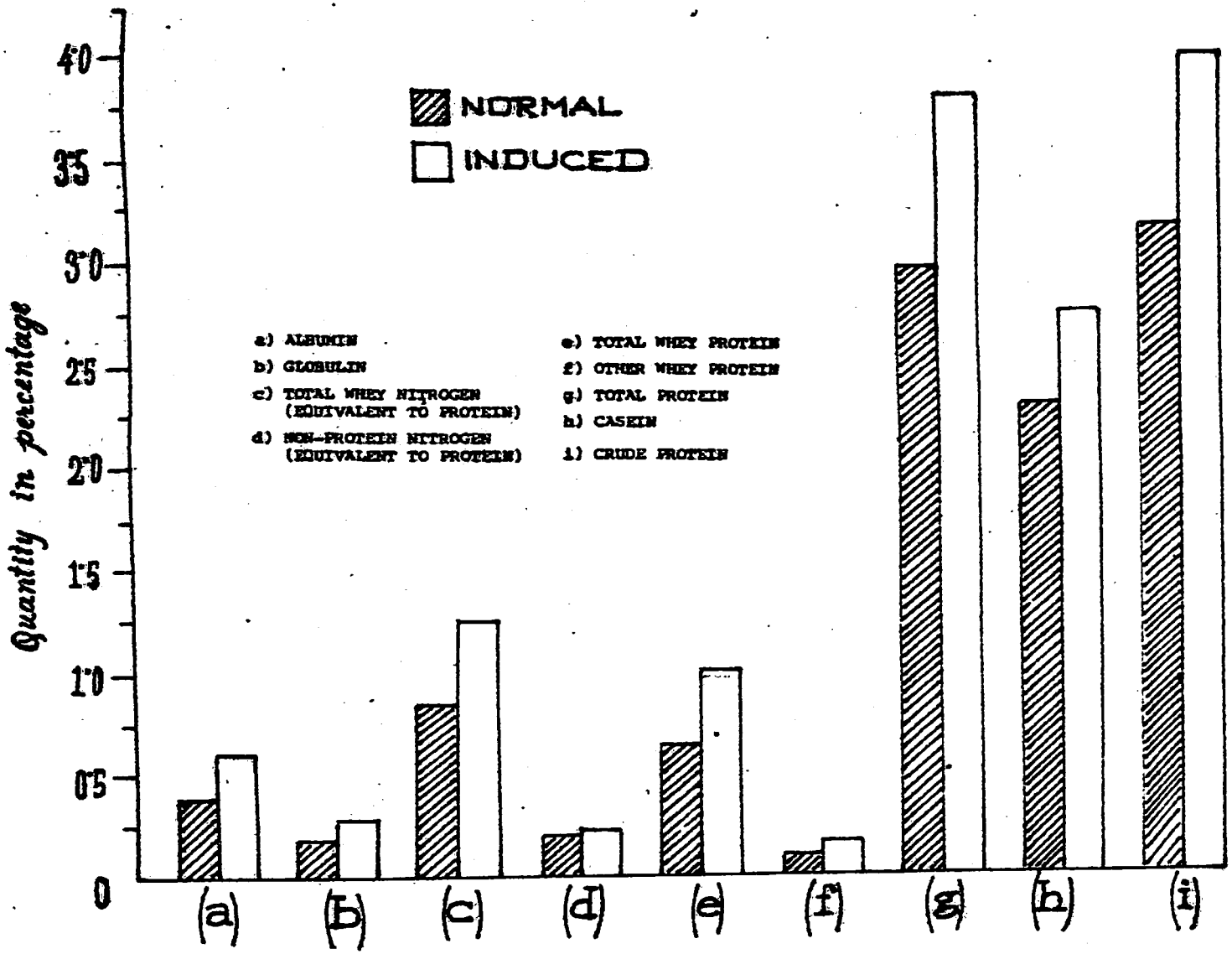
Component	% of total whey protein nitrogen	
	Normal	Induced
Albumin	39.60	57.66
Globulin	26.60	27.19
Other whey proteins	15.20	15.15

Table 22. Distribution of whey proteins in milk (as percentage of total protein)

Component	% of total protein	
	Normal	Induced
Albumin	12.90	15.91
Globulin	6.02	7.50
Other whey proteins	3.30	4.18
Total whey protein	22.22	27.59

