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VARIATIONS IN HEMATOLOGIC PARAMETERS AMONG HIGH-PRODUCING
DAIRY CATTLE ON HIGH AND LOW PROTEIN DIETS
I. EFFECTS OF PROTEIN INTAKE AND STAGE OF LACTATION
ON HEMATOLOGICAL CONSTITUENTS AND ACTIVITIES OF RED
BLOOD CELL ENZYMES OF HIGH-PRODUCING DAIRY CATTLE
II. HEMOGLOBIN-BINDING PROTEINS (HAPTOGLOBIN-Hp)
IN NORMAL HEALTHY DAIRY CATTLE SERA

by

KING A. N. ESIEVO

D.V.M., Ahmadu Bello University, Zaria, Nigeria, 1974

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Major Professor

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DEDICATION

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SECTION I

THE EFFECTS OF PROTEIN INTAKE AND STAGE OF LACTATION
ON HEMATOLOGICAL CONSTITUENTS AND ACTIVITIES OF RED BLOOD
CELL ENZYMES OF HIGH-PRODUCING DAIRY CATTLE.

INTRODUCTION

Few reports have been published relating blood composition in dairy cows to their stage of lactation or to pregnancy beyond the weeks close to parturition²¹ or to different levels of dietary protein. Protein is an essential material for erythrocyte production and nutritional deficiency anemia results from low dietary protein²².

The activity of rat liver glucose-6-phosphate dehydrogenase increases by fasting and refeeding a high carbohydrate diet with adequate amounts of protein¹⁷. There is however, very little information on the hexose-monophosphate shunt enzymes activities; viz Glucose-6-phosphate dehydrogenase (G6PD); 6-phosphogluconate dehydrogenase (6PGD); and also the non-protein sulfhydryl compound, reduced glutathione (GSH) in the red blood cells of high producing dairy cattle fed increased and reduced dietary protein. Deficiency of G6PD in erythrocytes is accompanied by a diminished ability to preserve GSH in the reduced state through the activity of the NADPH-dependent GSH reductase⁴; since GSH gives integrity to the red blood cell membrane, hemolytic disease results from oxidative stress, exposure to fava beans or infection⁴ or to certain drugs^{23,4,26} in man.

Although the National Research Council (NRC) recommended 16% protein intake for high producing cattle, protein requirements remain unspecified in spite of numerous experiments¹⁹. Some data indicate need approaches 16%¹⁶ while in other cases

12%^{19,25,5} protein was satisfactory, thus making the complete ration concept of feeding dairy cattle increasingly popular³.

This project was designed to evaluate hematologic parameters that may serve as indicators of dietary related anemia among high producing dairy cattle under standard management, to elucidate some mechanisms of nutritional anemia in high producing dairy cattle, and to attain proficiency in research techniques necessary for the study of nutritional and trypanosomic anemias in Nigerian livestock.

LITERATURE REVIEW

Lower concentrations of serum albumin in cows early in lactation have been reported^{10,9,21}. Concentrations of albumin rose during the first 90 days of lactation²¹, 100 days¹⁰, and 40-100 days post partum⁹. However, Hewett⁷ working with some herds in Sweden reported no change in albumin concentration with stage of lactation. Parker et al¹⁵ found no consistent correlation between digestible crude protein intake as a percentage of requirement (DCP I/R %) and plasma albumin.

It has been reported that both albumin and hemoglobin concentrations in cows in mid-lactation can indicate protein status¹²; these findings were confirmed by the results of Rowlands et al²¹. The latter workers also reported a decrease in hemoglobin concentrations with increasing milk yield. Parker et al¹⁵ reported a significant correlation in some herds between DCP (I/R %) and hemoglobin.

A decline in total protein concentrations during the weeks prior to calving followed by a slight rise after calving has been reported²¹.

Naik and Anderson¹³ working with several breeds of cattle including dairy breeds suggested a high frequency of G6PD deficiency in this species, by applying the Brilliant cresyl blue (BCB) decolorization technique of Motulsky and Campbell-Kraut. They speculated that cattle that are deficient in G6PD activity might be better adapted to areas that are endemic for trypanosomiasis, tick infestations, and tick-borne protozoon diseases.

Paniker and Beutler¹⁴ reported that the BCB reduction test for G6PD involves the action of two enzymes - G6PD and NADPH - diaphorase and a deficiency of either one of these enzymes in the red blood cells would result in the BCB remaining unchanged in the test, whereas the spectrophotometric method which follows the reduction of NADP to NADPH measures the G6PD activity. After verifying the findings of Naik and Anderson¹³ by applying the BCB method and the spectrophotometric determinations of G6PD and NADPH diaphorase activities, Paniker and Beutler¹⁴ concluded that Bovine species are not G6PD deficient.

MATERIALS AND METHODS

(a) Experimental Animals

The hematologic studies initially included nineteen high-producing Holstein-Friesian dairy cows that ranged from 4 to 9 years old. They were managed under standard conditions; artificially inseminated and recieved all routine vaccinations including Brucellosis, at calf age; Infectious Bovine Rhinotracheitis (IBR) and Para Influenza (PI₃), by nasal tube - February 1975; and Leptospirosis annually. They were at different stages of lactation and on different levels of protein diet. (13%, 15% and 17%). These cows were on a rotational schedule, that is, spending a complete lactation cycle on one of the three dietary protein levels. During dry periods, the cows were placed on a dry lot and fed ration I (13%) for economic reasons. Dry lot ration I differed from milking cows ration I in energy level from grains but not the percentage of protein.

One cow (#177) died after four months in the study, and cows (# 033, 078, 117, 146) completed their third cycle of laction before the end of the study.

(b) Sample Collection and Preparations

Twenty ml blood sample was collected monthly from each cow by jugular venipuncture with 20 ml sterile disposable syringes and sterile needles 18 gauge X 1½". Two 4 ml vacutainers with Ethylene diamine tetra-acetate (EDTA) anticoagulant were rapidly filled and mixed by inversion and a 15 ml

vacutainer was filled with blood. One 4 ml vacutainer was placed immediately in an ice bath pending assay of glutathione (GSH), Glucose-6-Phosphate dehydrogenase (G6PD) and 6-Phosphogluconate dehydrogenase (6PGD).

Serum samples were prepared by allowing clotted blood to settle with glass beads for one hour and centrifuged at 2000 g at room temperature for 15 minutes. After a second centrifugation, sera were frozen at -20°C in screw cap tubes until required.

Sample Analysis

Packed cell volume, Hemoglobin concentration, erythrocyte count^a, total leukocyte counts^a, differential counts and reticulocytes were determined by standard method²² within 2 hours after sample collection.

Total protein and serum albumin were performed on a 12 channel sequential multiple analyzer^b (SMA 12/60).

Serum iron and iron-binding capacity were determined by the method of Henry et al (1958) as described in Henry et al⁶ (1974).

Enzymes of hexose mono-phosphate shunt.

G6PD and 6PGD were determined by the increase in Absorbance (optical density) at 340 nm in a system containing Tris-HCl 1M; EDTA 5mM, PH.8; MgCl_2 0.1M; NADP 2mM; G-6-P 6mM, and

a Coulter Electronics Hialeah. Florida,

b Technicon Corporation, Tarrytown, N.Y.

6PGA 6mM at 37°C^{30,1}, with a Gilford spectrophotometer 2400 (including automatic sample selector and circulating water bath 37°C) with 1 cm light path cuvet. Enzymes were assayed on the second day after storage at 4°C.

Non-protein sulfhydryl compound: GSH was assayed on the day of blood collection by the 5, 5' - Dithiobis (2 - nitrobenzoic acid) (DTNB) method¹; absorbance was read at 412 nm with Gilford microsample spectrophotometer 300N.

Statistical Analysis

Due to the relatively short period involved in this study, and the fact that some cows, within the 9 -month period, were in late lactation, dry period and early lactation, while a few other cows remained on one lactation cycle and as such on one dietary protein level, there was difficulty in making standard statistical analysis of some of the results. The effects due to differences in cycle of lactation or pregnancy or seasonal changes also increased the variables, this would have required interpretation in combination with other inter-related effects. However, since most of the parameters being studied might show a delayed response to protein intake rather than a simultaneous relationship, the mean values of all parameters of all cow samplings in the herd were calculated so that comparisons could be made between dry herd fed 13% protein ration and lactating herds fed 13%, 15% and 17% protein rations.

RESULTS

Packed Cell Volume (PCV)

The range and mean PCV concentrations for the four dietary groups are presented in table I. The dry cows on 13% protein ration had the highest mean PCV concentrations. Among the lactating cows, the group on the reduced protein ration had the highest mean PCV concentrations, but the cows on the increased protein ration (17%) had higher mean PCV concentrations than the 15% dietary protein group. In February lactating cows on 17% dietary protein had a higher mean PCV concentration than the lactating cows on the reduced protein (13%) ration (see table I).

When all cows, irrespective of the levels of dietary protein, were grouped according to period of lactation, starting from parturition (table 2A) mean PCV concentrations decreased for a period of four months and rose thereafter.

Hemoglobin (Hb)

Dry cows had increased mean Hb concentrations throughout the sampling period, when compared to lactating cows; and among the lactating dietary groups, cows on 13% protein had higher mean Hb concentrations than cows on 17% protein ration. Lactating cows on 15% protein ration had reduced mean Hb concentrations (see table I).

Mean Hb concentrations of all lactating cows according to month of lactation are presented in table 2B. There was a decrease in mean Hb concentrations from the first month of lactation;

the mean Hb concentration was lowest in the second month of lactation; mean Hb concentration remained low until the fourth month and by the sixth month of lactation mean Hb concentrations had approached the mean concentrations for dry cows (see table 2B).

Red Blood Cells (RBC)

Changes in mean RBC counts/ul followed the pattern described above for PCV and Hb, except for the month of January when the lactating cows on 15% protein ration had higher mean RBC counts than the lactating cows on 17% protein ration (table 1). When pooled according to month of lactation mean RBC counts decreased during the first and second months of lactation and remained low through the fifth month (table 2C).

Total Protein, Serum Albumin and Globulin

The range and mean concentrations of total protein, serum albumin and globulin for all four dietary groups are presented in table 3. Mean total protein concentrations in all dietary groups were higher than normal concentrations as reported by Schalm et al²². There were corresponding increases in the serum globulin concentrations in dietary groups (table 3) when compared to the normal concentrations²².

Mean serum albumin concentrations were reduced below normal²² for a period of two months after parturition and then increased (table 4). Total protein and serum globulin mean concentrations were not affected by period of lactation.

Serum Iron (SI), Iron - binding capacity (IBC), Unsaturated -
Iron -binding capacity (UIBC), and Percent saturation

Mean concentrations for SI, IBC, UIBC and Percent saturation are presented in table 5. No significant differences were evident in these parameters among dietary groups. Mean concentrations for SI and IBC according to month of lactation are presented in table 6A and 6B respectively. There were decreases in serum iron and iron-binding capacity in the first month of lactation. Serum iron mean concentration was lowest in the third month of lactation, after which there was a gradual increase (table 6A).

Glucose-6-Phosphate-dehydrogenase, 6-Phosphogluconate-
dehydrogenase and reduced Glutathione

The range and mean concentrations of G6PD, 6PGD, and GSH for all dietary groups are presented in table 7.

There were no significant differences in these parameters among the dietary groups. Stage lactation had no significant effects on the enzymes activities and GSH concentrations.

Discussion

The observations in this study are the differences in PCV, Hb, and RBC concentrations between non-lactating and lactating cows (Figs 1B, 2B, & 3B); when these parameters were related to duration of lactation mean values were decreased during the first four months (Fig. 1A, 2A, & 3A). Dry cows were on a positive protein balance even at a lower protein intake, resulting in increases in these parameters. The protein balance of the lactating cows, that is, intake versus output might have caused reduction in these parameters. Although protein is essential for the production of hemoglobin and erythrocytes in animals²² and in man²⁹ increased protein intake did not result in increased concentrations of erythrocytes or Hemoglobin in this study. However dairy cows are relatively more docile and used to regular handling than feedlot steers, as such, release of erythrocytes into circulation as a result of splenic contraction during sampling of the steers might have caused higher PCV concentrations reported for feedlot steers²⁸. Blood loss that accompanies parturition might have caused declines on these three parameters early in lactation; although no evidence of excessive hemorrhage at parturition was observed for these animals. Since all cows were bled on the same day, some cows were bled too close off the date of parturition for any changes due to early lactation to be noticed. (eg. see table 2A, 2B, column 1 cow 100). This may indicate that no significant bleeding occurred at parturition.

Our results on PCV decline early in lactation agrees with

Manston et al¹² and that on Hb agrees with others^{12, 9, 21, 15}. It is surprising that Parker et al¹⁵ noted little tendency for PCV to change early in lactation. RBC was not as markedly reduced as Hb concentration. In this study, we did not find any significant correlation between protein intake and hemoglobin and this is contrary to the results of Parker et al¹⁵.

Reticulocytes were not found among cows in any dietary groups. None of the animals were significantly anemic and reticulocytosis was not expected since reticulocytes are not found in health in the peripheral blood of the cow²².

There was decreased albumin concentrations during the 1st and 2nd months of lactation (fig. 4) and this agrees with results of others^{10, 9, 21, 15}. Stage of lactation had no significant effects on total protein and globulin; however, there were increased concentrations of total proteins and corresponding increases in serum globulin when compared to normal values²². There were no evidences of hemoconcentration at sampling time. In feedlot cattle there is a positive correlation of total serum protein with time on feed and with protein content of ration²⁸. There were no significant differences among dietary groups of cattle in total protein, globulin and serum albumin. Parker et al¹⁵ also found no consistent correlation between DCP and plasma albumin.

There were no significant dietary differences found for serum iron, and iron binding capacity; but both serum iron, and iron-binding capacity decreased early in lactation

(Figs. 5 & 6). Hewett⁷ reported that stage of lactation has a significant effect on serum iron.

Since there is a high demand for the production of milk proteins, and increasing levels of β -2 and γ -1 globulins before parturition²² with simultaneous reduction in the synthesis of other proteins such as albumin¹⁰ and probably transferrin, due to the difficulty in perfectly covering the cows' requirements at this time, diminished concentrations of these constituents in the blood early in lactation may be easy to understand. Since iron is an integral part of the hemoglobin molecule and is therefore absolutely essential for hemoglobin synthesis, decreases in serum iron and IBC correlate well with decreased Hemoglobin concentrations and erythrocyte count early in lactation (Figs. 6, 2A, 3A) though RBC was not as markedly reduced as Hb concentration. However, there was no hypochromia or any other evidence for iron deficiency anemia. The precipitous drop in mean IBC in the 12th month of lactation was due to analysis of only 3 cows at this stage; one (cow # 131) had very low IBC and serum iron. No explanation could be offered for these findings in cow # 131. Chronic infection would result in such a decrease but there was no evidence for this.

Although failure of hemoglobin and serum albumin concentrations to rise at mid-lactation after decline early in lactation indicates low protein intake^{12, 21} the number of cows involved in this study did not allow for separation into dietary protein groups when assessing the effect of period of lactation on albumin and Hb concentrations. There were, however,

gradual increases in albumin and Hemoglobin concentrations in this study (Figs. 2A, 4) when all lactating cows were pooled according to month of lactation irrespective of the dietary protein level. This may indicate that although 13% protein diet may be less than optimum for production, it is adequate to maintain hematologic and serum protein concentrations.

Significant differences among groups were not evident for G6PD and 6PGD activities and GSH concentrations. G6PD results agree with those reported in the protein-calorie malnutrition studies of Verjee and Behal²⁷ for man. There was no evidence of G6PD deficiency in this dairy breed which agree with the results of Paniker and Beutler¹⁴. The concentrations of 2, 3 - diphosphoglycerate (DPG) a metabolite of the glycolytic pathway that influences the affinity of hemoglobin for oxygen, are inversely correlated with age in man⁸. GSH concentration has also been reported to decrease with increasing age in calf within the first twelve weeks of life¹¹. If this age influence applies to G6PD, it may explain why the figures in this study (table 7) on adult female cows were lower than those of Paniker and Beutler¹⁴ who worked with steers younger than 4 yrs old. However, the N'dama and Muturu cattle breeds are less susceptible to the pathogenic effects of infection with trypanosomes than the West African Zebu cattle, but the nature of the resistance is still incompletely understood²⁰, it will be worthwhile to study this enzyme G6PD in the red blood cells of cattle that survive in the areas endemic for trypanosomiasis including Nigeria.

The lactating cows on 15% protein diet which had the lowest mean Hb concentrations, had the highest mean GSH concentrations. Increases in GSH concentrations as a response to fall in Hb concentrations had been suggested to protect the hemoglobin molecule from further denaturation during anemia, however the pattern of the GSH curves seems to indicate that further GSH studies with this breed may reveal a seasonal variation similar to that reported in sheep² and hereford cows²⁴. In this study the number of lactating cows on 15% protein diet in September, October, November and December were too few to allow for a plot. However, during January to May, this dietary group had the lowest mean concentrations of Hb, PCV, and RBC counts, but all cows in this group were in early lactation period, and these parameters also decreased in other dietary groups during early lactation.

In conclusion, all of the parameters that declined early in lactation rose gradually to pre-lactation levels by mid-lactation. These include albumin, hemoglobin and IBC. There were no significant differences among dietary groups in total protein globulin, albumin, serum iron, IBC, G6PD, 6PGD and GSH. While dry cows on reduced protein ration (13%) had the highest mean concentrations for PCV, Hb and RBC, and lactating cows on 13% protein diet had increased mean values for these parameters, the lactating cows on the 15% protein ration had lower mean concentrations as compared with lactating cows on the increased (17%) protein ration, apparently no significance of correlation between these parameters and percent protein

intake. While reduced protein diet, (13%) may be less than optimum for production, it is adequate to maintain hematologic and serum protein concentrations of high producing dairy cows under standard management.

Table 1

Range and Mean concentrations of PCV, Hb, RBC of Dry cows on 13% protein diet									
N*	Sept '76 (7)	Oct '76 (6)	Nov '76 (8)	Dec '76 (8)	Jan '77 (4)	Feb '77 (4)	Mar '77 (4)	Apr '77 (3)	May '77 (2)
PCV %	31-39 (35.3)	32-40 (35.5)	28-40 (34.4)	28-40 (34.8)	33-36 (34.8)	34-39 (36)	36-45 (38.5)	34-42 (38)	35-39.5 (37.3)
Hb gm%	11-13.4 (12.6)	10.8-14.1 (12.7)	9.2-13.6 (11.7)	9.9-13.6 (12.1)	11.7-13.9 (13.2)	11.1-13.8 (12.3)	12-15 (13.4)	12.4-14 (13.3)	12-13 (12.5)
RBC X 10 ⁶	5.4-8.3 (6.7)	6.2-8.4 (7.1)	5.7-7.9 (6.8)	6.4-8.5 (7.1)	6.9-8.4 (7.7)	6-7.6 (6.9)	6.6-8.2 (7.3)	6.3-7.8 (7.2)	6.2-6.9 (6.5)
RETIC	-	-	-	-	-	-	-	-	-
* Range and Mean concentrations of PCV, Hb, RBC of lactating cows on 13% protein diet (I)									
N*	Sept '76 (5)	Oct '76 (5)	Nov '76 (5)	Dec '76 (5)	Jan '77 (4)	Feb '77 (4)	Mar '77 (4)	Apr '77 (4)	May '77 (3)
PCV %	31-36 (33.2)	30-37 (32.4)	29.5-34 (32.1)	28-35 (33.0)	31-35 (33.0)	32-34 (32.8)	27-34 (31.8)	25-38 (34)	25-34 (31)
Hb gm%	11-13.6 (12.5)	11.2-13.3 (12.1)	10.6-12.1 (11.5)	10.5-12.5 (11.9)	11.3-12.2 (11.7)	11.3-11.4 (11.4)	8.9-12.4 (11.1)	8.9-13.6 (11.8)	8.6-12 (10.7)
RBC X 10 ⁶	5.8-7.5 (6.6)	6-7.4 (6.6)	6-7.2 (6.8)	6.2-7.8 (7.2)	6.4-7.8 (7.1)	6.3-7.0 (6.6)	6.1-6.7 (6.5)	6-7.7 (6.7)	5.7-6.9 (6.4)
RETIC	-	-	-	-	-	-	-	-	-

* Numbers in paranthesis below month of sampling represent number of animals

Table 1 (Cont'd)

Range and Mean concentrations of PCV, Hb, RBC of lactating cows on 15% protein diet (II)

	* N	Sept'76 (2)	Oct'76 (2)	Nov'76 (1)	Dec'76 (1)	Jan'77 (3)	Feb'77 (3)	Mar'77 (3)	Apr'77 (3)	May'77 (3)
PCV %		29-34 (31.5)	29-34 (31.5)	39.0	27.0	28-31 (30.0)	28-32 (29.3)	25-29 (27.7)	26-32 (28.7)	26.5-34 (29.7)
Hb gm%		10.4-13.4 (11.9)	11.2-12.9 (12.1)	14.0	9.2	10.2-11.4 (11.4)	9.2-11.0 (9.8)	8.9-11.4 (10.6)	8.9-11.0 (9.7)	8.8-11.0 (9.7)
RBC		5.9-6.9 (6.4)	6.0-7.0 (6.5)	8.0	5.5	5.7-7.1 (6.4)	4.9-6.6 (5.8)	5.5-6.4 (5.8)	5.3-6.8 (5.8)	4.8-6.6 (5.7)
RETIC		-	-	-	-	-	-	-	-	-

Range and Mean concentrations of PCV, Hb, RBC of lactating cows on 17% protein diet (III)

	* N	Sept'76 (5)	Oct'76 (6)	Nov'76 (5)	Dec'76 (5)	Jan'77 (6)	Feb'77 (6)	Mar'77 (5)	Apr'77 (6)	May'77 (6)
PCV %		28-34 (31.2)	28-31 (29.8)	27-33 (30.4)	27-33 (31.2)	27-33 (31.2)	30-36 (33.2)	27-34 (30.8)	24-36 (31.8)	28-34 (31.2)
Hb gm%		9.9-12.6 (11.4)	9.9-11.7 (10.7)	9.9-11.4 (10.7)	9.1-11.9 (11.0)	9.8-11.9 (11.3)	9.9-12.0 (11.0)	9.4-12.5 (10.8)	8.9-13.6 (11.3)	9.2-11.4 (10.3)
RBC X 10 ⁶		5.2-7.0 (6.0)	5.4-7.0 (5.9)	5.4-7.5 (6.5)	5.3-7.3 (6.5)	5.5-6.7 (6.3)	5.8-7.0 (6.3)	5.6-7.0 (6.3)	5.1-6.6 (6.1)	5.3-6.9 (6.0)
RETIC		-	-	-	-	-	-	-	-	-

* Numbers in paranthesis below month of sampling represent number of animals.

TABLE 2A: Effects of Dry period and duration of lactation on PCV%

Month	D1	D2	1	2	3	4	5	6	7	8	9	10	11	12
Cow #														
033	-	-	-	-	-	-	-	-	34.0	33.0	33.0	34.0	-	-
058	37.0	36.0	-	-	-	-	-	-	-	-	34.0	34.0	39.0	-
163	33.0	-	33.0	27.0	25.0	25.0	-	-	34.0	31.0	31.0	33.0	-	-
016	36.0	-	30.0	27.0	27.0	27.0	33.0	34.0	35.0	34.0	-	-	-	-
078	-	-	-	-	-	-	-	-	-	-	32.0	30.0	31.0	31.0
146	-	-	-	-	-	-	31.0	31.0	33.0	35.0	35.0	34.0	34.0	38.0
051	36.5	35.0	31.0	28.0	29.0	28.0	26.5	-	-	-	-	-	-	-
055	32.0	28.0	27.0	31.0	32.0	29.0	32.0	34.0	-	-	-	-	-	-
020	32.0	33.0	28.0	28.0	25.0	26.0	28.5	-	-	-	-	-	-	-
100	-	-	34.0	30.0	29.5	28.0	31.0	32.0	32.0	38.0	34.0	-	-	-
131	-	-	-	-	-	-	-	31.0	31.0	31.0	34.0	33.0	32.0	34.0
118	32.0	-	30.0	30.0	32.0	32.0	36.0	33.0	36.0	33.0	-	-	-	-
023	39.0	40.0	-	-	-	-	-	-	-	-	-	-	-	-
177	31.0	32.0	-	-	-	-	-	-	-	-	-	-	-	-
165	32.5	28.0	33.0	30.0	27.0	30.0	30.5	-	-	-	-	-	-	-
117	-	-	-	28.0	30.0	33.0	33.0	33.0	34.0	33.0	32.0	-	-	-
015	37.0	37.0	30.0	-	-	-	-	-	-	-	-	-	-	-
095	36.0	35.0	30.0	30.0	27.0	24.0	28.0	-	-	-	-	-	-	-
139	35.0	36.0	34.0	31.0	-	-	-	36.0	37.0	34.0	34.0	33.0	-	-
Mean	34.5	34.0	30.9	29.1	28.4	28.2	31.0	33.0	34.0	33.6	33.2	33.0	34.0	34.3
SD.	+2.6	+3.8	+2.3	+1.6	+2.6	+2.9	+2.8	+1.7	+1.9	+2.1	+1.3	+1.4	+3.6	+3.5

D₁D₂ represent 1st and 2nd dry months before parturition.

Table 2B: Effect of duration of lactation on Hemoglobin (gm%)

Month Cow #	D1	D2	1	2	3	4	5	6	7	8	9	10	11	12
033	-	-	-	-	-	-	-	-	12.8	13.3	11.7	12.5	-	-
058	13.5	13.9	-	-	-	-	-	-	-	-	13.4	12.9	14.0	-
163	11.7	-	11.4	8.9	-	8.6	-	-	12.6	11.7	11.2	11.2	-	-
016	13.2	-	10.3	9.9	9.1	9.8	11.0	11.4	13.6	11.4	-	-	-	-
078	-	-	-	-	-	-	-	-	-	-	11.6	11.2	11.4	11.4
146	-	-	-	-	-	-	11.0	11.4	11.7	11.9	12.0	11.4	12.4	13.2
051	12.4	12.1	12.4	9.2	11.4	9.2	8.8	-	-	-	-	-	-	-
055	11.7	9.2	9.2	11.7	11.0	11.4	11.0	11.0	-	-	-	-	-	-
020	11.0	11.2	10.2	9.2	8.9	8.9	9.4	-	-	-	-	-	-	-
100	-	-	13.6	11.3	10.6	10.5	11.3	11.3	11.4	13.6	11.4	-	-	-
131	-	-	-	-	-	-	-	12.2	11.2	11.2	12.1	12.2	11.3	11.7
118	12.0	-	10.6	10.0	11.4	11.6	12.0	12.5	12.2	11.0	-	-	-	-
023	13.4	14.1	-	-	-	-	-	-	-	-	-	-	-	-
177	11.0	10.8	-	-	-	-	-	-	-	-	-	-	-	-
165	11.3	9.9	11.0	9.9	9.5	9.8	10.0	-	-	-	-	-	-	-
117	-	-	-	9.9	10.6	11.0	11.9	11.9	11.4	11.0	12.0	-	-	-
015	13.5	13.6	10.2	-	-	-	-	-	-	-	-	-	-	-
095	12.8	12.5	11.9	9.9	9.4	8.9	9.2	-	-	-	-	-	-	-
139	11.1	12.0	11.0	10.2	-	-	-	13.0	13.3	12.1	12.5	11.3	-	-
Mean	12.2	11.9	11.1	10.0	10.1	10.0	10.6	11.8	12.2	11.9	12.0	11.8	12.3	12.1
SD.	+1.0	+1.7	+1.2	+0.8	+1.0	+1.1	+1.1	+0.7	+0.9	+1.0	+0.7	+0.7	+1.3	+1.0

D₁D₂ (1st and 2nd Dry months)

Table 2C: Effect of duration of lactation on RBC ($\times 10^6$)

Month Cow #	D1	D2	1	2	3	4	5	6	7	8	9	10	11	12
033	-	-	-	-	-	-	-	-	6.47	6.87	6.79	7.34	-	-
058	7.84	8.03	-	-	-	-	-	-	-	-	6.88	6.95	7.98	-
163	7.30	-	6.98	6.13	5.74	5.69	-	-	6.96	6.96	7.24	7.25	-	-
016	6.47	-	5.35	5.44	5.33	5.50	6.35	6.68	6.63	6.28	-	-	-	-
078	-	-	-	-	-	-	-	-	-	-	5.88	5.91	6.05	6.27
146	-	-	-	-	-	-	5.76	6.36	7.06	7.34	7.05	6.57	6.67	7.28
051	6.42	6.53	7.07	4.91	5.48	5.29	4.83	-	-	-	-	-	-	-
055	6.21	5.66	5.48	6.28	6.59	6.39	6.77	6.60	-	-	-	-	-	-
020	6.34	6.68	5.74	5.76	5.61	5.26	5.56	-	-	-	-	-	-	-
100	-	-	7.49	6.00	6.03	6.19	6.42	6.52	6.66	7.71	6.65	-	-	-
131	-	-	-	-	-	-	-	6.47	6.48	6.82	7.77	7.76	6.28	6.55
118	5.40	-	5.38	6.29	6.39	6.59	6.96	7.00	6.47	6.85	-	-	-	-
023	7.90	7.87	-	-	-	-	-	-	-	-	-	-	-	-
177	5.76	6.22	-	-	-	-	-	-	-	-	-	-	-	-
165	6.49	6.35	6.69	5.93	5.64	5.97	6.12	-	-	-	-	-	-	-
117	-	-	-	5.18	5.80	7.50	7.02	6.73	6.59	6.44	6.18	-	-	-
015	7.52	7.44	5.54	-	-	-	-	-	-	-	-	-	-	-
095	7.36	7.30	6.27	5.78	5.59	5.13	5.27	-	-	-	-	-	-	-
139	6.04	6.61	6.06	5.66	-	-	-	6.83	7.39	7.19	7.25	6.98	-	-
Mean	6.70	6.87	6.19	5.76	5.82	5.95	6.11	6.65	6.75	6.94	6.85	6.97	6.75	6.70
SD	+0.80	+0.76	+0.77	+0.44	+0.40	+0.74	+0.74	+0.21	+0.32	+0.44	+0.57	+0.59	+0.86	+0.52

D₁D₂ (1st and 2nd Dry months)

TABLE 3
Range and mean concentrations of total protein, serum albumin
and globulin for Dry cows on 13% protein diet (I).

Normal Values	* N	Sept 76 (7)	Oct 76 (6)	Nov 76 (8)	Dec 76 (8)	Jan 77 (4)	Feb 77 (4)	Mar 77 (4)	Apr 77 (3)	May 77 (2)
Tot. Protein	7.6gm%	7.6-9.5 (8.4)	7.6-8.9 (8.5)	7.5-9.1 (8.2)	6.2-9.4 (8.2)	8.0-9.3 (8.5)	8.3-9.1 (8.6)	7.8-8.9 (8.5)	8.4-8.9 (8.7)	8.2-8.3 (8.3)
Albumin	3.63gm%	3.1-4.0 (3.69)	3.5-3.9 (3.7)	3.1-3.9 (3.64)	3.5-4.1 (3.74)	3.4-4.0 (3.73)	3.5-4.1 (3.85)	3.6-4.0 (3.78)	3.7-4.2 (4.0)	3.8-3.9 (3.85)
Globulin	3.97gm%	3.6-6.4 (4.69)	3.8-5.4 (4.75)	3.6-5.4 (4.51)	2.7-5.7 (4.43)	4.4-5.9 (4.8)	4.2-5.6 (4.78)	4.2-4.9 (4.7)	4.3-5.2 (4.7)	4.3-4.5 (4.4)

Range and mean concentration of total protein, serum albumin
and globulin for lactating cows on 13% protein diet (I)

Normal Values	* N	Sept 76 (5)	Oct 76 (5)	Nov 76 (5)	Dec 76 (5)	Jan 77 (4)	Feb 77 (4)	Mar 77 (4)	Apr 77 (4)	May 77 (3)
Tot. Protein	7.6 gm%	6.5-8.1 (7.6)	7.8-8.3 (8.1)	7.3-8.2 (7.9)	7.8-8.9 (8.3)	7.8-8.6 (8.3)	7.7-9.2 (8.5)	7.5-8.8 (8.3)	8.8-9.3 (9.0)	7.6-8.6 (8.2)
Albumin	3.63gm%	3.1-4.0 (3.68)	3.5-4.0 (3.78)	3.7-4.1 (3.92)	3.8-4.1 (3.88)	3.8-4.1 (3.9)	3.8-4.0 (3.93)	3.5-3.8 (3.58)	3.6-4.4 (3.95)	3.5-3.9 (3.67)
Globulin	3.97gm%	3.4-4.2 (3.88)	4.0-4.6 (4.3)	3.6-4.4 (4.02)	4.0-5.1 (4.44)	3.9-4.8 (4.38)	3.7-5.2 (4.53)	3.7-5.3 (4.7)	4.5-5.3 (5.03)	4.0-5.1 (4.5)

* N Figures below month of sampling represent number of animals.

Table 3 Cont'd Range and mean concentrations of total protein, serum albumin and globulin for lactating cows on 15% protein diet (II)

	Normal Values N	Sept 76 (2)	Oct 76 (2)	Nov 76 (1)	Dec 76 (1)	Jan 77 (3)	Feb 77 (3)	Mar 77 (3)	Apr 77 (3)	May 77 (3)
Tot. Protein 7.6 gm%		7.1-8.1 (7.6)	7.4-8.0 (7.7)	8.6	8.3	7.6-8.1 (7.9)	7.8-8.5 (8.2)	8.0-8.8 (8.5)	7.7-9.7 (8.8)	8.2-9.3 (8.7)
Albumin 3.63gm%		3.6-4.2 (3.9)	3.7-4.0 (3.85)	4.1	3.3	3.5-3.8 (3.63)	3.7-4.0 (3.87)	3.4-3.8 (3.6)	3.3-4.0 (3.77)	3.1-3.4 (3.27)
Globulin 3.97gm%		3.5-3.9 (3.7)	3.7-4.0 (3.85)	4.5	5.0	3.8-4.6 (4.3)	3.8-4.7 (4.37)	4.2-5.4 (4.87)	3.7-6.4 (5.0)	5.1-6.0 (5.4)

Range and mean concentrations of total protein, serum albumin and globulin for lactating cows on 17% protein diet (III)

	Normal Values N	Sept 76 (5)	Oct 76 (6)	Nov 76 (5)	Dec 76 (5)	Jan 77 (6)	Feb 77 (6)	Mar 77 (5)	Apr 77 (6)	May 77 (6)
Tot Protein 7.6gm%		8.3-9.4 (8.9)	7.9-10.1 (8.6)	5.2-8.7 (7.8)	7.7-9.2 (8.6)	7.5-9.5 (8.4)	8.0-9.9 (8.8)	7.8-9.7 (8.5)	8.2-9.5 (8.8)	8.2-9.4 (9.0)
Albumin 3.63gm%		3.0-4.1 (3.78)	2.8-3.9 (3.47)	1.9-4.0 (3.5)	3.2-4.0 (3.72)	3.4-4.0 (3.77)	3.5-4.3 (3.97)	3.4-4.0 (3.68)	3.1-4.1 (3.67)	3.0-3.9 (3.4)
Globulin 3.97gm%		4.4-5.8 (5.08)	4.3-7.3 (5.17)	3.3-4.8 (4.3)	4.5-5.3 (4.86)	4.0-5.8 (4.65)	4.2-5.9 (4.87)	4.0-6.3 (4.78)	4.6-5.9 (5.17)	4.3-6.2 (5.55)

* N Figures below month of sampling represent number of animals.

TABLE 4: Changes in Mean Albumin Concentration During Lactation

Month	D1	D2	1	2	3	4	5	6	7	8	9	10	11	12
Cow #														
033	-	-	-	-	-	-	-	-	4.0	3.7	3.8	3.8	-	-
058	4.1	4.0	-	-	-	-	-	-	-	-	4.2	4.0	4.1	-
163	3.5	-	3.9	3.5	3.6	3.5	-	-	3.9	3.6	3.8	3.6	-	-
016	4.0	-	3.6	4.0	4.0	3.9	4.3	3.7	3.9	3.3	-	-	-	-
078	-	-	-	-	-	-	-	-	-	-	4.1	3.9	3.9	3.9
146	-	-	-	-	-	-	4.0	3.9	4.1	3.8	3.8	3.8	3.5	3.8
051	3.9	3.5	3.8	4.0	3.8	4.0	3.4	-	-	-	-	-	-	-
055	3.8	3.1	3.3	3.6	3.9	3.6	4.0	3.1	-	-	-	-	-	-
020	3.6	3.8	3.5	3.7	3.4	3.3	3.3	-	-	-	-	-	-	-
100	-	-	3.1	3.5	3.7	3.8	3.9	4.0	3.8	4.4	3.6	-	-	-
131	-	-	-	-	-	-	-	3.8	3.8	4.0	4.1	4.1	4.0	3.5
118	3.1	-	2.8	1.9	3.2	3.7	3.8	3.4	3.5	3.5	-	-	-	-
023	3.9	3.9	-	-	-	-	-	-	-	-	-	-	-	-
177	3.7	3.7	-	-	-	-	-	-	-	-	-	-	-	-
165	3.5	3.5	3.7	4.1	3.8	3.8	3.9	-	-	-	-	-	-	-
117	-	-	-	3.0	3.5	3.9	3.9	4.0	4.1	4.0	4.1	-	-	-
015	3.6	3.7	3.2	-	-	-	-	-	-	-	-	-	-	-
095	3.6	3.7	3.4	3.5	3.5	3.1	3.0	-	-	-	-	-	-	-
139	3.7	3.6	3.6	3.5	-	-	-	3.5	4.0	4.0	3.9	3.8	-	-
Mean	3.7	3.7	3.4	3.5	3.6	3.7	3.8	3.7	3.9	3.8	3.9	3.9	3.9	3.7
SD.	+0.26	+0.25	+0.33	+0.61	+0.25	+0.29	+0.39	+0.32	+0.19	+0.33	+0.20	+0.16	+0.26	+0.21

(D₁D₂) represent 1st and 2nd Dry months before parturition.