Illustrations

FIG. 2 MILK PROTEINS FROM NORMAL AND INDUCED LACTATION



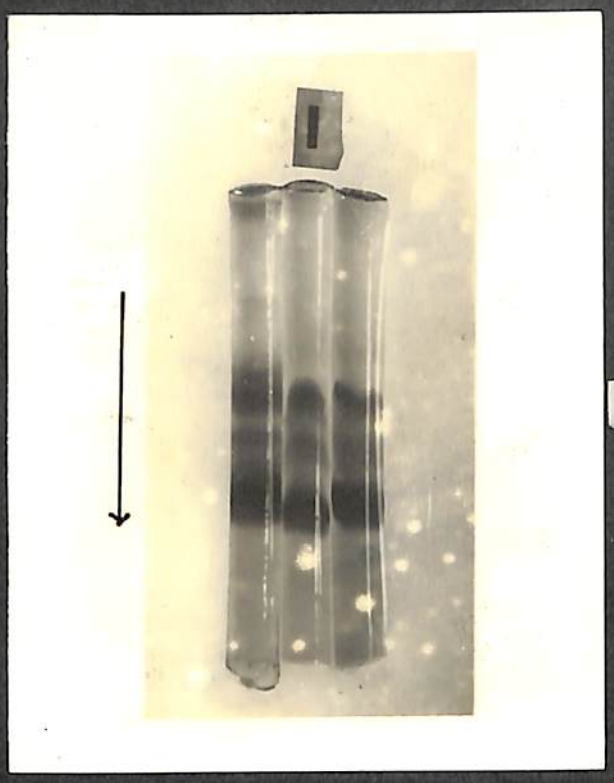


Plate I

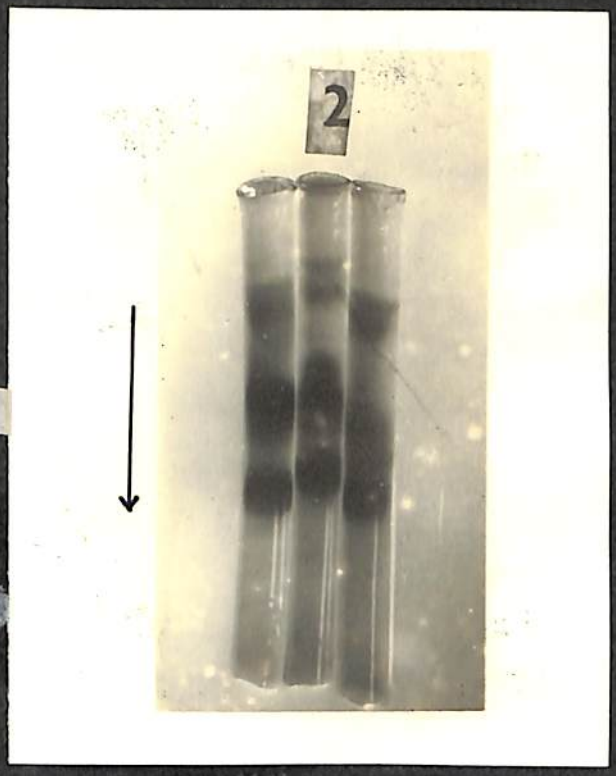
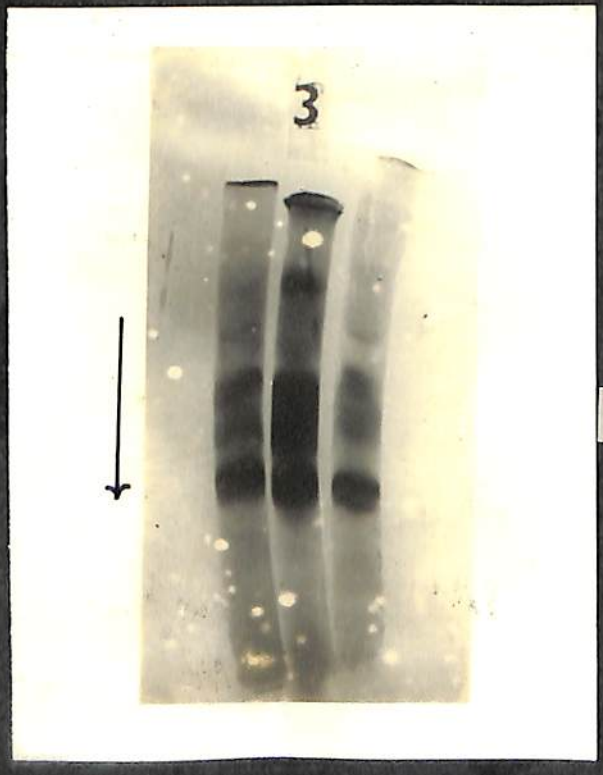