TABLE 5

Dry cows 13% protein diet (I)

	Sept '76 (4)	Oct '76 (6)	Nov '76 (8)	Dec '76 (8)	Jan '77 (4)	Feb '77 (4)	Mar '77 (4)	Apr '77 (3)	May '77 (2)
Serum Iron ug/dl	22.4-265.5 (170.6)	101.8-194.3 (134.4)	125.8-200.5 (159.5)	103.3-206.6 (173)	109.6-149.1 (126.5)	113.5-127.8 (120)	97.2-162.3 (122.8)	130.3-162.3 (143.8)	124.1-154 (139)
IBC ug/dl	34.7-304.4 (227.3)	146.6-387.5 (246.6)	184.6-324.6 (233.4)	198.6-281.3 (246.2)	146.4-230.4 (177.1)	206.1-243.0 (227.6)	185.9-234.4 (212.3)	183.0-231.5 (201.0)	190.3-233.2 (212)
UIBC ug/dl	12.3-125.1 (56.7)	31.6-193.2 (112.3)	36.6-124.2 (73.9)	9.4-140.9 (73.3)	22.9-120.9 (50.6)	92.6-117.8 (107.6)	72.1-108.8 (89.5)	44.2-69.2 (57.3)	66-79 (72.5)
% Saturat.	58.9-95.7 (73.2)	46.9-78.4 (56.5)	61.1-80.1 (69.3)	49.7-95.4 (71.2)	47.7-85.4 (74)	49.2-55.4 (52.8)	52.3-69.2 (57.4)	69.0-75.8 (71.6)	65-66 (65.7)

Lactating cows 13% protein diet (I)

	Sept '76 (2)	Oct '76 (5)	Nov '76 (5)	Dec '76 (5)	Jan '77 (4)	Feb '77 (4)	Mar '77 (4)	Apr '77 (4)	May '77 (3)
Serum Iron ug/dl	151.9-171.8 (161.8)	113.7-186.3 (138.1)	102.3-170.1 (136.0)	142.6-260.7 (199.7)	101.7-164.4 (131.7)	110.9-163.0 (129.5)	61.9-196.4 (106.0)	44.8-145.2 (110.8)	56-143 (107.7)
IBC ug/dl	165.7-238.9 (202.3)	238.8-320.5 (276.6)	184.5-340.5 (246.3)	224.2-381.1 (281.2)	170.3-260.1 (202.5)	141.5-261.4 (216.1)	102.9-263.0 (167.2)	102.6-190.7 (161.2)	156-201 (179.7)
UIBC ug/dl	13.8-67.1 (40.4)	90.1-175.4 (143.5)	36.6-170.5 (110.3)	29.1-168.0 (81.5)	58.8-95.8 (70.7)	11.1-150.6 (86.6)	16.5-81.1 (61.3)	36.7-62.0 (50.5)	58-100 (72)
% Saturat.	71.9-91.7 (81.8)	39.3-67.4 (48.9)	49.9-80.2 (56.8)	55.9-87.1 (72.2)	59.7-71.5 (65)	42.4-92.2 (63.5)	43.3-84.0 (62.9)	43.7-77.9 (66.1)	35.9-71.1 (58.4)

* Range and means of Serum Iron, Iron-binding capacity, unsaturated iron binding capacity, and per cent saturation. Figures in parenthesis below month of sampling represent number of cows.

Table 5 (Cont'd) Lactating cows 15% protein diet (II)

	Sept '76 (1)	Oct '76 (2)	Nov '76 (1)	Dec '76 (1)	Jan '77 (3)	Feb '77 (3)	Mar '77 (3)	Apr '77 (3)	May '77 (3)
Serum Iron ug/dl	144.4	104.4-208.8 (156.6)	165.9	137.7	103.3-121.3 (111.3)	87.4-134.4 (112.2)	68.3-80.1 (72.6)	80.1-115.3 (98.2)	98-113 (104)
IBC ug/dl	439.2	326.7-348.7 (337.7)	429.7	266.2	122.5-179.3 (154.9)	172.2-236.8 (194.8)	105.8-185.8 (141.0)	111.5-165.8 (146.8)	133-171 (154)
UIBC ug/dl	294.8	117.9-244.3 (181.1)	263.8	128.5	12.9-59.9 (43.6)	41.0-122.0 (82.6)	37.5-105.7 (68.4)	12.2-85.7 (48.5)	32-60 (50)
% Saturat.	32.9	29.9-63.9 (46.9)	38.6	51.7	63.2-89.4 (73.4)	48.5-76.6 (58.6)	43.1-64.6 (53.5)	48.3-89.0 (69.3)	62.0-75.9 (68.0)

Lactating cows 17% protein diet (III)

	Sept '76 (5)	Oct '76 (6)	Nov '76 (5)	Dec '76 (5)	Jan '77 (6)	Feb '77 (6)	Mar '77 (5)	Apr '77 (6)	May '77 (6)
Serum Iron ug/dl	47.3-211.6 (141.2)	64.8-190.3 (134.1)	107.8-174.2 (150.7)	180.3-280.3 (211.9)	41.7-176.1 (116.5)	78.3-169.6 (124.1)	37.4-135.6 (88.2)	94.0-156.9 (120.6)	63.0-148.0 (111.5)
IBC ug/dl	177.2-358.3 (273.5)	103.7-345.6 (244.6)	191.4-407.4 (289.3)	204.4-312.5 (268.6)	80.7-248 (185.2)	153.8-375.2 (249.6)	102.9-288.8 (183.0)	137.8-368.9 (236.9)	106-236 (188.3)
UIBC ug/dl	91.6-188.2 (132.3)	38.9-191.0 (110.5)	37.5-233.2 (138.6)	24.1-121.4 (56.7)	22.2-146.2 (68.7)	26.0-205.7 (125.5)	35.7-185.2 (94.8)	41.5-274.9 (116.3)	37-139 (76.8)
% Saturat.	26.7-65.4 (49.8)	38.3-74.8 (56.8)	42.4-80.4 (54.7)	60-89.7 (79.6)	41.0-88.0 (62.8)	36.4-83.1 (52.6)	35.9-68.0 (49.8)	25.5-69.7 (55.5)	40.9-75.5 (60.4)

* Range and means of Serum Iron, Iron-binding capacity, Unsaturated iron binding capacity, and per cent saturation. Figures in parenthesis below month of sampling represent number of cows.

Table 6 A: Effect of duration of lactation on serum iron concentration (ug/dl)

Month	D ₁	D ₂	1	2	3	4	5	6	7	8	9	10	11	12
Cows														
033	-	-	-	-	-	-	-	-	172	114	140	143	-	-
058	139	116	-	-	-	-	-	-	-	-	144	104	166	-
163	134	-	130	79	45	56	-	-	212	155	174	182	-	-
016	266	-	190	108	180	176	136	136	157	148	-	-	-	-
078	-	-	-	-	-	-	-	-	-	-	170	120	149	205
146	-	-	-	-	-	-	-	186	170	187	113	163	196	129
051	200	182	103	87	80	99	101	-	-	-	-	-	-	-
055	132	155	138	110	115	68	115	113	-	-	-	-	-	-
020	126	207	121	134	69	80	98	-	-	-	-	-	-	-
100	-	-	-	119	120	195	102	111	62	124	124	-	-	-
131	-	-	-	-	-	-	-	-	147	148	261	147	113	86
118	-	-	65	169	-	123	120	76	96	114	-	-	-	-
023	162	154	-	-	-	-	-	-	-	-	-	-	-	-
177	179	139	-	-	-	-	-	-	-	-	-	-	-	-
165	169	177	94	78	89	94	96	-	-	-	-	-	-	-
117	-	-	-	120	108	153	280	102	113	104	126	-	-	-
015	123	130	135	-	-	-	-	-	-	-	-	-	-	-
095	148	202	42	128	37	143	63	-	-	-	-	-	-	-
139	113	109	108	113	-	-	-	152	115	102	213	164	-	-
Mean	158	157	113	113	94	119	123	125	138	133	163	146	156	140
S.D.	±43	±34	±41	±26	±44	±47	±62	±36	±46	±28	±48	±27	±35	±60

D₁D₂ represent 1st and 2nd dry months before parturition.

Table 6 B: Effect of duration of lactation on IBC (Ug/dl)

Month	D ₁	D ₂	1	2	3	4	5	6	7	8	9	10	11	12
Cows #														
033	-	-	-	-	-	-	-	-	239	289	274	224	-	-
058	280	146	-	-	-	-	-	-	-	-	439	349	430	-
163	157	-	141	160	103	156	-	-	324	346	407	303	-	-
016	277	-	254	255	204	203	228	206	198	204	-	-	-	-
078	-	-	-	-	-	-	-	-	-	-	358	251	315	254
146	-	-	-	-	-	-	-	276	341	277	173	237	263	166
051	325	224	163	172	186	112	133	-	-	-	-	-	-	-
055	245	201	266	123	237	106	163	171	-	-	-	-	-	-
020	201	230	179	175	132	166	158	-	-	-	-	-	-	-
100	-	-	-	239	236	224	170	261	143	186	182	-	-	-
131	-	-	-	-	-	-	-	-	320	185	299	206	225	103
118	-	-	104	278	-	206	221	112	138	151	-	-	-	-
023	231	233	-	-	-	-	-	-	-	-	-	-	-	-
177	304	273	-	-	-	-	-	-	-	-	-	-	-	-
165	215	281	188	215	206	369	235	-	-	-	-	-	-	-
117	-	-	-	212	283	191	313	248	304	289	212	-	-	-
015	232	189	236											
095	185	272	81	154	103	270	106	-	-	-	-	-	-	-
139	206	197	235	198	-	-	-	166	273	197	381	260	-	-
Mean	238	225	185	198	188	200	192	206	253	239	303	261	308	174
S.D.	±50	±43	±63	±47	±63	±77	±63	±60	±78	±65	±99	±49	±89	±76

D₁D₂ represent 1st and 2nd dry months before parturition.

TABLE 7

13% protein diet (I)

	Sept. 76 (7)	Oct. 76 (6)	Nov. 76 (8)	Dec. 76 (8)	Jan. 77 (4)	Feb. 77 (4)	Mar. 77 (4)	Apr. 77 (3)	May 77 (2)
DRY COWS									
G6PD uM/gm Hb/min	2.8-4.7 (3.9)	4.4-7.2 (5.7)	3.7-5.8 (4.8)	4.9-7.0 (6.3)	5.1-7.0 (5.9)	5.7-6.5 (6.0)	5.6-6.5 (5.9)	6.3-6.6 (6.5)	7.2-7.5 (7.3)
6PGD uM/gm Hb/min	1.5-3.3 (2.6)	1.1-2.0 (1.5)	1.1-1.9 (1.6)	1.6-2.7 (2.1)	1.3-1.6 (1.4)	1.0-2.2 (1.5)	1.4-2.2 (1.7)	1.5-2.0 (1.7)	1.7-2.0 (1.8)
GSH uM/gm Hb	6.8-10.2 (8.3)	8.3-11.2 (10.1)	7.0-10.0 (8.3)	7.5-10.6 (8.9)	8.1-9.1 (8.5)	6.4-7.7 (7.1)	7.3-9.8 (8.2)	7.5-8.7 (8.1)	9.7-9.8 (9.8)
13% protein diet (I)									
LACTATING COWS									
G6PD uM/gm Hb/min	3.4-5.9 (4.3)	5.0-8.4 (6.3)	3.8-6.0 (4.7)	5.1-6.4 (5.9)	4.9-6.6 (6.1)	5.7-7.0 (6.4)	5.8-6.4 (6.1)	5.0-7.6 (6.4)	4.6-7.9 (6.5)
6PGD uM/gm Hb/min	2.0-4.2 (2.8)	1.0-2.4 (1.6)	0.7-2.7 (1.6)	1.3-2.9 (1.9)	1.2-2.1 (1.6)	1.2-1.5 (1.4)	1.5-2.1 (1.7)	1.2-2.5 (1.7)	1.3-2.3 (1.7)
GSH uM/gm Hb	6.2-8.0 (6.7)	7.9-9.4 (8.6)	7.4-8.5 (7.8)	8.1-8.9 (8.5)	7.3-9.1 (8.3)	6.7-8.4 (7.6)	8.2-8.8 (8.4)	7.6-8.6 (8.2)	8.3-10.1 (9.2)

Ranges and Means values of G6PD, 6PGD and GSH of Dry and Lactating cows on Ration I.

Figures in parenthesis below month of sampling represent number of cows.

Table 7 Cont'd 15% protein diet (II)

	Sept. 76 (2)	Oct. 76 (2)	Nov. 76 (1)	Dec. 76 (1)	Jan. 77 (3)	Feb. 77 (3)	Mar. 77 (3)	Apr. 77 (3)	May 77 (3)
LACTATING COWS II									
G6PD uM/gm Hb/min	2.1-4.9 (3.5)	4.2-7.0 (5.3)	5.1	6.5	5.8-6.8 (6.2)	6.3-7.2 (6.8)	5.1-7.3 (6.6)	6.5-7.6 (7.2)	7.9-8.6 (8.3)
6PGD uM/gm Hb/min	2.1-2.4 (2.3)	1.3-1.5 (1.4)	1.7	2.4	1.3-2.2 (1.8)	1.2-2.1 (1.6)	1.4-2.5 (1.9)	1.9-2.7 (2.2)	2.2-2.9 (2.5)
GSH uM/gm Hb	6.4-8.5 (7.5)	8.1-9.8 (9.0)	7.3	10.1	8.4-13.2 (10.7)	7.4-11.8 (9.7)	8.3-11.4 (10.1)	7.7-10.9 (9.5)	8.5-11.6 (11.1)

17% protein diet (III)

	Sept. 76 (5)	Oct. 76 (6)	Nov. 76 (5)	Dec. 76 (5)	Jan. 77 (6)	Feb. 77 (6)	Mar. 77 (5)	Apr. 77 (6)	May 77 (6)
LACTATING COWS III									
G6PD uM/gm Hb/min	2.8-7.6 (4.7)	4.7-7.8 (6.8)	4.4-5.6 (5.1)	5.6-7.2 (6.2)	4.5-7.3 (6.1)	4.4-9.3 (6.0)	4.7-8.3 (6.0)	5.8-7.8 (6.5)	6.8-9.1 (7.7)
6PGD uM/gm Hb/min	1.1-3.3 (2.4)	0.8-1.9 (1.4)	0.9-1.7 (1.4)	1.2-2.0 (1.7)	1.0-2.1 (1.5)	0.7-2.0 (1.4)	0.9-2.5 (1.7)	1.0-1.9 (1.5)	1.0-2.1 (1.7)
GSH uM/gm Hb	6.2-8.6 (7.2)	7.6-10.6 (8.7)	5.2-8.9 (7.5)	7.3-9.6 (8.4)	6.3-10.3 (8.6)	7.2-10.1 (8.4)	8.8-9.6 (9.2)	7.5-9.4 (8.7)	7.2-10.8 (9.3)

Ranges and Mean values of G6PD, 6PGD and GSH of Lactating cows on Rations II and III.

Figures in parenthesis below month of sampling represent number of cows.