Plate II

Plate No. I-III
Electrophorograms of whey proteins from induced lactation



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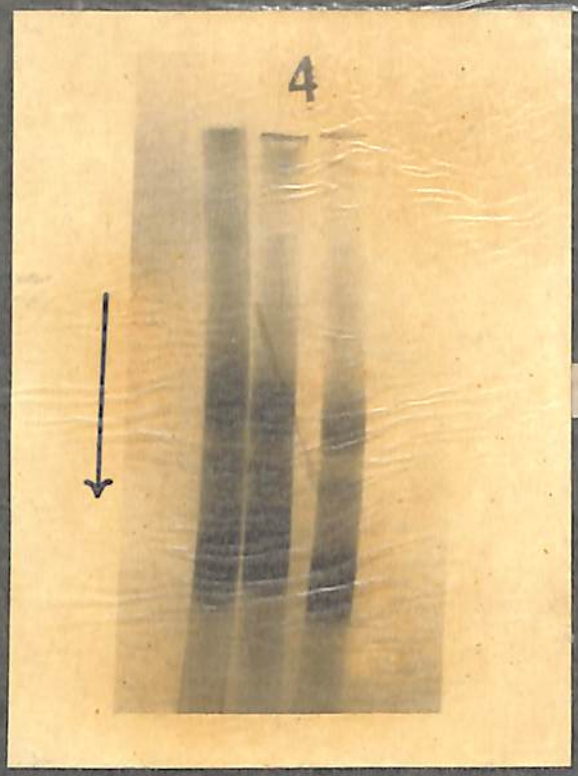

Plate IV

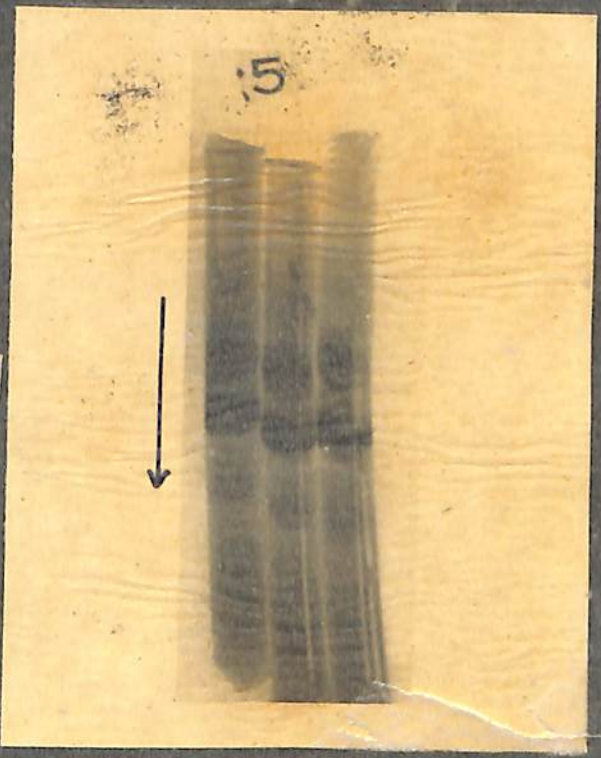

Plate V

Plate No. IV-VI
Electrophorograms of whey proteins from normal lactation

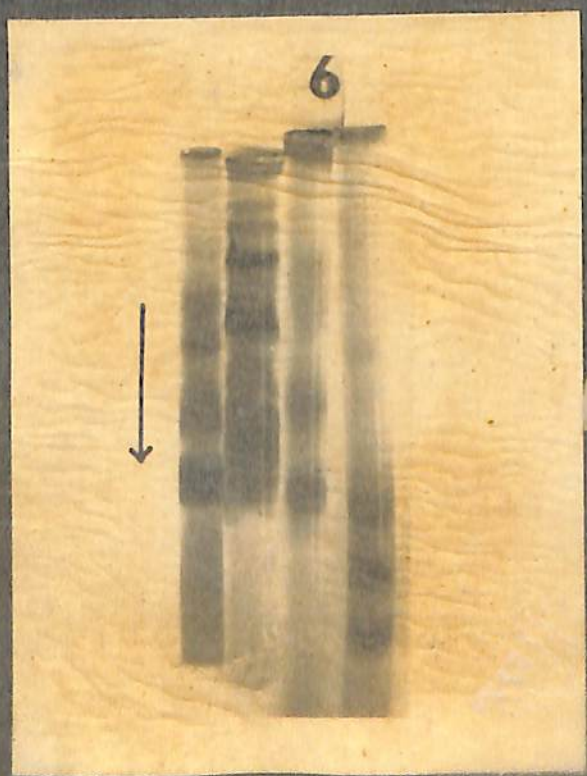

Plate VI

Discussion

DISCUSSION

Earlier, investigations on udder development and induction of lactation in the bovine were conducted by the scientists using (i) estrogen alone, (ii) estrogen plus progesterone together and (iii) estrogen-progesterone combined with other similar drugs. Duration was prolonged in many occasions. After Smith and Schanbacher (1973, 1974), it was convincing that the dose and the duration recommended by them for induction of lactation with estrogen-progesterone were useful. This was followed in the present study. Injections were given daily once instead of dividing the daily dose into two.