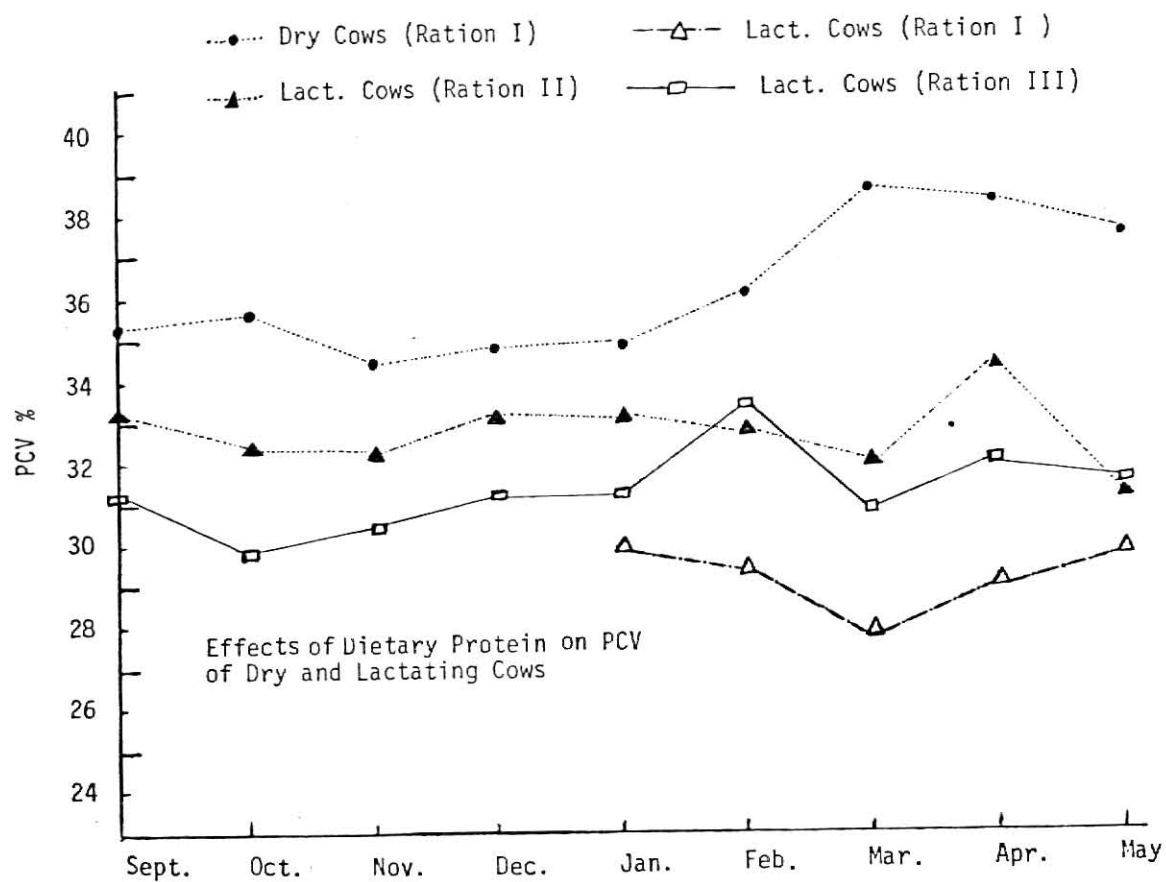
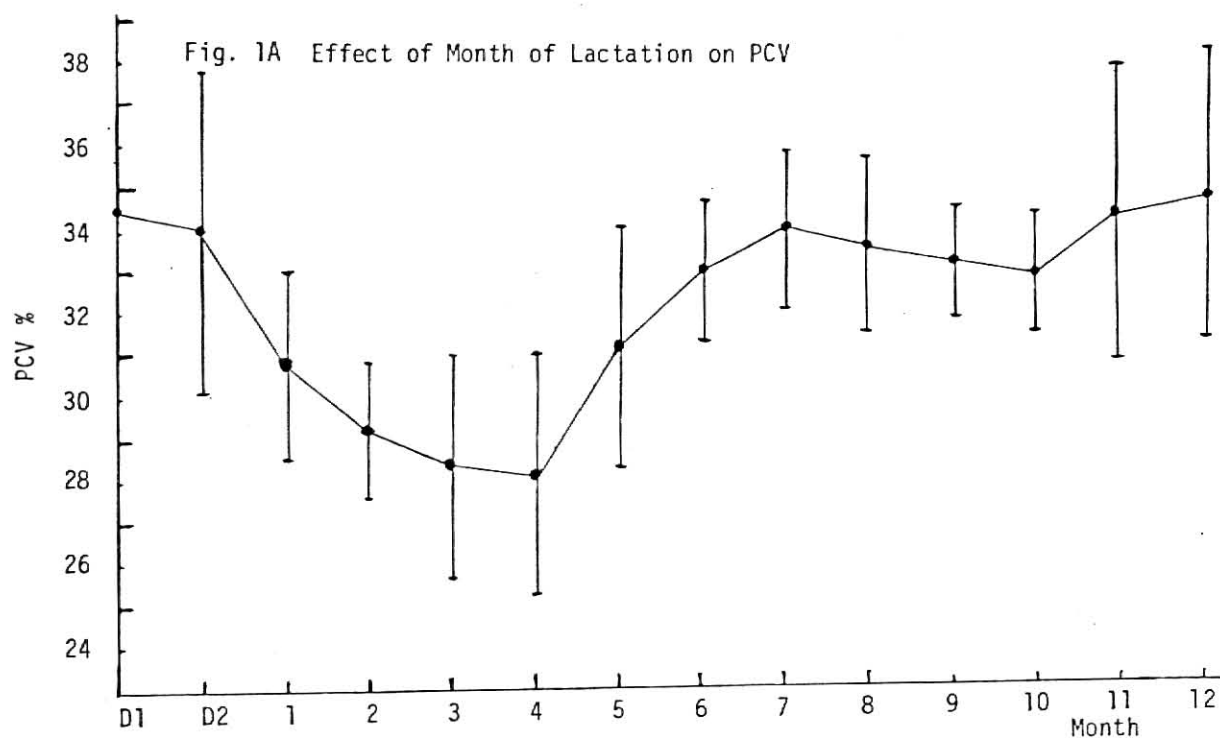


Fig. 1B

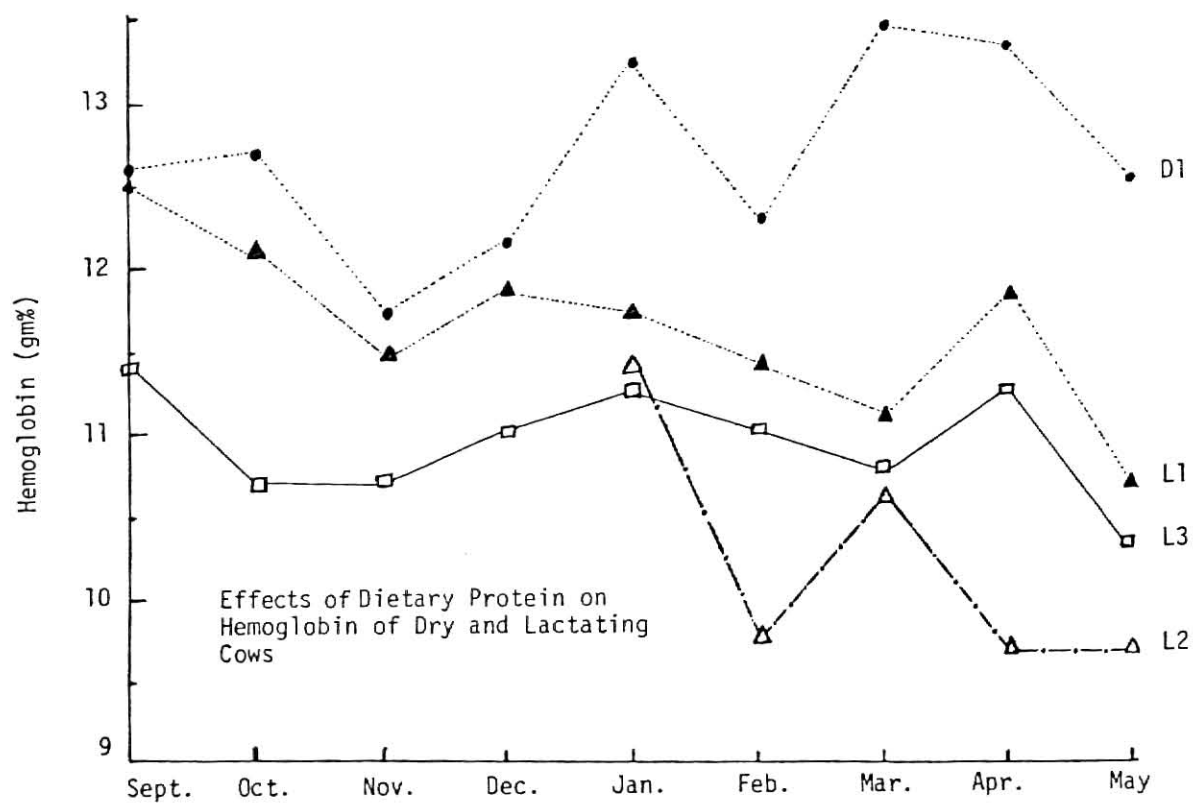
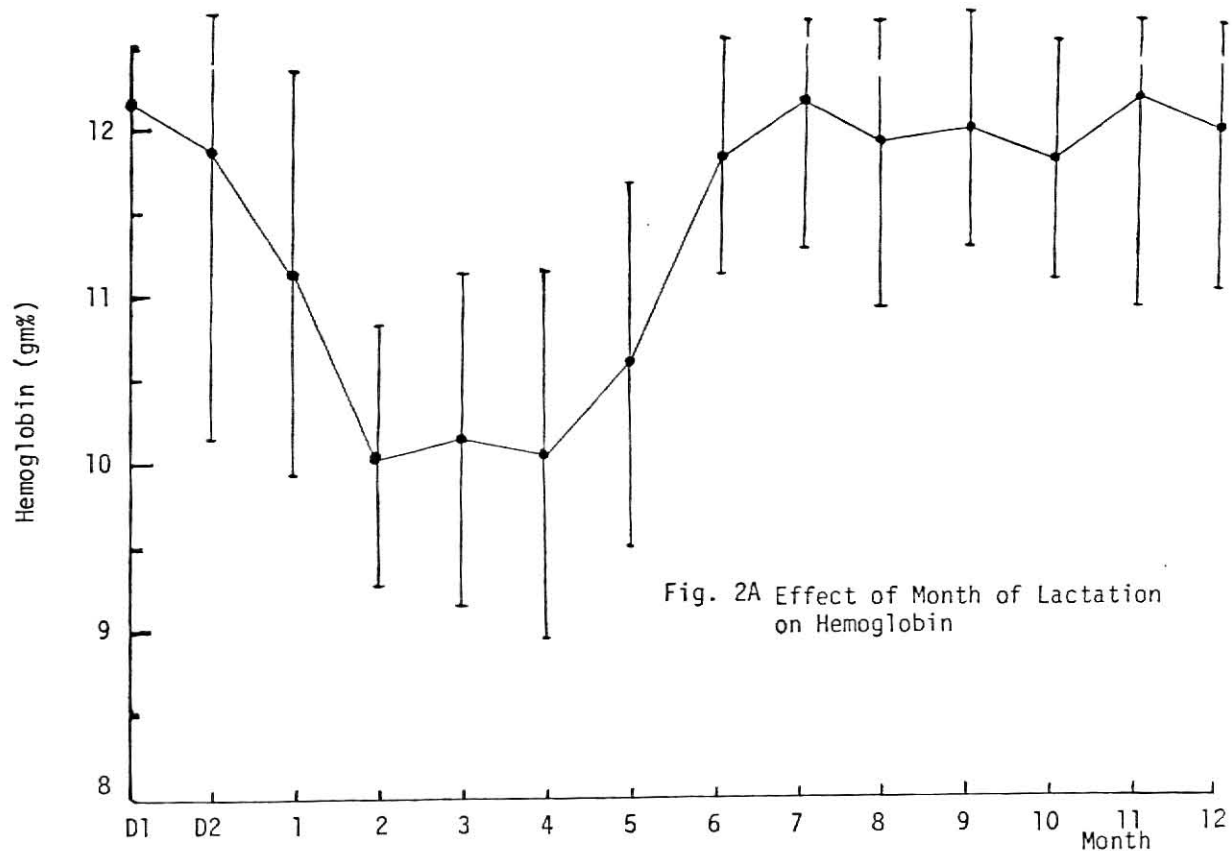


Fig. 2B

Fig. 3A Effect of Month of Lactation on RBC

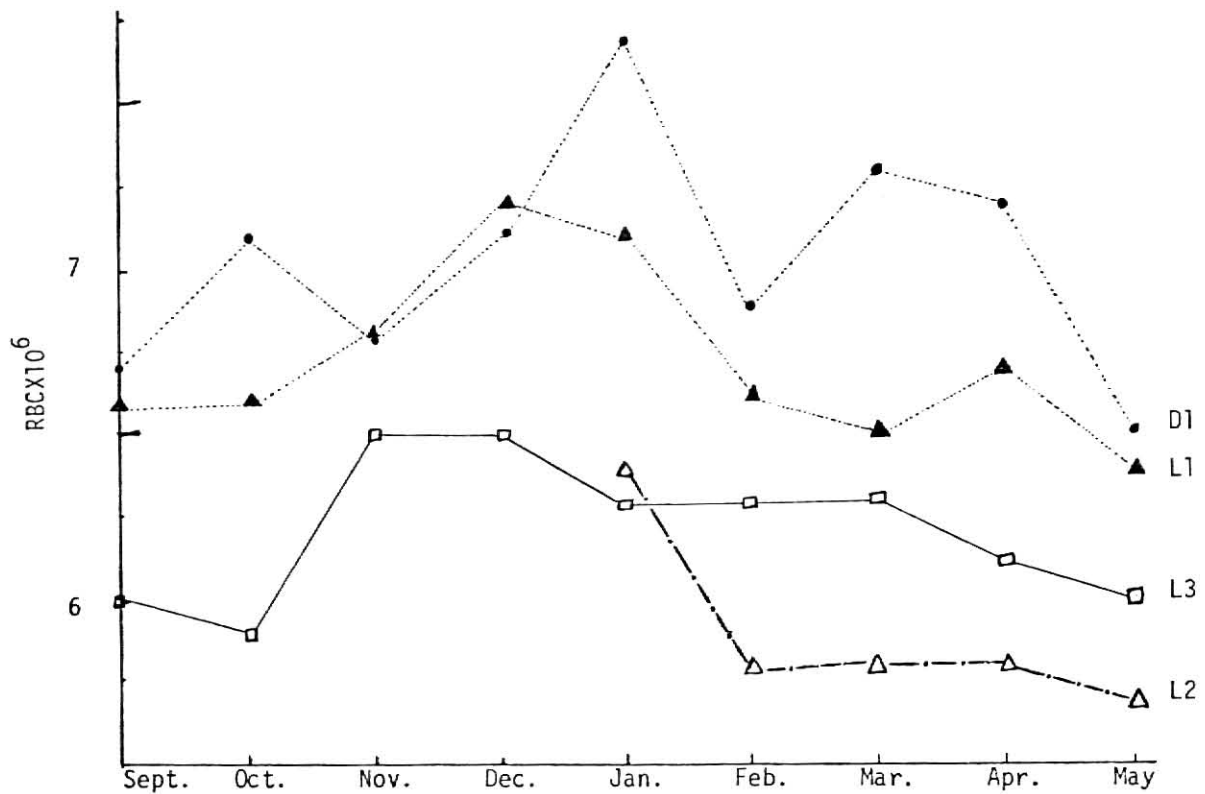
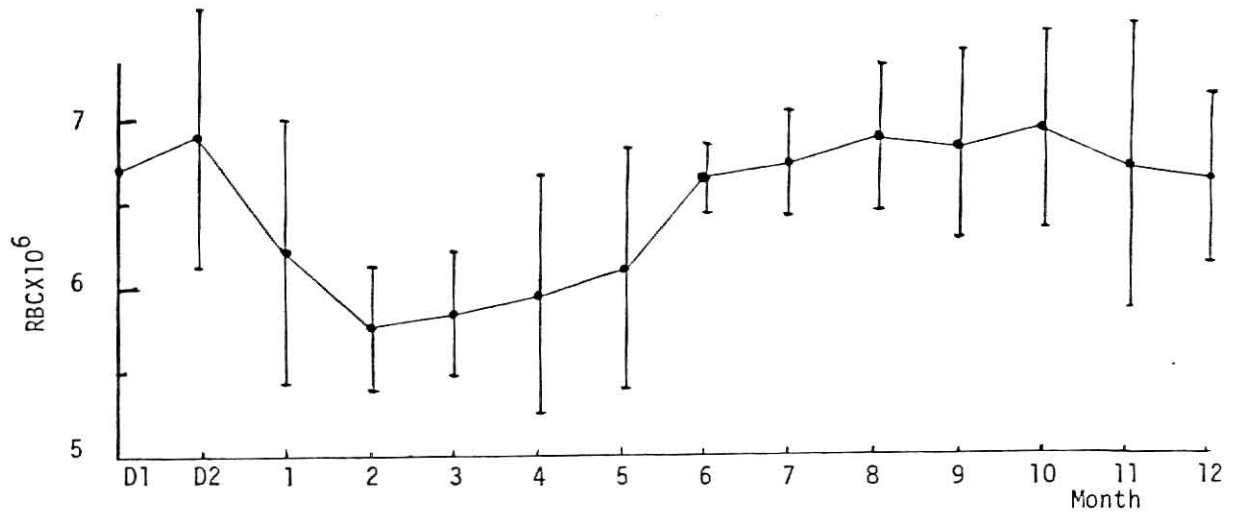


Fig. 3B Effects of Dietary Protein on RBC of Dry and Lactating Cows

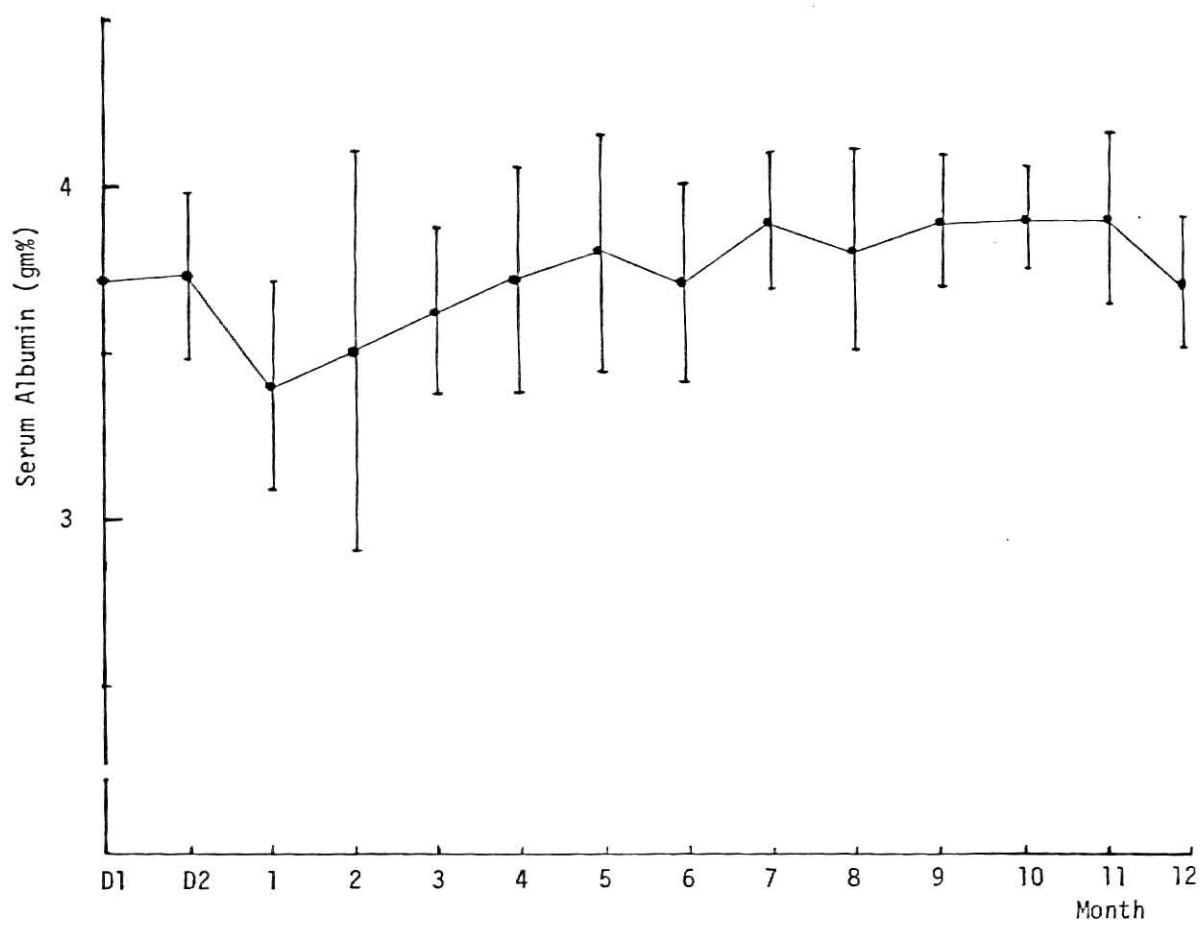


Fig. 4. Effect of Stage of Lactation on Serum Albumin

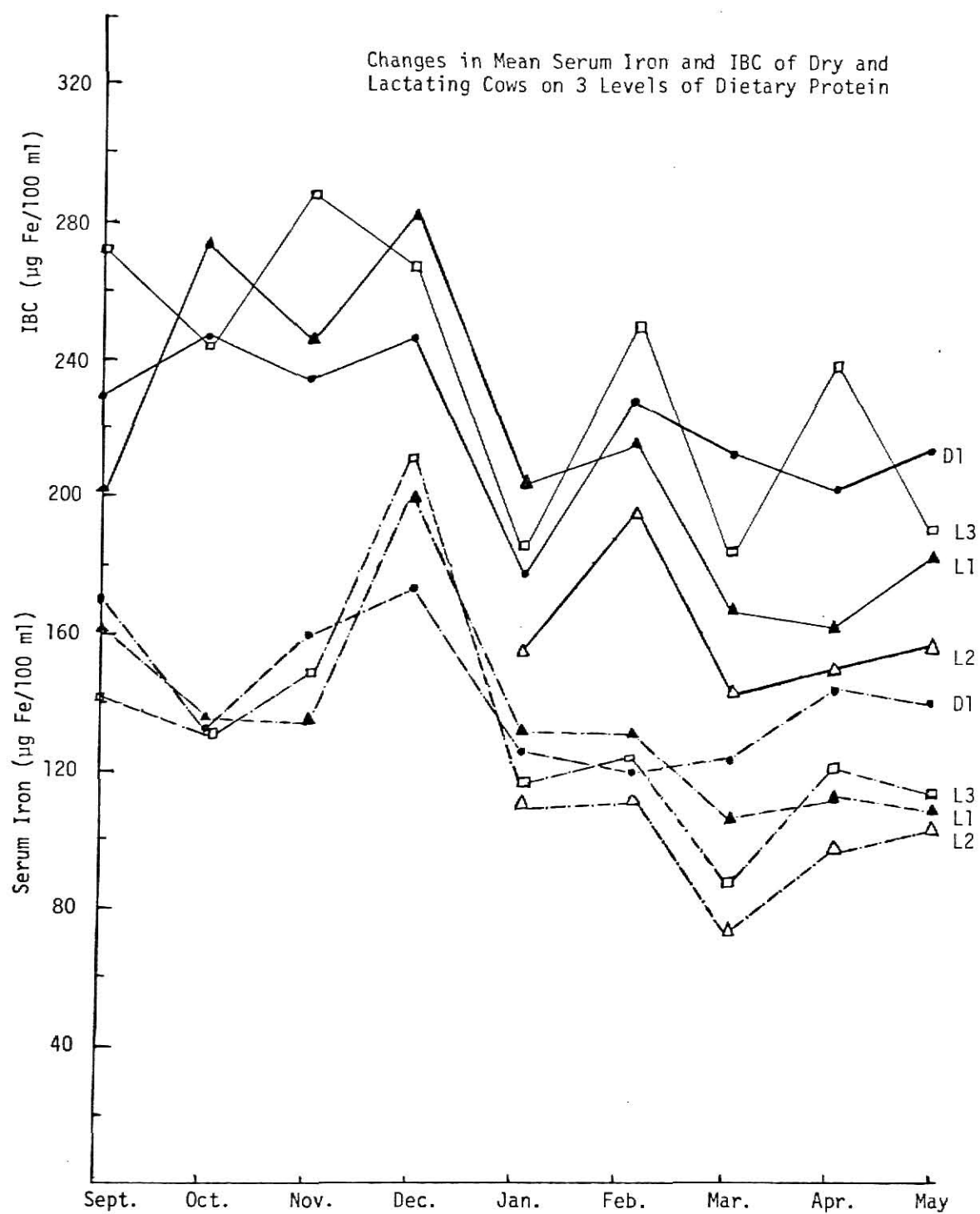


Fig. 5

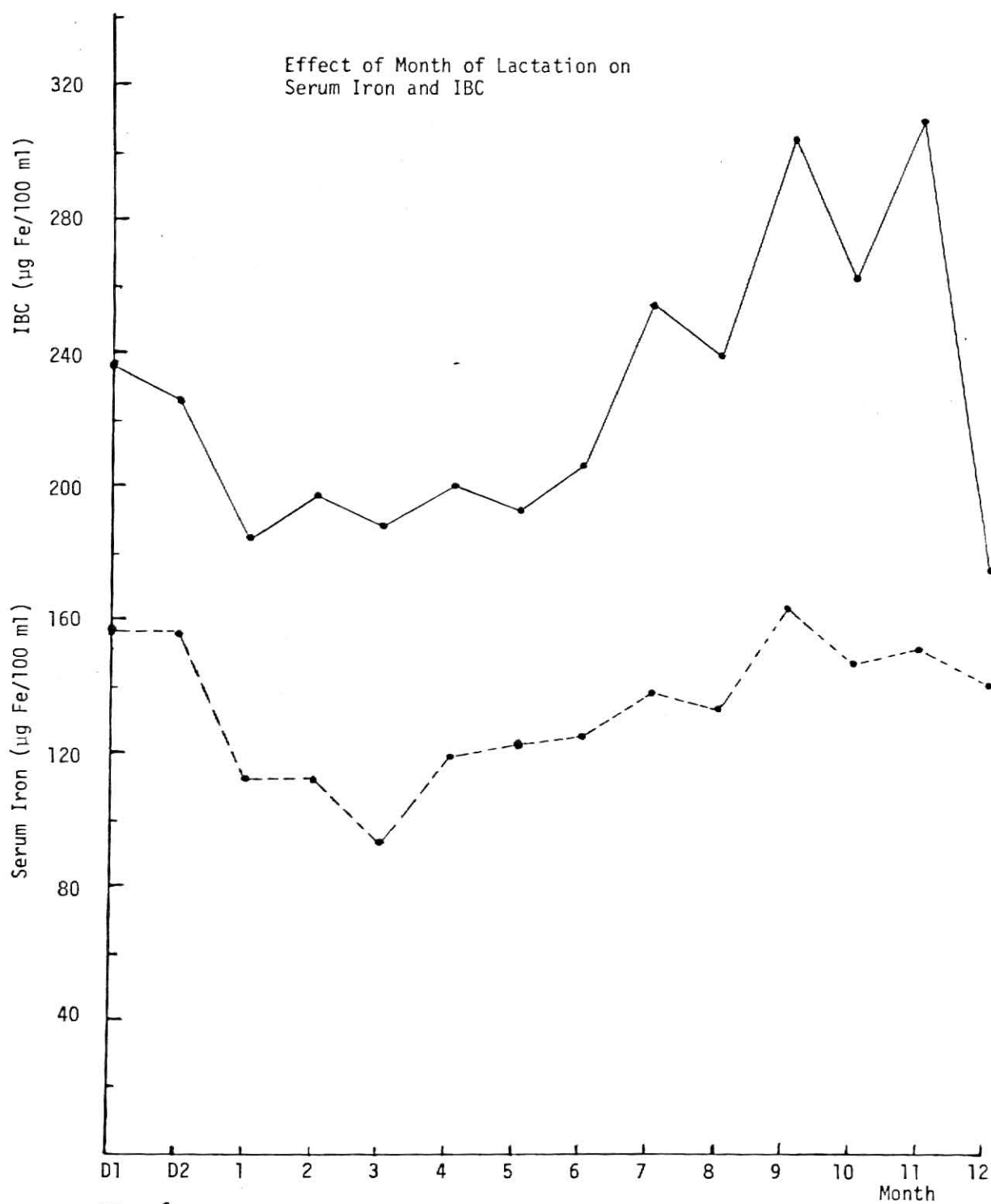
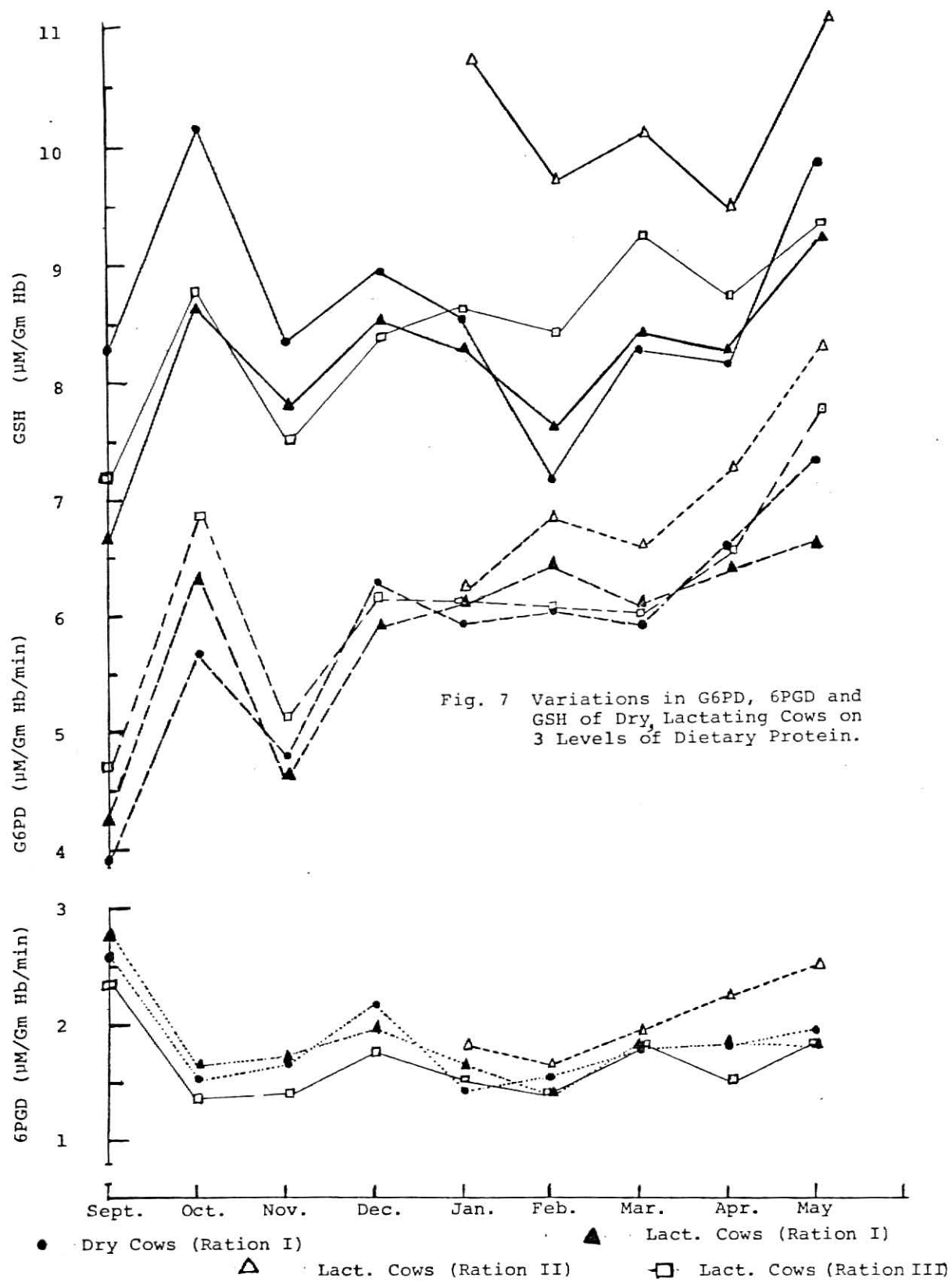


Fig. 6



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SECTION II

SERUM HAPTOGLOBINS:HEMOGLOBIN-BINDING PROTEINS (HAPTOGLOBIN -
HP) IN NORMAL HEALTHY DAIRY CATTLE SERA

SUMMARY

Nineteen clinically healthy high-producing dairy cattle sera were assayed for haptoglobin monthly over a 9month period. Haptoglobin was determined by cellulose acetate strip electrophoresis, and quantitated densitometrically. A range of 11-86 mg/dl was found. There were no significant differences among dietary and lactating groups. There was no evidence of stress clinically.

INTRODUCTION

Haptoglobin (Hp), a group of glycoproteins in the α -2 globulin fraction, binds and forms a stable complex with hemoglobin and the resultant complex is rapidly removed from circulation by the reticuloendothelial system^{12, 4}.

The estimation of serum haptoglobin levels was reported to be a very useful diagnostic test in clinical medicine¹⁵. A low level of serum haptoglobin usually would indicate recent intravascular hemolysis in man^{13, 12, 11} and elevated levels usually followed inflammatory diseases of man¹⁴.

Little work on Haptoglobin in cattle had been reported, possibly because of the apparent failure to detect a hemoglobin-binding component in most cattle sera². Bremner³ indicated that it was difficult to detect any haptoglobin in normal cattle, and that hemoglobin binding capacity of bovine plasma must be considered a less sensitive indicator of hemolytic disease in cattle than is the case in the human. This lack of detectable

serum hemoglobin-binding proteins would appear to be a normal feature of ruminants⁹ or the concentration of the protein was too low to permit detection on acrylamide gel by available methods⁵. However, animals under certain forms of stress showed polymeric series of high-molecular weight hemoglobin-binding proteins in relatively high concentrations. Stress from tissue damage in cattle^{3, 5} and sheep⁹ induced by subcutaneously injecting oil of turpentine; or administration of cortisone, adrenocorticotrophic hormone (ACTH) and diabetogenic drug-alloxan - into sheep⁹ had all resulted in elevated levels of this protein in the serum.

The exact site of hemolysis in trypanosomiasis is open to further investigations. Jennings et al¹⁰ indicated that extravascular destruction of red blood cells is a more likely explanation for the anemia of rats and mice infected with trypanosomes, since no evidence of rapid intravascular hemolysis such as jaundice and increased bilirubin had been found as a consistent feature. However, they reported cases of hemoglobinuria and hemoglobinemia in rats infected with trypanosomes¹⁰. At low rates release of hemoglobin into plasma, all of the hemoglobin is found to be attached to the haptoglobin in man¹⁸, but hemoglobinuria occurs when the binding capacity of haptoglobin is exceeded^{16, 18}. There is no reported information on Hp levels of bovine trypanosomiasis or tick-borne protozoon diseases.

The purpose of the present study was to reinvestigate Hp

levels of bovine species, and to study the variability in Hp among high producing dairy cattle on increased and reduced dietary protein.

MATERIALS AND METHODS

Experimental Animals - As in section 1 of the project.

Sample Collection and Preparation - As in section 1 of the project.

Analysis:

Total leukocyte count (Coulter Counter Model ZBI)^a

Differential Counts and Hemoglobin were determined by standard methods¹⁶.

Haptoglobin (Hp) : Serum Hp was determined by the method of Wybenga, Ibbott and Pileggi as described by Henry et al⁸ with the following modifications.

(1) O-Diansidine stock stain was made by adding 1.36 g to 1 litre of 95% ethanol in an Erlenmeyer Flask (instead of 1.43 g to 1 litre of absolute ethanol) and the working stain comprised 147 ml of the stock stain, 20 ml acetate buffer, 4 ml 3% H₂O₂ and 29 ml water.

(2) 200 ul of serum sample was spiked with 5 ul of approximately 7.5% hemolysate (instead of 10 ul of 7.5% hemolyzate). Hemolysate was prepared⁸ from the blood of a cow different from the experimental group. In March a new 6% hemolysate was prepared from the same cow.

(3) Electrophoresis of spiked samples was run for 12 minutes (Gelman instrument) at 300 volts (instead of 110 volts)

a Coulter Electronics, Hialeah, Florida.

and approximately 40 ma. Electrophoretic chambers contained 100 mls of 0.05M phosphate buffer PH7.0. Strips were dried at 52°C for 15 minutes and scanned by a densitometer^b (Quick Scan Densitometer) using a 445 nm filter.

Calculation

$$\text{mg haptoglobin/100 ml serum (as hemoglobin-binding capacity)} \\ = \frac{\text{Area Hb - bound}}{\text{Area Hb-free} + \text{Area Hb-bound}} \times F$$

$$\text{where F, in hemolysis free serum} = G \times \frac{100}{0.2}$$

G = mg Hb in 5 ul of approximately 7.5% hemolysate

$$F, \text{ in serum with hemolysis} = G \times \frac{100}{0.2} + H$$

H = mg Hb/100 ml of sample showing hemolysis.

Also to determine electrophoretic migratory characteristics of haptoglobin, serum samples from two experimental cows were electrophoresed concurrently with hemoglobin spiked samples but stained with Ponceau S. and scanned on an integrating densitometer using a 570 nm filter.

Also to determine if the protein being measured is actually a hemoglobin-binding component, serum samples from two experimental cows 146, 058.(200 ul at a time), were spiked with varying concentrations of Hemoglobin (see table 6).

^b HELENA Laboratories Beaumont, Texas.

RESULTS

Two major bands were visible on the strips, the free hemoglobin band close to the origin and the hemoglobin-Haptoglobin complex migrating further away from the origin towards the anode (Figure 1). Only one band was seen when hemoglobin was applied at the origin and no band was produced when serum was applied. On scanning the bands two peaks were produced, the free hemoglobin and hemoglobin-haptoglobin peaks (Fig. 2A). A scan of the single band produced by applying only hemoglobin reproduced only one peak (Fig. 2B). The numbers obtained from the integrating densitometer represent the areas under the peaks and are used in the calculations.

The Range and means of all four dietary groups are presented in Tables 1, 2, 3, and 4. Total leukocytes and Differential counts are also presented in the respective tables.

When these means were statistically compared there were no significant differences in serum haptoglobin among groups due to the effect of different dietary protein levels or duration of lactation. During the months of November, December, January and February, dry and lactating cows on the lowest protein ration (13%) had non-consistently higher mean serum haptoglobin, than the lactating cows on the highest protein ration (17%). In March mean serum haptoglobin levels of all dietary groups of dry and lactating cows decreased significantly with dry cows having the lowest concentrations. All groups produced gradual and sharp increases in mean serum haptoglobin

values in April and May respectively (Tables 1, 2, 3, and 4; Figure 3).