In the present study, the mode of development of the udder following treatment was similar to those in most of the previous works (Turner et al., 1956; Erb et al., 1973; Smith and Schanbacher, 1973, 1974; Fleming et al., 1977; Joseph, 1977, etc.) one of the cows (No.670) which gave highest yield (4.5 litre/day) showed the development earlier than the other two; response was prompt in this heifer comparatively. In general, the development was appreciable only after a week's treatment was over. It was also possible to milk all the 3 animals regularly from the 21st day after the commencement of injection. This was a similar phenomenon as that of Smith and Schanbacher (1973)

and Joseph (1977). In the observation of Erb et al. (1973) also, the milk yield was obtained from 10-21 day of the first injection, there was lacteal secretion in the study of Piper and Williams (1974). They have also used the same hormones and treated for 7 days. In the study of Smith and Schanbacher (1973), lactation started from 11-21 days after the first injection.

There were variations in respect of milk yield in the present study. The highest yield in a day from one animal (No.670) was 4,500 ml whereas the lowest was 200 ml in a day from another (No.032). Response was variable also in the reported values in this aspect. The authors in the respective reports, however, explained the possible reasons based upon their observations.

The variability in mammary cell development and milk yield under steroid therapy was found to be associated with some other additional lactogenic factors according to Howe et al. (1975). They undertook a study on the histology of induced bovine lactogenesis. They speculated that a stimulation of milk withdrawal was necessary for continued mammary gland enlargement. They further observed that the mammary tissue from all cows was not equally developed.

Although, in the normal cases, elevation of estrogen preceded the formation of alveoli at 150 days of pregnancy,

there was the appearance of alveolar epithelial cells by one week after administration of oestradiol-17 beta and progesterone in several attempts before (Malouin et al., 1980). The general belief is that the decreased concentration of progesterone in plasma at the end of pregnancy is one of the events leading to copious milk synthesis by the mammary gland. The mammary development followed a linearity with the course of treatment during the study of Fleming et al. (1977) also. As in the observation of Meredden et al. (1969), the exotic inheritance in the animals with difference of intensity might have played a crucial role in the expression of different traits concerned with the lactation.

During the normal lactogenesis in the bovine, the progesterone level was low and the estrogen level high (Smith et al., 1973). Lack of control of internal progesterone synthesis as speculated by Smith and Schanbacher (1973) may be attributed to the variabilities in the lactogenesis.

Higher concentration of prolactin associated with the high yielders (Chakriyarat et al., 1976; Reninger and Bauman, 1979), seasonal effect (Reninger and Bauman, 1979), period of treatment etc. might have influenced to result in the variations.

Albumin

The classical "lactalbumin" or the albumin fraction of milk protein which includes beta-lactoglobulin, alpha-lactalbumin and serum albumin together (Rolleri et al., 1956) was found to be 0.383 ± 0.0097 per cent on an average in the normal cows taken for this study. The value in the milk of induced animals was 0.603 ± 0.066 per cent (Table 2). On statistical analysis, there was no significant difference between the two values (Table 3, Fig.2).

Albumin nitrogen, on an average, contributed as 12.09 per cent of the total nitrogen in normal milk. But it was 15.044 per cent in the induced lactation milk (Table 20). Albumin was also 12.9 per cent and 15.907 per cent out of total protein in the normal and induced lactation milk respectively (Table 22). Albumin nitrogen contributed almost 60% of the total whey protein nitrogen in both the types of lactation.

In normal milk, albumin was reported to be 0.402% (Schneider et al., 1948). It was 0.4% on an average as reported by Barry, 1961). Rolleri et al. (1956) and Mariani (1975) observed a significant difference between seasons and between breeds in respect of albumin content. The albumin was variable throughout the year depending upon season (Ghosh and Anantakrishnan, 1963) and they found it within a range of 0.23% to 0.30%.

The present finding in respect of normal lactation was in agreement with most of the values reported so far. However, Ghosh and Anantakrishnan (1963) reported a lower value. The value obtained in the present investigation was close to the reported value of Hambraeus (1962). The milk of induced lactation had a higher content (0.603±0.0661 per cent) on an average, though there was no significant difference from the normal lactation on statistical analysis. There might be enhanced synthesis and secretion under the influence of induction of lactation which brought about the higher albumin content. There was an indication of a marked variation of secretion of albumin between individuals; because there was high significant difference on statistical analysis. However, the higher content of albumin in induced milk is an added advantage so far as the nutritive value is concerned. An elaborate study with a very large number of animals in this regard is necessary to establish this variation among individuals.

Globulin

Average of globulin content in the present study was found to be 0.1736 ± 0.01 per cent in the milk of normal cows. The value in induced lactation was found to be higher (0.2841 ± 0.051 per cent). Statistical analysis did not indicate significant difference either between groups or between individuals (Fig. 2).