Mean serum haptoglobin values of all lactating cows, irrespective of level of dietary protein, are presented on Table 5. The lowest mean serum haptoglobin concentration was observed during the first month, with a gradual increase up to the tenth month of lactation, after which a sharp decrease in mean serum haptoglobin concentrations were observed. The gradual increases were interrupted during fourth, seventh and ninth months respectively. (Table 5 and Figure 4).

Representative electrophoretic tracings of normal serum and hemoglobin-spiked serum were compared; this hemoglobin-binding protein migrated in the α -2 globulin fraction (Fig. 5). Varying the amounts of the hemoglobin concentrations added to the serum samples made differences to the pattern of haptoglobins. (see table 6 and Fig. 6; the haptoglobin concentrations presented in table 6 were not calculated from the electrophoretogram presented in Fig 6).

A graph relating the hemoglobin concentrations to serum hemoglobin-binding capacity, and percent bound is presented in Fig. 7. Hb-binding capacity appears linear while % bound is relatively constant.

The Haptoglobin values obtained from all experimental animals within a nine month period ranged from 11-86 mg Haptoglobin/100 ml of serum (as Hemoglobin-binding capacity).

Dry cows had increases in mean Band neutrophils in September, October, November and December, higher than lactating cows on highest protein ration during the same periods (Table 1, 2, 3, and 4).

DISCUSSION

This method is based on the property of hemoglobin to bind stoichiometrically with haptoglobin to form a complex that has peroxidase-like activity¹¹. Though the reaction of haptoglobin with hemoglobin has a broad species specificity⁷ bovine Hb was used in this study and serum samples were visibly free of Hb. Decreasing the Hb-solution to 5 ul increased the Hb-Hp peak in relation to Hb-peak.

Eosinopenia of physical and emotional stress has been reported¹⁶ due to elevated levels of adrenocorticosteroids and catecholamines like epinephrine. Lymphopenia and Neutrophilia also accompany stress. Marked differences between individual cows were apparent; variability in these total and differential counts was influenced by the little disturbance involved in bleeding some of the cattle¹⁶, the fact that few of the cattle had persistently high total counts, (see Appendix table 1) and the differences in time of blood sampling from date of parturition; the cows calved at different dates. Guidry et al⁶ had reported that, at parturition, changes occurred in all circulating cell concentrations and that these changes were preceded by sharp increases in corticosteroid values. Circulating neutrophils increased

from $3362/\text{mm}^3$ 12 days before parturition to $5889/\text{mm}^3$ on day of parturition in the 1st lactation studied and from $2441/\text{mm}^3$ to $9413/\text{mm}^3$ in the 2nd⁶. Eosinophils decreased 12 days before, to 5 days after parturition, and lymphocytes decreased for 5 days after parturition and increased to prepartum levels by postpartum day 15⁶. However, few samplings, within the study period fell in this time span as to affect the results. The high mean Bands values for dry cows on Ration I (13%) during the months of September to December is probably due to the fact that the cows were dry for a month or two before becoming fresh, and increases in circulating neutrophils before parturition are accompanied by increases in immature neutrophils, all from the bone marrow and not from a marginal pool⁶. Diurnal variations in eosinophil count has been demonstrated, with highest count at midnight and the lowest at noon, related to the corresponding variations in endogenous corticosteroid level¹⁶. This has been avoided by bleeding all cows same day and time - 10 a.m. In effect the experimental animals throughout the nine-month period had no evidence for clinical stress at the times of sampling. Individual cows with lymphocyte counts of about $3000/\text{ul}$ did not have increased levels of Hp. (see appendix table I).

Although cows on Ration I (13%) had higher mean serum haptoglobin during November, December, January and February when compared to the cows on Ration III (17%), lactation had no influence on Hp levels among cows on Ration I. Probably the ration (17% protein) of lactating cows was very adequate

hence the steady mean values between September and February. The number of lactating cows on Ration II (15%) did not allow for a plot during September to December (Table 3). The precipitous drop in Haptoglobin during March in all cows - Dry or Lactating of all rations may be seasonally induced (Fig. 3); February/March being the end of the winter period and the beginning of a new season with increasing environmental temperatures. The change to 6% hemolysate in March had little effect on the precipitous drop in mean Hp concentrations, since the same 6% hemolysate was applied in April and May (Fig. 3). Further studies may indicate seasonal variation in Haptoglobin.

When all lactating cows were grouped according to month of lactation, one would have expected first month of lactation to have the highest mean Hp values. Since this would include cattle assayed during the period when endogenous concentrations of corticosteroids are highest in the serum, that is, immediately following parturition. However, the endogenous concentrations of corticosteroids following parturition may not have been high enough to cause increased Hp as indicated by the study of Jarret⁹ using exogenous corticosteroid administration in sheep, or many animal samplings were not obtained immediately after parturition. Although the highest mean Hp concentration occurred during the tenth month of lactation, length of lactation has no significant effect on Hp (Fig. 4). The sharp decrease in the 12th month was due to analysis of only three cows at this stage and one (cow # 131) had low

Hp concentration. (see section I serum Iron and IBC).

This Hb-binding protein migrated in the α -2 globulin fraction (Fig. 5) as already reported for man¹². The fact that addition of varying amounts of hemoglobin to the serum made differences to the pattern of haptoglobins (table 6, Fig. 6) indicated that this is a hemoglobin-binding protein.

In humans, Hp concentration range is 21-195 mg/dl⁸; in horses, dogs, and cats, 20-190 mg cyanmethemoglobin binding capacity/dl⁷ and dogs 0-234 mg/dl¹. In our present study a range of 11-86 mg/dl was found by applying total hemoglobin concentration for routine determination¹⁷ for man.

Linearity of the Hb-binding capacity with concentration of Hb added to the serum indicates that the available Hp in the serum was not saturated and suggests that Hb-binding capacity of the bovine Hp is related to the free Hb concentration added to the serum. Generally the cow's serum is more yellowish than the human serum due to plant pigments - Xanthophil and Carotenes and probably contains certain amount of hemoglobin concentrations from sampling procedures, which may be less than 50 mg/dl of serum and as such have been unnoticed.

The quantitative haptoglobin method in this study of adult bovine serum is simple, rapid and reproducible, and may be applied in evaluating levels of intravascular hemolysis in the adult bovine species. However, further investigations need to determine the optimum Hb concentration that will saturate bovine Hp, if it has saturability characteristics

like human Hp¹⁷ and so evaluate the optimum spiking Hb concentration that will increase sensitivity with negligible interference from unnoticed serum hemoglobin.

TABLE 1: Haptoglobin Concentrations, Total Leukocytes (ul) and Differential Counts of Dry Cows on 13% Dietary Protein (I)

	Sept. 76 (7)	Oct. 76 (6)	Nov. 76 (8)	Dec. 76 (8)	Jan. 77 (4)	Feb. 77 (4)	Mar. 77 (4)	Apr. 77 (3)	May 77 (2)
Hp mg/dl	22.8-79.9 (47-19)	25.7-77.6 (41-19)	11.1-82.6 (50-26)	34.6-56.1 (45-7)	42.4-57.0 (49-6)	42.1-53.8 (46-5)	23.9-31.5 (28-4)	28.7-40.6 (34-6)	43.2-44.8 (44-1.0)
WBC x 10 ³	6.1-17.7 (9.3-4.0)	7.7-12.4 (9.0-1.8)	4.8-11.3 (7.8-2.1)	7.8-18.7 (10.7-3.9)	7.1-18.6 (11.5-5.0)	6.1-10.3 (8.0-1.9)	8.0-11.8 (9.7-1.8)	9.9-13.5 (11.8-1.8)	6.1-8.1 (7.1-1.4)
Basop.	0	0-248 (85)	0-156 (37)	0-178 (56)	0-186 (64)	0-69 (17)	0	0-119 (40)	0
Eosinop.	61-1239 (600)-437	0-539 (244-182)	0-624 (302-205)	0-902 (401-303)	186-710 (381-249)	414-1056 (757-266)	354-1470 (890-521)	675-4158 (1928-1936)	976-1620 (1298-455)
Bands	0-1224 (348-425)	372-624 (459-90)	0-336 (207-140)	82-1296 (445-430)	0-186 (88-77)	0-206 (117-86)	0-236 (121-103)	0	0-122 (61-86)
Segs.	1714-5841 (3017-1339)	1738-4464 (3042-1120)	1680-4068 (2881-827)	2624-9911 (4556-2833)	2485-9300 (4887-3032)	2196-4635 (3000-1149)	2520-6254 (3876-1640)	1972-7560 (3903-3168)	2349-2440 (2395-64)
Lymph.	2142-9912 (4998-2495)	3588-6448 (4798-991)	2064-6102 (4005-1527)	3354-7128 (4829-1404)	3408-8556 (5919-2259)	2562-5280 (3715-1240)	2940-6195 (4275-1406)	3069-5831 (4542-1390)	2257-4131 (3194-1325)
Mono.	164-444 (269-103)	156-744 (317-218)	0-904 (345-291)	0-935 (341-302)	95-284 (169-87)	264-488 (377-95)	315-590 (513-133)	0-540 (345-300)	0-305 (153-216)

Range and means + SD

* Figures in Parenthesis below month of sampling represent number of animals.

TABLE 2: Haptoglobin Concentrations, Total Leukocytes (ul) and Differential Counts of Lactating Cows on 13% Dietary Protein (I)

	Sept. 76 (5)	Oct. 76 (5)	Nov. 76 (5)	Dec. 76 (5)	Jan. 77 (4)	Feb. 77 (4)	Mar. 77 (4)	Apr. 77 (4)	May 77 (3)
Hp mg/dl	24.6-39.2 (34+6)	20.9-69.5 (44+28)	28.7-86.3 (50+23)	42.3-54.2 (49+4)	31.5-59.6 (46+12)	44.2-53.8 (49+5)	22.4-41.1 (31+8)	23.7-40.3 (32+7)	40.5-43.0 (41+1)
WBC x 10 ³	5.1-11.2 (7.5+2.4)	5.6-8.2 (6.7+1.3)	5.6-9.8 (7.8+2.0)	7.1-9.4 (8.5+0.9)	5.6-11.9 (7.8+2.8)	4.6-9.8 (7.3+2.2)	5.7-8.7 (7.6+1.4)	5.4-9.9 (8.3+2.0)	4.9-14.9 (8.7+5.4)
Basoph	0-112 (22)	0-80 (16)	0-57 (11)	0	0-67 (31)	0-138 (79)	0-87 (40)	0-91 (23)	49-298 (137)
Eosinoph	0-1680 (563+650)	0-420 (188+166)	87-368 (221+100)	92-609 (352+217)	0-392 (217+164)	0-1104 (396+490)	72-696 (328+303)	0-693 (395+289)	539-745 (617+112)
Bands	0-336 (185+133)	0-177 (101+73)	87-644 (296+213)	0-328 (130+140)	56-119 (78+28)	46-294 (156+115)	57-360 (213+164)	0-182 (81+76)	49-149 (108+52)
Segs	1292-3486 (1952+873)	1121-2400 (1673+593)	1456-3496 (2249+780)	1491-3384 (2398+817)	1512-3451 (2600+804)	1104-5586 (2811+1971)	2109-4698 (2887+1227)	1566-4095 (3153+1126)	1568-5215 (3080+1901)
Lymph.	3162-7056 (4579+1551)	3060-6314 (4447+1228)	2850-6958 (4670+1738)	4611-6808 (5312+901)	3082-7735 (4385+2245)	2139-5226 (3343+1365)	2262-6206 (3708+1701)	3024-5742 (4202+1154)	2548-8046 (4518+3062)
Nono.	83-336 (178+95)	112-480 (303+131)	174-490 (353+127)	0-470 (271+188)	345-595 (448+107)	294-552 (467+122)	172-609 (324+196)	297-616 (423+146)	98-298 (174+108)

Range and means $\pm$ SD

* Figures in parenthesis below month of sampling represent number of animals.

TABLE 3: Haptoglobin Concentrations, Total Leukocytes (ul) and Differential Counts of Lactating Cows on 15% Dietary Protein (II)

	Sept. 76 (2)	Oct. 76 (2)	Nov. 76 (1)	Dec. 76 (1)	Jan. 77 (3)	Feb. 77 (3)	Mar. 77 (3)	Apr. 77 (3)	May 77 (3)
Hp mg/dl	32.60-59.6 (46.1)	50.8-68.6 (59.7)	46.5	44.7	37.6-54.0 (44.9)	43.6-48.5 (46.2)	28.6-38.2 (34.5)	31.1-39.3 (36.5)	41.5-45.8 (44.2)
WBC x 10 ³	9-10.0 (9.5)	7.9-9.1 (8.5)	9.1	6.4	6.7-8.9 (8.0+1.2)	6.1-7.8 (7.0+0.9)	7.0-9.9 (8.0+1.6)	6.8-9.1 (7.8+1.2)	6.1-8.5 (7.0+1.3)
Basop.	0	0-9.1 (46)	0	0	0-89 (30)	0-69 (43)	0	0-152 (96)	0
Eosinop.	100-720 (410)	395-637 (516)	546	448	267-603 (432+168)	305-552 (442+126)	198-630 (420+216)	76-728 (449+336)	252-1275 (651+547)
Bands	100-180 (140)	158-364 (261)	182	640	0	122-483 (254+199)	198-280 (231+43)	0	63-122 (90+30)
Segs	2200-2790 (2495)	1738-2184 (1961)	2093	960	2546-3655 (2987+586)	1830-2886 (2400+533)	2304-5148 (3277+1620)	1972-3185 (2758+681)	2805-3294 (3125+277)
Lymph	4950-7100 (6025)	5372-5460 (5416)	5915	4224	3417-5162 (4305+873)	2760-3822 (3312+532)	3570-3960 (3803+206)	3672-4823 (4149+600)	2135-4080 (2933+1019)
Mono.	360-500 (430)	237-273 (255)	364	128	85-623 (281+297)	414-468 (436+28)	140-396 (299+139)	304-476 (381+87)	63-170 (118+54)

Range and means + SD

* Figures in parenthesis below month of sampling represent number of cattle.

TABLE 4: Haptoglobin Concentrations, Total Leukocytes (ul) and Differential Counts of Lactating Cows on 17% Dietary Protein (III)

	Sept. 76 (5)	Oct. 76 (6)	Nov. 76 (5)	Dec. 76 (5)	Jan. 77 (6)	Feb. 77 (6)	Mar. 77 (5)	Apr. 77 (6)	May 77 (6)
Hp mg/dl	28.2-64.4 (41+15)	22.6-55.9 (43+13)	17.2-76.6 (43+22)	21.1-58.7 (43+14)	36.3-49.7 (42+6)	35.1-48.6 (45+5)	26.9-37.7 (31+4)	30.4-39.9 (33+4)	36.6-46.5 (41+4)
WBC x 10 ³	6.9-10.4 (8.1+1.3)	6.0-18.3 (9.3+4.6)	7.2-17.2 (10.1+4.1)	7.8-15.3 (10.9+3.1)	6.5-17.3 (11.6+4.6)	6.0-17.7 (9.6+4.3)	6.3-18.0 (10.5+5.3)	4.2-16.7 (9.6+4.8)	5.3-12.9 (7.9+3.0)
Basop.	0-79 (20)	0-183 (83)	0	0-226 (69)	0-164 (76)	0-177 (47)	0-180 (92)	0-262 (51)	0-129 (38)
Eosinop.	300-728 (528+213)	270-671 (505+156)	172-656 (421+214)	306-678 (442+145)	0-2952 (893+1210)	0-783 (429+295)	126-1620 (905+670)	0-3013 (709+1146)	212-1485 (687+476)
Bands.	158-416 (289+126)	60-366 (201+133)	72-1032 (416+367)	0-791 (267+324)	0-164 (48+75)	0-240 (100+97)	0-143 (82+56)	0-220 (91+87)	0-159 (27+65)
Segs.	2175-3224 (2549+464)	1020-3477 (2103+892)	1944-3956 (2835+820)	2262-3002 (2625+335)	585-7564 (3679+2618)	1440-368 (2751+903)	2112-4680 (3235+1188)	924-6930 (2871+2071)	1378-3354 (2583+710)
Lymph.	3519-5512 (4492+829)	3294-12993 (5893+3593)	4212-11524 (6095+3068)	4266-11628 (7082+2953)	3224-13321 (6303+3607)	3280-13275 (5580+3848)	3328-10440 (5743+3197)	2310-13694 (5304+4422)	2679-8514 (4281+2273)
Mono	138-375 (246+116)	244-732 (436+189)	164-516 (373+148)	158-720 (351+218)	74-1116 (568+349)	420-1062 (683+213)	126-1080 (462+371)	183-1100 (567+320)	129-495 (285+129)

Range and means + SD

* Figures in parenthesis below month of sampling represent number of animals.

TABLE 5: Effect of Duration of Lactation on Haptoglobin (mg/dl)

	1	2	3	4	5	6	7	8	9	10	11	12
G-100	38.32	20.87	86.33	42.32	31.50	44.18	30.28	28.09	40.48	-	-	-
118	30.40	76.61	42.95	37.34	48.64	37.71	33.81	42.48	-	-	-	-
016	52.25	17.15	21.06	49.68	47.23	26.93	30.75	41.10	-	-	-	-
055	36.48	44.65	54.03	48.51	38.22	39.26	43.66	-	-	-	-	-
051	34.58	40.16	43.58	28.56	31.0	45.75	-	-	-	-	-	-
095	39.99	47.02	32.89	39.91	43.60	-	-	-	-	-	-	-
G-20	37.64	45.37	35.04	38.64	41.52	-	-	-	-	-	-	-
165	36.32	43.60	27.35	30.40	46.53	-	-	-	-	-	-	-
163	45.35	29.90	35.71	43.00	-	-	64.39	48.94	35.02	51.86	-	-
139	34.78	37.73	-	-	-	39.18	24.85	56.46	54.19	51.27	-	-
117	-	46.33	55.93	49.45	58.66	47.41	48.25	32.22	31.10	-	-	-
033	-	-	-	-	-	-	24.60	62.35	33.79	51.97	-	-
058	-	-	-	-	-	-	-	-	59.64	68.58	-	-
078	-	-	-	-	-	-	-	-	28.2	46.57	35.42	38.64
177	-	-	-	-	-	-	64.07	-	-	-	-	-
G-131	-	-	-	-	-	35.27	43.00	45.90	49.93	42.76	53.68	22.40
146-E	-	-	-	-	33.5	69.46	28.65	48.68	59.62	53.81	41.10	40.27
015	36.62	-	-	-	-	-	-	-	-	-	-	-
# of cows	(11)	(11)	(10)	(10)	(10)	(9)	(11)	(9)	(9)	(7)	(3)	(3)
Mean	38.4	40.9	43.3	40.8	42.0	43.0	40	45	44	52	43	34
SD.	+6	+16	+18	+7	+9	+12	+14	+11	+12	+8	+9	+10

(Figures in parenthesis represent number of animals)

Table 6. Hemoglobin - Binding Experiment

Sample #	Hb solution	mg Hb = G	Area Hb-bound	Area Hb-Free + Area Hb-Bound	% Bound	mg Hp/dl
146-1	20 ul	1.104	39.3	119.3	33	181.8
2	10 ul	0.552	22.2	83.2	27	73.6
3	7 ul	0.3864	25.8	81.3	32	61.3
4	6 ul	0.3312	25.5	74.7	34	56.5
5	5 ul	0.2760	19.1	59.6	32	44.2
6	(1:2 dil) 5 ul	0.1380	15.5	37.0	42	28.9
158-1	20 ul	1.104	26.8	105.0	26	140.9
2	10 ul	0.552	24.5	93.5	26	72.3
3	7 ul	0.3864	23.4	77.9	30	58.0
4	6 ul	0.3312	20.0	75.5	26	43.9
5	5 ul	0.2760	11.0	42.5	26	35.7
7	5 ul (1:2 dil)	0.1380	17.0	51.3	33	22.9
7	5 ul (1:4 dil)	0.0690	16.4	45.9	36	12.3

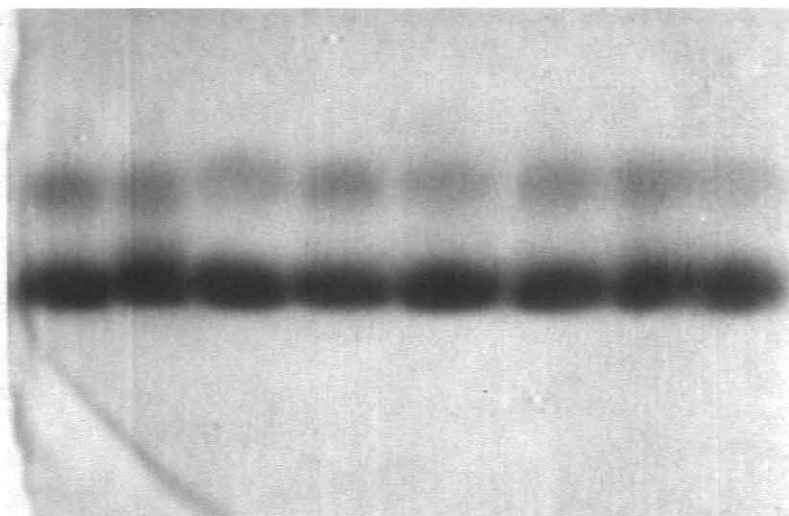


Fig. 1. Cellulose Acetate Strip Electrophoresis. Bands of Free Hemoglobin and Hemoglobin-Haptoglobin Complex.

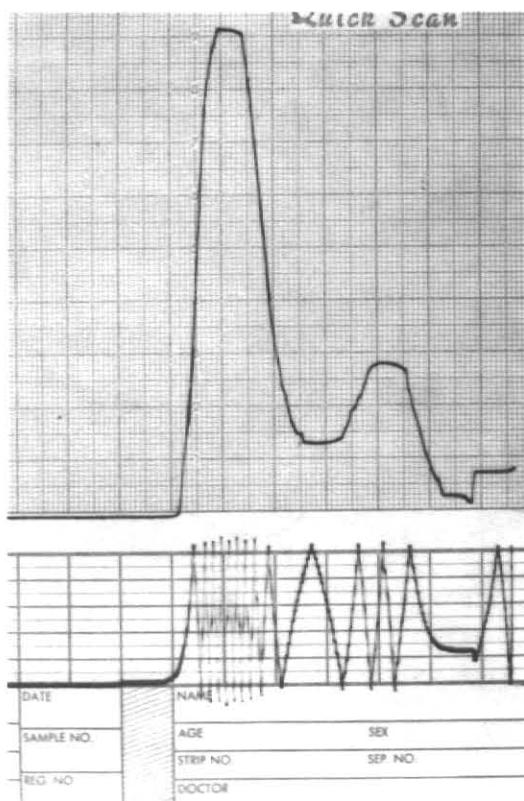


Fig. 2A. Free Hemoglobin and Hemoglobin-Haptoglobin Peaks.

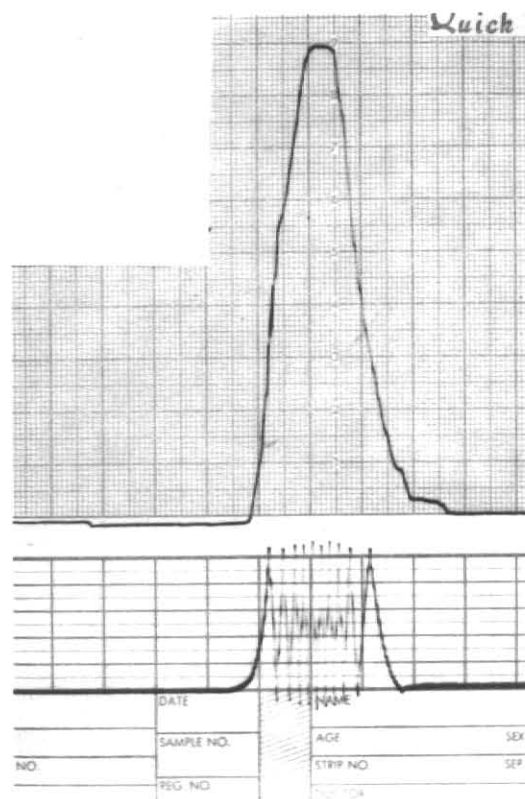
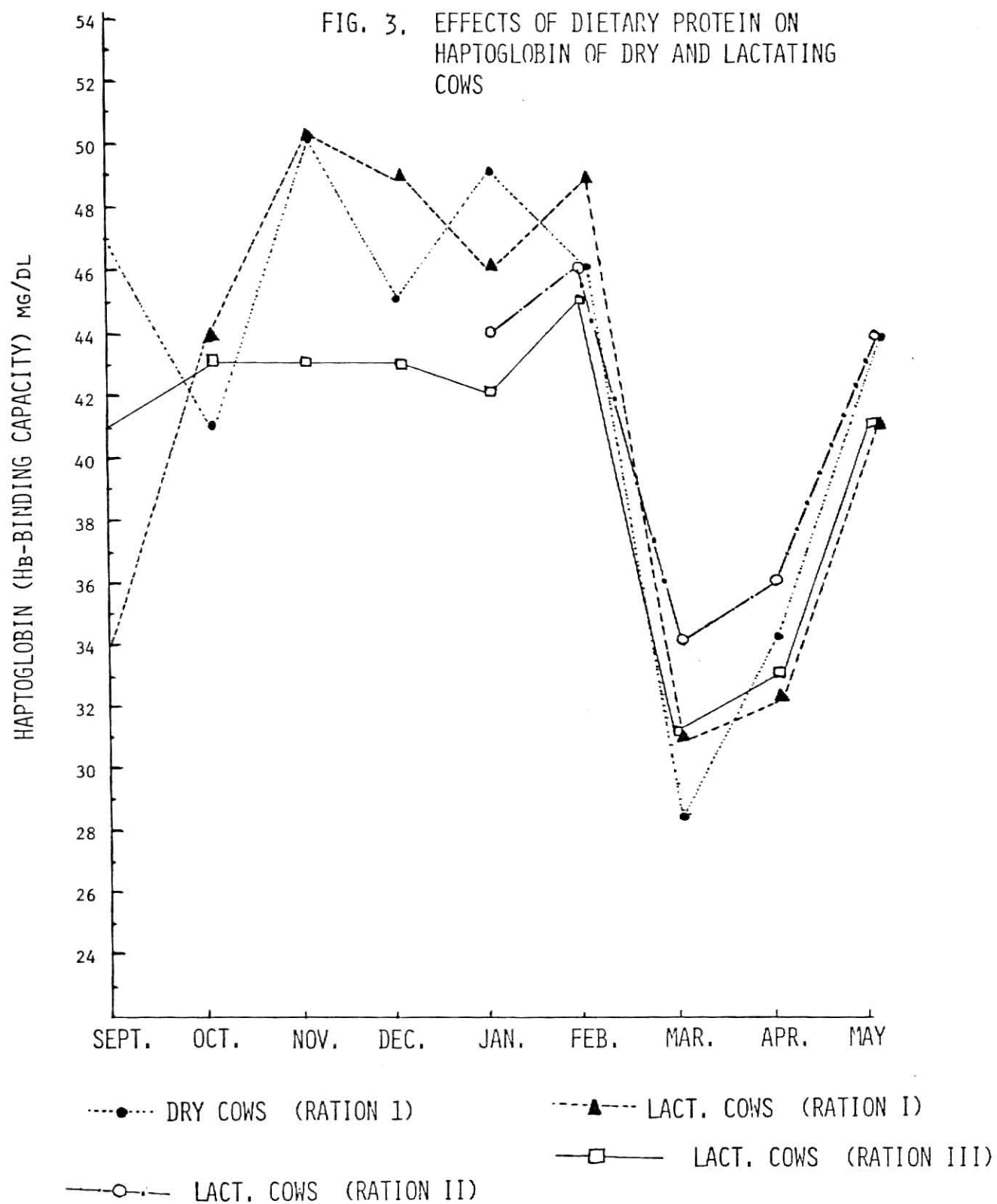


Fig. 2B. Free Hemoglobin Peak

(Arrows indicate direction of migration)

FIG. 3. EFFECTS OF DIETARY PROTEIN ON
HAPTOGLOBIN OF DRY AND LACTATING
COWS



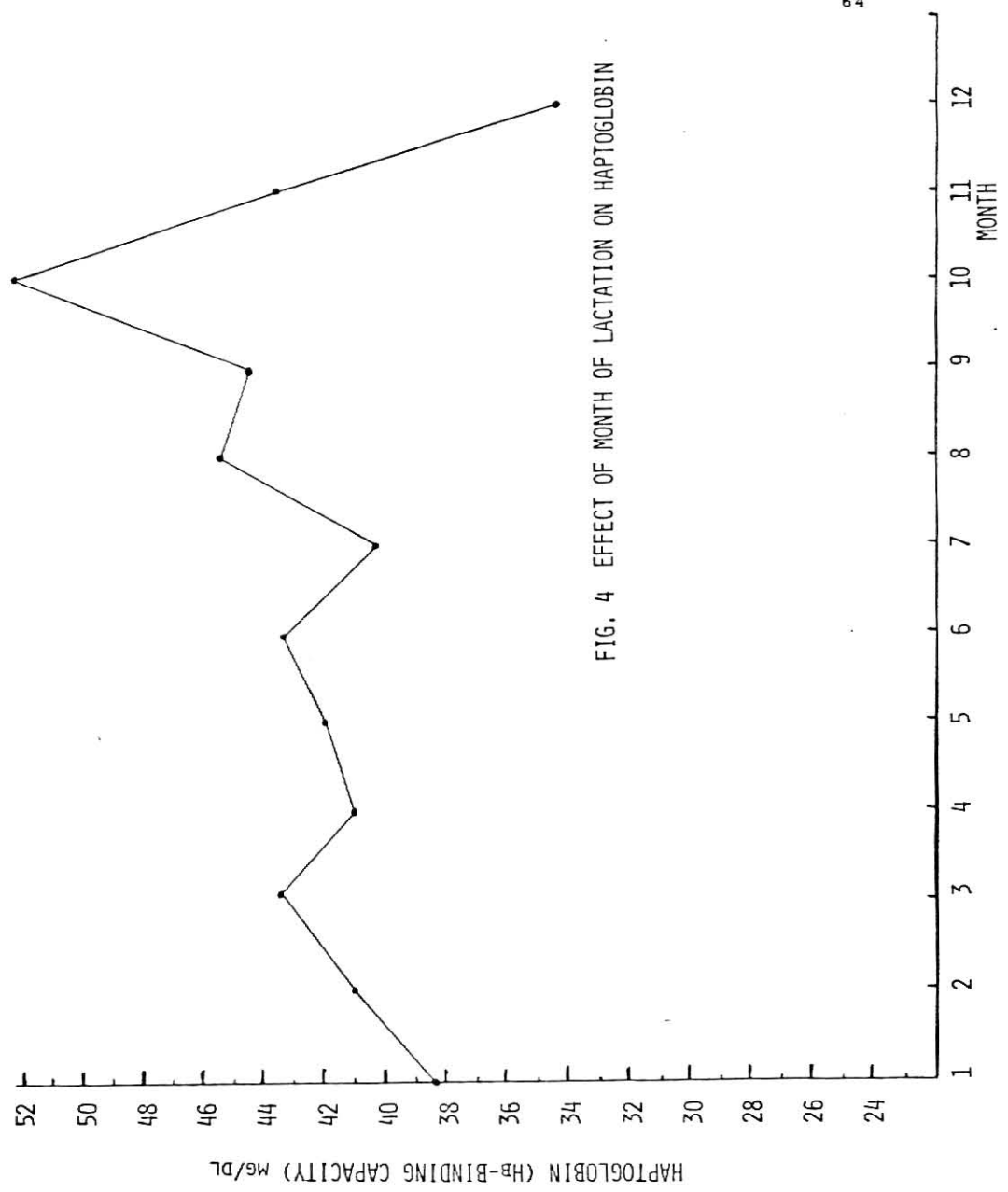


FIG. 4 EFFECT OF MONTH OF LACTATION ON HAPTOGLOBIN

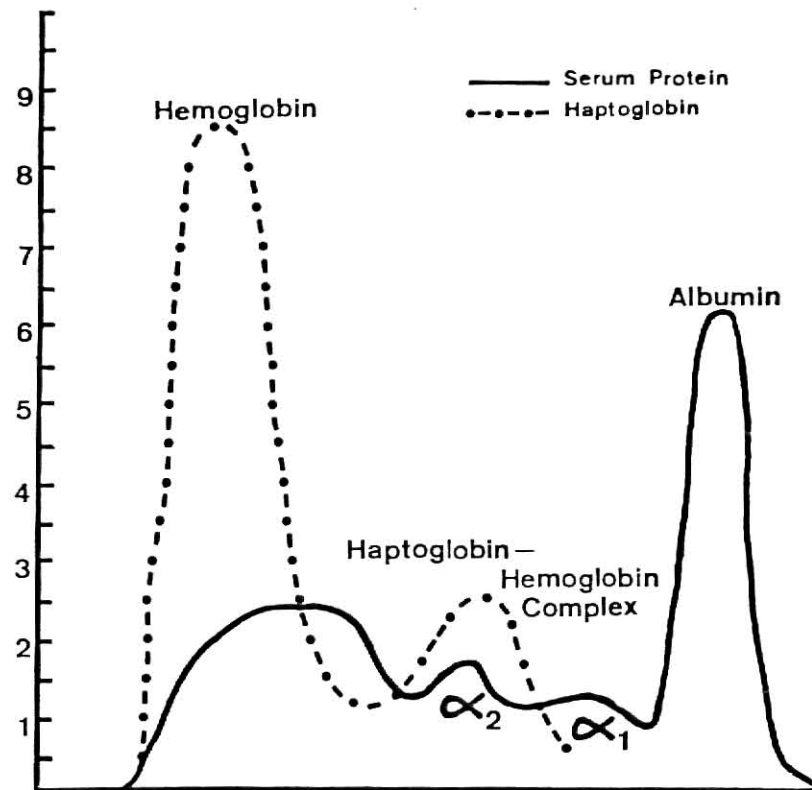


Fig. 5. Electrophoretogram of the haptoglobin-hemoglobin complex compared with that of normal total protein to indicate migratory characteristics of the complex.