Values in both the sets of animals in the present study was higher than the ones reported by Rolleri et al. (1956) and Brunner et al. (1960) in the normal cow. In the present study, globulin nitrogen was found to contribute 5.64 per cent and 7.09 per cent of the total nitrogen in the normal and the induced lactation respectively; these were higher than the reported value (3.4-4.0 per cent) of Reinart and Nesbitt (1956). There was 28 mg globulin nitrogen per 100 g milk in the present observation as against 20 mg per 100 g in the observation of Jenness and Patton (1969) who found that globulin was 0.13 per cent in milk as against 0.1786 ± 0.01 per cent in the present study. The values obtained in this study was found to be higher than the findings (0.066 per cent) of Hambraeus (1982). Aboul-Khier (1983) reported that globulin comprised of 13.5 per cent of whey protein of cows' milk. As compared to the value in the present finding (27 per cent) it was exactly double the reported value (Table 21). But the values in both the normal and the induced lactation lie around the range (0.6-1 g/litre milk) given by Swaisgood (1982). Again, the present finding was lower than what Barry (1961) has reported. It was also within the range observed (0.15-0.24 per cent) by Ghosh and Anantakrishnan (1963) in the normal cows except that the value in the induced lactation exceeded slightly (by 0.044 per cent).

The globulin in induced lactation maintained a higher level than in the normal lactation. But the secretory process might have limited the value within a range around normal. The earlier reports were limited to mostly the multiparous cows. The pattern of synthesis and secretion during the early lactation might have some similarities with that in the induced lactation for which the tendency prevailed for higher level of globulin. It is yet to be established whether the globulin level during the first lactation could be higher than in the subsequent lactations. However, higher value of globulin is desirable. Detailed study in future may help to reveal the difference.

Total whey nitrogen

The total whey nitrogen was obtained when the casein nitrogen was eliminated out from the total nitrogen in milk. Here, the non-protein nitrogen and total whey protein nitrogen are accounted together. For the purpose of comparison in the present study, the equivalent protein value was calculated as in the case of other proteins.

In terms of protein, average values were 0.841 ± 0.01 per cent in the normal and 1.263 ± 0.078 per cent in the milk of induced lactation (Table 6). On statistical analysis, there was a significant difference between groups and a highly significant difference between individuals (Table 7, Fig.2).

Total whey nitrogen out of total nitrogen in milk were found to be 26.526 per cent and 31.524 per cent on average in normal and induced lactation respectively (Table 20). On an average, the total whey nitrogen was 131.8 mg and 197.97 mg per 100 g milk in the normal and the induced lactation respectively. The values were higher than the reported values of Bellamonica et al. (1965). There was a highly significant difference between individuals which indicated that there was a wide variation in the secretary process of the individual animals. The indications lead to believe that this non-casein nitrogen as a whole has got accelerated rate of synthesis in the udder of hormonally induced lactation.

Non-protein nitrogen (NPN)

In the present study, the NPN content was 31.68 mg/100 g milk of normal cows. In the induced milk, the value was 34.11 mg/100 g. Expressed in terms of protein for comparison, the values on average were 0.202 ± 0.0093 per cent and 0.2175 ± 0.0072 per cent in normal and induced milk respectively (Table 8).

On statistical analysis, there was no significant difference either between groups or between individuals in respect of NPN (Table 9, Fig.2). It was 6.376 per cent in normal milk and 5.432 per cent in induced milk out of total nitrogen in milk (Table 20).

The present values of NPN from both sets of animals were in the range of values reported by Harland et al. (1955). The values are also in agreement with those observed by Dellamonica et al. (1965). But Mariani (1974) reported a lower value of 16.2 mg/100 ml milk. NPN as percentage of total nitrogen in the present study was found to be higher than the one reported by Reinart and Nesbitt (1956) The NPN contents were 6.376 and 5.432 per cent of total nitrogen in normal and induced milk respectively (Table 20). These values nearly corresponded to those (4.4-6.0 per cent of total nitrogen) observed by Reinart and Nesbitt (1956) who investigated the distribution of NPN in 6 different breeds.

In terms of protein (Table 8), NPN was found slightly lower than the maximum values (0.14 per cent) obtained by Ghosh and Anantakrishnan (1963).

Most of the reported values correspond to the present value with close approximation. This is an indication of undisturbed secretory process in the udder in respect of these compounds in the present observation. The process of synthesis and secretion in this aspect might have a similar character in the induced lactation as that in the normal. Lactational drain of nitrogen from plasma as described by Menon, et al. (1969) is supposed to be usual

Total whey protein

This is the combination of all the whey proteins viz., albumin, globulin and rest other whey proteins. While estimating it, the non-protein nitrogen was eliminated from total whey nitrogen. This can, therefore, be considered as true whey protein also.

On an average, it was found to be 0.64 ± 0.014 per cent in the milk from normal lactation. But, in induced lactation it was 1.045 ± 0.079 per cent (Table 10). There was a significant difference between the two sets of animal with respect to total whey protein. Between individuals, the differences were highly significant (Table 11, Fig.2). It was seen that whey protein content as a fraction of total protein was 22.22 per cent in the normal and 27.59 per cent in the induced lactation (Table 22).

As per the report of Swaisgood (1982), whey protein in bovine milk was 5-7 g/l. The value in the present study falls within this range as far as normal milk is concerned. It is beyond that range in the induced milk. Albumin + globulin alone was 0.64 ± 0.04 per cent in the observation of Sumonic-Bijelac (1977) which was 0.561 per cent in the present observation. So, the present value would be corresponding to the observation of the preceding report. The present value was found to be higher than the reported value of Gone (1973) in the case of normal milk.

In this study, the percentage value of total whey protein out of total protein in normal cows' milk (22.22%) was seen as nearly the same value (20%) given by McMeekin (1954). Whey protein was 17 and 18 parts of total protein in normal milk in two different situations (Gone, 1973); both the values were lower than the present finding.

There are indications to believe that there might be marked differences between the secretory pattern in respect of the whey proteins as a whole between normal and induced lactation. It is also noticed that animals differ greatly from one another in respect of their individual capacity for synthesis and secretion of whey protein. At the same time hormonal induction of lactation is found to bring about an acceleration for the production of whey protein.

Other whey proteins

If albumin and globulin are excluded from total whey protein, the rest will include proteose-peptone, iron-binding protéins, ceruloplasmins, lysozymes, etc. These whey protein components under this heading was collectively obtained by the difference only.

The values were very low. From the normal milk, there was 0.10 ± 0.0178 per cent and from induced lactation, 0.158 ± 0.0185 per cent whey proteins other than albumin

plus globulin (Table 12). There was no significant difference between the two sets of animals in respect of this fraction (Table 13, Fig.2). As percentage of total protein, the other whey proteins mentioned here were 3.3 and 4.18 in the milk of normal and induced lactation (Table 22) respectively. As percentage of total whey protein nitrogen the other whey protein nitrogen was almost close to each other (Table 21). As percentage of total nitrogen in milk the value of other whey protein nitrogen were 3.095 and 3.954 in the normal and induced milk respectively (Table 20).

In normal lactation, the maximum value of proteose peptone alone in the report of Bellamonica et al. (1965) exceeded the value of other whey proteins observed here; the lowest value of the authors' observation (11.84 mg/100 g milk) however, was lower than the observed value (15.67 mg/100 g milk). Proteose peptone alone was 0°06-1.8 g/litre of normal cows' milk in the report of Swaisgood (1982). Other whey proteins as a whole in the present study contributed only 3.3% and 4.18% to total protein in the normal and the induced lactation respectively. In the present study, the percentage of other whey proteins was found to be lesser in quantity as compared to the values reported. Therefore, it can be concluded that for the synthesis and secretion of these proteins, there might be restriction inherently in the animals, or other physiological differences might have existed.

Total protein

On an average, the total protein was 2.97 ± 0.048 per cent in normal milk and 3.789 ± 0.155 per cent in induced lactation milk (Table 14). These values were significantly different from each other on statistical analysis (Table 15, Fig.2). Total protein content of milk in individual animals were also significantly different from each other.

The average total protein content in normal cows was in agreement with the finding of Thibier et al. (1976). But the value was lower compared to these observed by Sumenic-Bijelic (1977) and Chakravarty et al. (1981).

The total protein nitrogen as percentage of total nitrogen of the milk was 93.623 and 94.570 per cent in normal and induced lactation respectively. These values were lower than the values reported by Mariani (1974).

Possibly, the genetic potentiality or low protein ration were determining factors. Of course, the possibility of higher protein in the milk of subsequent lactations cannot be ruled out. Because, Joseph (1977) reported a higher value in such cases. The present study was confined to the first lactation only. By this time, udder development might have not taken place to a maximum extent. Also individual animals might not have possessed similar capacity for the protein synthesis which lead to marked differences among them.

Casein

The casein content on an average was 2.33 ± 0.053 and 2.77 ± 0.126 per cent in normal and induced lactation respectively (Table 16). On statistical analysis, difference among individuals was significant ($P < 0.05$). Nitrogen distribution in normal milk was 73.47% and the same in the induced milk was 68.22%.

The casein nitrogen was lower than the reported value (78% of total nitrogen) of Nishikawa et al. (1976).

There was a lower value of casein in normal lactation in this study compared to those (3.05±0.06% in early and 2.71 ± 0.05% in mid lactation) observed by Chakravarty et al. (1981). The value was also lower than that reported by Swaisgood (1982). The reported average value (2.89 ± 0.51%) of Gorebulis and Farrell (1975) was also a higher one than the present observation; but it was close to the value in the milk obtained by induced lactation. The present study indicated a lower value of casein in both the sets of animal. The non-significant difference between groups indicated that with regard to synthesis and secretion of casein, induced lactation did not accelerate the process. This tends to be parallel to each other unlike the case between individuals.

The whey protein/casein ratio (22:78) observed in the present study in normal lactation appeared to be slightly

different to these observed by Conc, 1973 (18:82) and Hambraeus, 1982 (25:75). Still the value lied in between. In the case of induced lactation the ratio (27: 73) indicated higher proportion of whey protein than any of the two.

The ratio in the normal and the induced lactation in the present study could otherwise be expressed as 1:3.64 and 1:2.65 respectively.

Crude protein

This was a calculated value from the total nitrogen in milk. There was 496.86 mg total nitrogen per 100 g milk in normal lactation on an average. The value in the induced lactation was 628.0 mg/100 g milk. Corresponding value for protein was 3.17 ± 0.052 and 4.007 ± 0.159 per cent respectively (Table 18). There was a highly significant difference between the crude protein of the two sets of animal (Table 19, Fig.2).

The present finding in normal lactation was lower than the average value (3.72±0.63 per cent) given by Cerolulis et al. (1975).

In a report for nitrogen in mg/100 ml milk, it was 549.0 in minimum and 586.4 in maximum (Mariani, 1974). The present value in normal lactation was lower than the reported values, but in induced lactation it exceeded all these values.

The crude protein of normal lactation was in agreement with that reported by Schneider et al. (1948). The value in induced lactation was higher.

It was clearly indicated that there was a tendency for higher value of nitrogen in the milk from induced lactation. In the present study, the protein in the ration could not be a determining factor since the ration was common for both the groups. High significant differences, therefore, indicated that individual differences for assimilation of nitrogenous compound in the ration might be existing.

In the present study, there was an indication that the significant difference between the total protein (Table 15) in milk between the normal and the induced lactation was mostly brought about by the whey proteins. The role if any, of casein was little because there was no significant difference in respect of casein between the normal and induced lactation (Table 17) whereas it did in respect of whey protein between the two groups (Table 11). Again, the casein nitrogen (in percentage of total nitrogen) was not higher in the induced lactation (Table 20) but at the same time the total protein was significantly different. On the other hand, the whey proteins in induced milk was higher in percentage out of total protein as against those in normal milk (Tables 2, 4, 6, 10).

Further investigations pertaining to induction of lactation will be needed to evaluate the reasons for the observed differences in milk proteins, interrelationship of the different components and physiological implications.

The resolution of whey proteins into a minimum of 6 different fractions was seen in the present study (Plate No.I to VI) using polyacrylamide gel and borate buffer system (pH 8.3). This gel system was adopted for getting extremely high resolution for the separation of components as claimed by Garvey et al. (1977). But the Tris-acetate buffer they suggested did not work well. Hence, Borate buffer was adopted with satisfactory results. Identification of different fractions of whey proteins as shown by Melachouris (1968), Thompson (1970) and Argyle et al. (1976), etc. was not done as the individual components in pure form were not available. The use of standards along with the elution technique could help to ascertain the differences of whey proteins quantitatively.

Summary

SUMMARY

A study was undertaken on the comparative aspect of different components of milk proteins in general and whey proteins in particular between first lactation of post-parturient cows and hormonally induced lactation in infertile heifers, all being crossbred animals.

It was intended to study the difference if any, between the two types of lactation in respect of the milk proteins and possible secretory process involved therein.

In response to the 7 days' treatment with oestrogen and progesterone accompanied by massage on the udder, there was gradual development of the same in experimental heifers. It was more conspicuous between the period of termination of the treatment and the commencement of first milking i.e., on the 21st day after the first injection. Before milking, every day, engorgement of the udder was appreciable. The mammary secretion, in appearance, was colostral at the beginning for about 4 days and resembled normal milk thereafter. The daily yield varied from 200 ml to 4,500 ml.

Quantitative estimation of different components of milk proteins from normal as well as induced lactation was done by chemical analysis in which Kjeldahl nitrogen estimation method was adopted. On an average, albumin, globulin and other whey proteins (whey proteins excluding albumin

plus globulin) in normal animals accounted for 0.383 ± 0.0097, 0.1786 ± 0.01 and 0.1 ± 0.0178 per cent, respectively. Their corresponding values in induced lactation were 0.603± 0.066, 0.2841 ± 0.051 and 0.158 ± 0.0184 per cent, respectively. Total whey nitrogen (or the non-casein nitrogen) and non-protein nitrogen (NPN) in the normal milk were found to be 131.8 and 31.68 mg per 100 g milk respectively: and their corresponding values for protein were 0.84 ± 0.0103 and 0.202 ± 0.0093 per cent respectively. On the contrary, the total whey nitrogen and NPN contents in induced milk were 197.971 and 34.11 mg per 100 g milk in induced lactation; and the corresponding protein values were 1.263±0.78 and 0.2175 ± 0.0072 per cent respectively. Total whey protein, total protein, casein and crude protein contents on an average were found to be 0.64 ± 0.0142, 2.97 ± 0.0484, 2.33 ± 0.053 and 3.17 ± 0.052 per cent respectively in the normal milk. The corresponding values in the induced milk were 1.045 ± 0.079, 3.79 ± 0.155, 2.773 ± 0.126 and 4.007 ± 0.159 per cent respectively. Whey protein:Casein ratio was found to be 22:78 in normal and 27:73 in induced milk.

Statistical analysis of the data revealed that there was no significant difference between the milk of normal and induced lactation in respect of albumin, globulin, other whey proteins (whey proteins other than albumin plus globulin) as well as casein and non-protein nitrogen. There was no

significant difference between individuals in a group in respect of globulin, other whey proteins and non-protein nitrogen. Contrary to this, albumin, total whey nitrogen (expressed in terms of protein), total whey protein, casein and crude protein were found to have highly significant difference between individuals. Differences between the two sets of animals in respect of total whey nitrogen (equivalent to protein), total whey protein and total protein were significant.

No significant difference in the NPN content of milk in the two sets of animals suggests that the process of secretion is similar in both sets of animals. All the protein components in induced lactation seemed to have a tendency to be higher than those in the normal lactation. But the values never exceeded the normal range. It was also observed that the whey proteins contributed greatly in bringing about such a difference.

In addition to chemical analysis, an attempt was also made for the fractionation of whey proteins in milk from both the normal and the induced lactation animals. In polyacrylamide gel system and borate buffer (pH 8.3) it was possible to get at least 6 bands from the milk of all animals. But the mode of separation was different and it was more true between groups.

Thus, a general trend for an increase in the protein content was seen in the milk of induced lactation that was mostly brought about by whey proteins. This may be considered as a reflection of the secretory pattern involved with cows of the normal and the induced lactation. More amount of detailed work in future may be necessary to elucidate this further.

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STUDIES ON THE WHEY PROTEINS OF COWS' MILK IN INDUCED LACTATION

By

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ABSTRACT

A comparative study on the milk proteins in general and the whey proteins in particular was conducted between cows of first lactation and infertile heifers brought into lactation by hormonal treatment. It was intended to study the differences, if any, between the two sets of cows in respect of the milk proteins and the possible secretory process involved therein.

An exhaustive review of literature has been presented on the levels of various components of whey proteins and other related aspects.

The methods of analysis of various components of milk have been detailed.

The cows used in the study were crossbred ones, randomly selected from the University Livestock Farm, Mannuthy and maintained under similar farm conditions.

The experimental group of animals, the heifers, were given oestrogen (0.1 mg/kg body weight) and progesterone (0.25 mg/kg body weight) together in a single injection daily for 7 days consecutively.

Gradual development of the udder during the course of treatment was noticed. Between the termination of the treatment and first milking, the development was more conspicuous.

Regular milking, twice in a day, was started from the 21st day after the first injection. Secretion was initially colostral, and gradually within a period of 5 days, it resembled normal milk in appearance. The minimum yield from one animal in a day was 200 ml whereas the maximum reached was 4,500 ml.

From the 16th day of first milking, chemical analysis was started with in the case of experimental group. Animals in the control group began with the 3rd month of their lactation when the sample collection was started for analysis. Milk samples from both the groups were collected every 6th day and thus 6 samples from each animal were used for analysis.

Both chemical analysis and electrophoresis were used to study the milk components. Estimation of nitrogen was done by the Kjeldahl method.

Average contents (in percentage) of albumin, globulin, total whey protein and other whey proteins were 0.383, 0.1786, 0.64 and 0.10 respectively in the milk of normal lactation; the values in the induced lactation being 0.603, 0.284, 1.045 and 0.158 respectively. Out of the total nitrogen in milk, the total whey nitrogen and the non-protein nitrogen were 26.526 and 6.376 per cent respectively. in normal lactation. But in induced lactation, the values were 31.524 and 5.432 per cent respectively.

Average total protein, casein and crude protein contents in normal milk were 2.97, 2.33 and 3.17 per cent respectively. But in induced milk, the values were 3.79, 2.773 and 4.007 per cent respectively.

The ratio of whey protein:casein was 22:78 in normal lactation whereas in induced lactation it was 27:73. The contribution of whey protein to the higher total protein content of induced milk is more than that of casein.

Differences between the whey components between the two groups were noticed. On statistical analysis, albumin, globulin, non-protein nitrogen and the other whey proteins together (whey proteins excluding albumin plus globulin) did not have significant difference between the two groups. So also was the casein. There was, however, significant difference between the two groups in respect of total whey nitrogen, total whey protein and total protein. Individual differences were highly significant in respect of albumin, globulin, total whey protein, total protein and crude protein. The last one had highly significant difference between the groups also. Globulin, non-protein nitrogen and other whey proteins, on the other hand, did not indicate any significant difference neither between groups or between individuals.

The whey protein was subjected to electrophoretic separation in Acrylamide gel system with Borate buffer. In this attempt, it could be fractionated into six different bands in each case. But the mode of fractionation between groups in particular was found different from each other. A general trend for an increase in the protein content was thus seen in the milk of induced lactation that was mostly brought about by whey proteins. This may be a reflection of the secretory pattern involved with the normal and the induced lactation. Further investigations in detail may elucidate this in future.