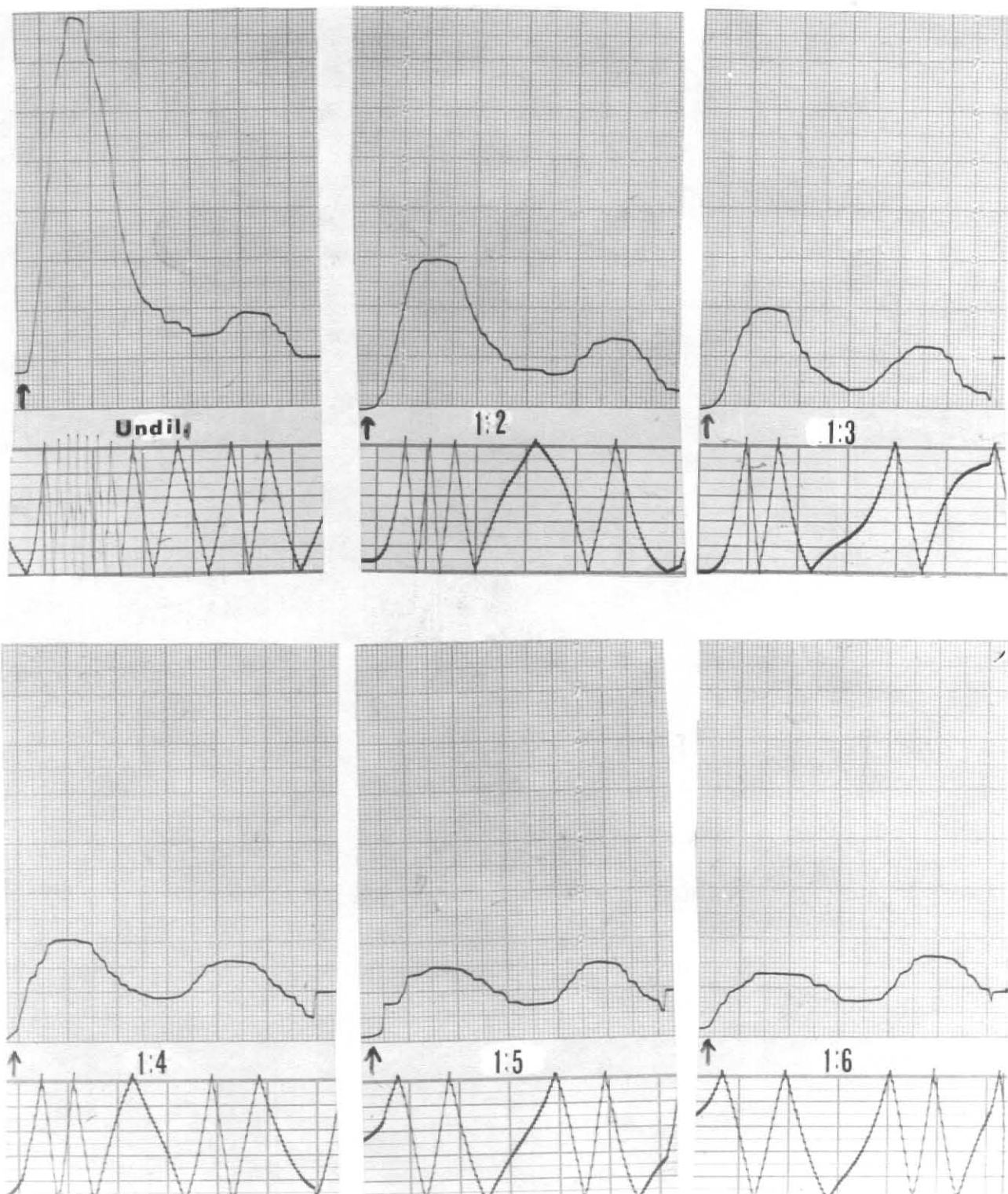
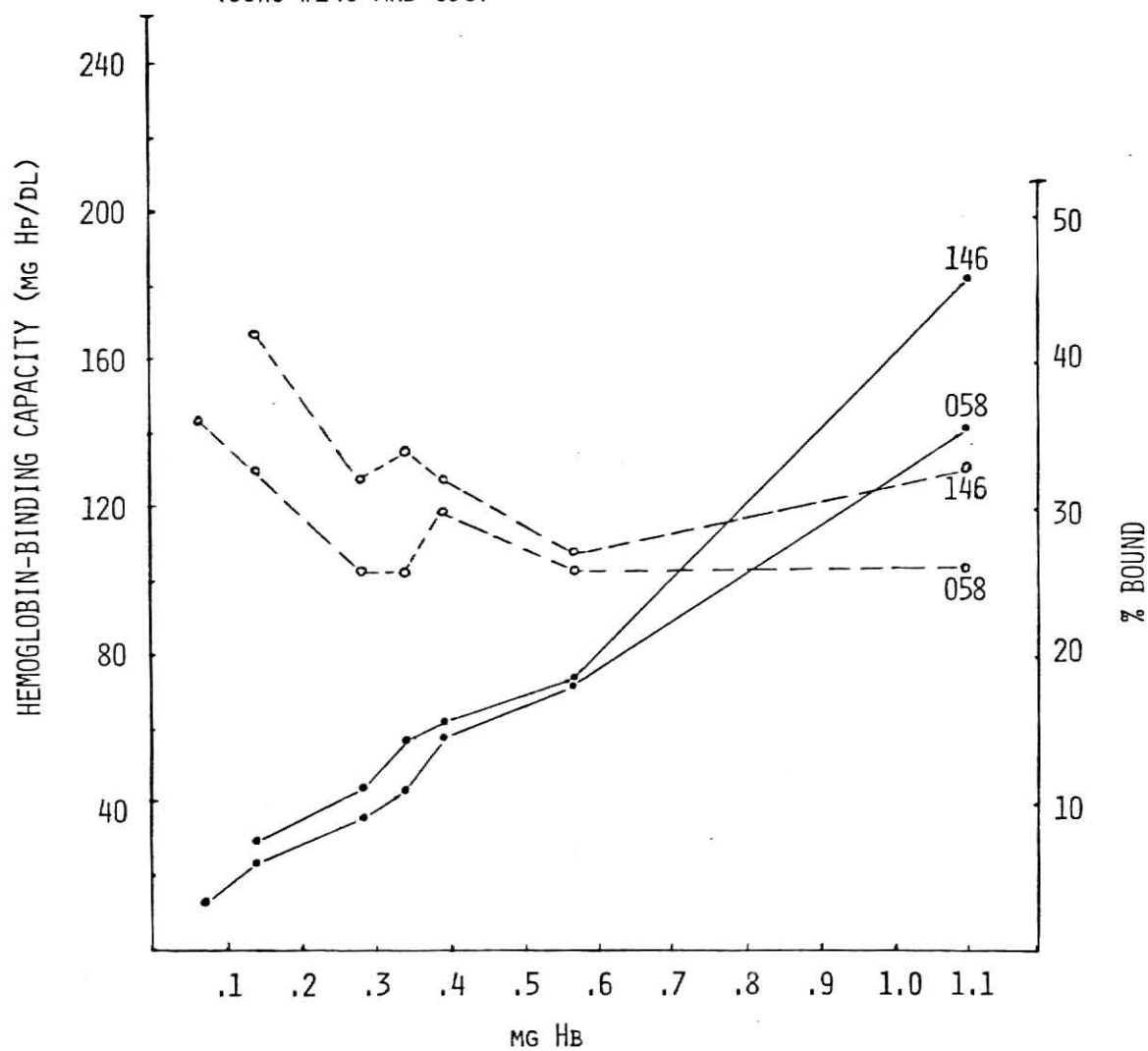


Fig. 6. Changes in Haptoglobin Patterns with Varying Amounts of Hemoglobin.
Arrows indicate points of application.

FIGURE 7

RELATIONSHIPS BETWEEN H_B-BINDING CAPACITY (—•—),
% BOUND (---○---) AND INCREASING H_B CONCENTRATIONS.
(COWS #146 AND 058)



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Lea and Febiger , Philadelphia. Pg. 205.

APPENDIX

INDIVIDUAL ANIMAL DATA ON HIGH
PRODUCING DAIRY CATTLE

No.	Determinations	Units
1	PCV	Vol. %
2	Hemoglobin	gm %
3	RBCx10 ⁶	μ l
4	Leukocytes	μ l
5	Basophil	μ l
6	Eosinophil	μ l
7	Band	μ l
8	Neutrophil	μ l
9	Lymphocyte	μ l
10	Monocyte	μ l
11	Total protein	gm/dl
12	Albumin	gm/dl
13	Haptoglobin	mg/dl
14	Serum Iron	μ g/dl
15	IBC	μ g/dl
16	G6PD	μ M/gm Hb/min
17	GPGD	μ M/gm Hb/min
18	GSH	μ M/gm Hb
19	Glucose	mg/dl
20	Sodium	meq/L
21	Potassium	meq/L
22	Total CO ₂	meq/L
23	B. Urea Nitrogen	mg/dl
24	Alk. Phos.	m μ /ml
25	GPT	m μ /ml
26	Calcium	mg/dl
27	Phosphorus	mg/dl
28	Creatinine	mg/dl
29	SDH	I.U.
30	Chloride	meq/L

Table I

Individual Animal Data on high-producing dairy cattle

Animal No. 033 Date of parturition 2/23/76

	Date of Sampling											
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/24/77	March 3/30/77	April 4/26/77	May 5/25/77			
1.	34.0	33.0	33.0	34.0	-	-	-	-	-	-	-	-
2.	12.8	13.3	11.7	12.5	-	-	-	-	-	-	-	-
3.	6.47	6.87	6.79	7.34	-	-	-	-	-	-	-	-
4.	6,800	5,900	8,700	8,200	-	-	-	-	-	-	-	-
5.	-	-	-	-	-	-	-	-	-	-	-	-
6.	408	-	87	164	-	-	-	-	-	-	-	-
7.	-	177	87	328	-	-	-	-	-	-	-	-
8.	1,292	1,121	2,436	2,050	-	-	-	-	-	-	-	-
9.	4,964	4,307	5,916	5,494	-	-	-	-	-	-	-	-
10.	136	295	174	164	-	-	-	-	-	-	-	-
11.	8.1	8.3	8.2	8.9	-	-	-	-	-	-	-	-
12.	4.0	3.7	3.8	3.8	-	-	-	-	-	-	-	-
13.	24.6	62.4	33.8	52.0	-	-	-	-	-	-	-	-
14.	172	114	140	143	-	-	-	-	-	-	-	-
15.	239	289	274	224	-	-	-	-	-	-	-	-

Table I (Cont'd)
Animal No 033 Date of parturition 2/23/76

		Date of Sampling						
		Sep	Oct	Nov	Dec	Jan	Feb	March
		9/27/76	10/19/76	11/23/76	12/15/76	1/20/77	2/24/77	3/30/77
								April
								4/26/77
								May
								5/25/77
16.	5.9	7.0	4.1	5.9	-	-	-	-
17.	2.5	1.7	1.3	1.6	-	-	-	-
18.	6.4	9.2	8.5	8.4	-	-	-	-
19.	60	72	60	65	-	-	-	-
20.	142	142	137	144	-	-	-	-
21.	4.1	4.4	4.1	4.7	-	-	-	-
22.	30	39	31	28	-	-	-	-
23.	17	17	18	19	-	-	-	-
24.	30	30	25	25	-	-	-	-
25.	17	15	20	16	-	-	-	-
26.	8.5	8.4	8.6	9.4	-	-	-	-
27.	7.2	8.0	6.7	6.7	-	-	-	-
28.	0.9	0.8	0.9	1.0	-	-	-	-
29.	-	-	-	-	-	-	-	-
30.	-	-	-	-	-	-	-	-

Table I (Cont'd)
Individual Animal Data on high-producing dairy cattle
Animal No. 058 Date of parturition 1/1/76

	Date of sampling									
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/24/77	March 3/30/77	April 4/26/77	May 5/25/77	
1.	34.0	34.0	39.0	37.0	36.0	36.0	37.0	34.0	35.0	
2.	13.4	12.9	14.0	13.5	13.9	12.8	13.2	12.4	12.0	
3.	6.88	6.95	7.98	7.84	8.03	6.83	6.72	6.31	6.21	
4.	10,000	9,100	9,100	10,800	10,900	8,800	10,500	11,900	8,100	
5.	-	91	-	108	-	-	-	119	-	
6.	100	637	546	432	436	1,056	1,470	952	1,620	
7.	100	364	182	216	-	-	-	-	-	
8.	2,200	2,184	2,093	2,808	3,488	2,200	2,520	4,998	2,349	
9.	7,100	5,460	5,915	7,128	6,867	5,280	6,195	5,831	4,131	
10.	500	273	364	108	109	264	315	-	-	
11.	8.1	8.0	8.6	8.8	8.4	8.8	8.7	8.4	8.2	
12.	4.2	4.0	4.1	4.1	4.0	4.1	3.9	4.1	3.9	
13.	59.6	68.6	46.5	56.1	57	53.8	23.9	40.6	44.8	
14.	144	104	166	139	116	113	102	139	124	
15.	439	349	430	280	146	231	234	183	190	

Table I (Cont'd)
Animal No. 058 Date of parturition 1/1/76

	Date of Sampling									
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/24/77	March 3/30/77	April 4/26/77	May 5/25/77	
16.	4.9	7.0	5.1	6.0	6.1	6.1	5.9	6.6	7.2	
17.	2.1	1.5	1.7	1.8	1.4	1.3	1.5	1.7	1.7	
18.	6.4	8.1	7.3	7.9	8.5	7.7	7.9	8.1	9.7	
19.	60	80	60	67	65	65	66	63	60	
20.	137	139	136	137	144	140	140	144	142	
21.	3.4	3.2	3.4	3.3	3.4	3.2	3.7	3.9	3.5	
22.	34	33	30	30	38	33	36	36	30	
23.	34	39	40	34	35	32	36	29	26	
24.	42	51	50	40	35	35	37	35	35	
25.	20	25	30	22	32	43	25	26	20	
26.	7.0	9.3	9.3	9.3	9.3	9.0	9.2	10.0	10.0	
27.	5.8	5.5	5.7	5.3	5.6	5.6	6.5	7.1	4.7	
28.	1.1	1.1	1.2	1.5	1.2	1.3	1.5	1.4	1.6	
29.	-	-	-	-	-	9.9	8.6	5.3	6.5	
30.	-	-	-	-	-	97.0	97.0	100.0	101.0	

Table I (Cont'd)

Animal No. 163 Date of parturition 3/11/76, 2/5/77

	Date of Sampling									
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/20/77	March 3/30/77	April 4/26/77	May 5/25/77	
1.	34.0	31.0	31.0	33.0	33.0	33.0	27.0	25.0	25.0	
2.	12.6	11.7	11.2	11.2	11.7	11.4	8.9	8.9	8.6	
3.	6.96	6.96	7.24	7.25	7.30	6.98	6.13	5.74	5.69	
4.	10,400	8,600	7,800	7,900	9,500	9,800	7,200	8,800	14,900	
5.	-	86	-	-	-	98	72	-	298	
6.	728	516	546	474	190	-	72	-	745	
7.	416	86	234	-	95	294	360	88	149	
8.	3,224	2,838	3,496	3,002	4,275	5,586	2,592	3,784	5,215	
9.	5,512	4,386	4,212	4,266	4,845	3,430	3,672	4,312	8,046	
10.	312	602	312	158	95	294	288	616	298	
11.	9.4	8.2	8.3	8.5	8.0	8.5	8.2	8.9	8.6	
12.	3.9	3.6	3.8	3.6	3.5	3.9	3.5	3.6	3.5	
13.	64.4	48.9	35	51.9	47.4	45.4	29.9	35.7	43.0	
14.	212	155	174	182	134	130	79	45	56	
15.	324	346	407	303	157	141	160	103	156	

Table I (Cont'd)

Animal No. 163 Date of parturition 3/11/76, 2/5/77

	Date of Sampling							April 4/26/77	May 5/25/77
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/24/77	March 3/30/77		
16.	5.9	7.1	5.6	7.2	7.0	7.0	.6.4	7.6	7.9
17.	3.1	1.4	1.6	1.9	1.6	1.2	1.5	1.8	2.3
18.	6.2	8.8	7.6	8.2	8.4	8.1	8.2	8.2	9.2
19.	50	65	60	62	63	25	55	55	55
20.	-	139	136	142	142	143	140	138	137
21.	-	3.8	4.0	4.0	4.1	4.0	4.5	4.6	4.1
22.	-	27	28	28	31	28	30	28	31
23.	21	25	26	28	19	10	13	20	20
24.	25	30	30	34	35	38	30	31	30
25.	15	17	20	10	20	23	15	20	22
26.	9.0	9.0	9.0	10.4	9.4	8.6	8.9	9.1	8.3
27.	6.1	7.8	5.3	5.2	5.7	5.4	5.5	6.4	6.9
28.	0.7	0.8	1.0	1.2	1.0	1.1	0.9	0.9	0.9
29.	-	-	-	-	-	15.9	9.2	8.9	10.9
30.	-	-	-	-	-	97.0	101.0	97.0	95.0

Table I (Cont'd)
Animal No. 016 Date of parturition 10/2/76

	Date of Sampling									
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/24/77	March 3/30/77	April 4/26/77	May 5/25/77	
1.	36.0	30.0	27.0	27.0	27.0	33.0	34.0	35.0	34.0	
2.	13.2	10.3	9.9	9.1	9.8	11.0	11.4	13.6	11.4	
3.	6.47	5.35	5.44	5.33	5.50	6.35	6.68	6.63	6.28	
4.	17,700	18,300	17,200	15,300	17,300	17,700	18,000	16,700	12,900	
5.	-	183	-	-	-	177	180	-	129	
6.	1,239	366	172	306	-	531	1,620	167	774	
7.	531	366	999	306	-	-	-	-	-	
8.	5,841	3,477	3,989	2,754	3,633	2,655	4,680	2,338	3,354	
9.	9,912	12,993	11,524	11,628	13,321	13,275	10,440	13,694	8,514	
10.	177	732	516	306	346	1,062	1,080	501	129	
11.	7.6	7.9	8.2	8.5	8.1	8.8	8.7	9.5	8.5	
12.	4.0	3.6	4.0	4.0	3.9	4.3	3.7	3.9	3.3	
13.	79.9	52.3	17.2	21.2	49.7	47.2	26.9	30.8	41.1	
14.	266.0	190	108	180	176	136	136	157	148	
15.	277	254	255	204	203	228	206	198	204	

Table I (Cont'd)

Animal No. 016 Date of parturition 10/2/76

	Date of Sampling									
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/24/77	March 3/30/77	April 4/26/77	May 5/25/77	
16.	4.4	7.4	5.3	6.0	7.3	9.3	8.3	7.8	9.1	
17.	3.3	1.4	1.3	1.6	1.7	1.9	2.5	1.9	2.1	
18.	8.6	10.6	8.7	9.3	8.3	8.3	8.8	9.2	10.8	
19.	75	60	65	65	75	65	71	60	70	
20.	141	140	137	138	142	141	142	140	142	
21.	4.2	4.0	4.0	4.0	4.0	4.2	4.1	4.5	4.5	
22.	27	32	33	28	32	32	31	27	27	
23.	18	16	22	23	21	23	19	23	23	
24.	40	28	20	20	38	40	45	38	35	
25.	10	10	10	8	16	40	25	25	20	
26.	8.7	8.3	8.0	8.4	8.2	8.4	8.3	8.8	8.4	
27.	5.1	6.5	5.8	6.1	8.0	6.6	6.7	7.1	7.4	
28.	1.0	0.8	0.9	0.8	0.8	0.7	0.9	0.8	0.9	
29.	-	-	-	-	-	10.9	9.6	8.8	9.6	
30.	-	-	-	-	-	99.0	104.0	103.0	100.0	

Table I (Cont'd)
Animal No. 078 Date of parturition 1/5/76

	Date of sampling									
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/24/77	March 3/30/77	April 4/26/77	May 5/25/77	
1.	32.0	30.0	31.0	31.0	32.0	36.0	-	-	-	-
2.	11.6	11.2	11.4	11.4	11.3	12.0	-	-	-	-
3.	5.88	5.91	6.05	6.27	6.26	6.22	-	-	-	-
4.	7,900	9,000	8,200	12,000	7,400	8,700	-	-	-	-
5.	-	90	-	120	74	-	-	-	-	-
6.	-	270	656	360	370	783	-	-	-	-
7.	-	360	328	240	-	174	-	-	-	-
8.	-	1,890	2,378	2,280	1,850	3,045	-	-	-	-
9.	-	6,030	4,674	8,160	5,032	4,089	-	-	-	-
10.	-	360	164	720	74	609	-	-	-	-
11.	9.4	8.9	8.6	9.0	9.1	9.9	-	-	-	-
12.	4.1	3.9	3.9	3.9	3.9	4.0	-	-	-	-
13.	28.2	46.6	35.4	38.6	39.4	35.1	-	-	-	-
14.	170	120	149	205	163	170	-	-	-	-
15.	358	251	315	254	185	375	-	-	-	-

Table I (Cont'd)
Animal No. 078 Date of parturition 1/5/76

	Date of Sampling									
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/24/77	March 3/30/77	April 4/26/77	May 5/25/77	
16.	3.7	6.2	5.4	5.6	6.0	5.5	-	-	-	-
17.	1.6	1.4	1.4	1.6	1.5	1.2	-	-	-	-
18.	6.7	9.1	8.9	9.6	9.9	10.1	-	-	-	-
19.	60	70	62	65	70	60	-	-	-	-
20.	139	139	141	138	145	140	-	-	-	-
21.	4.5	4.2	4.4	3.9	3.5	3.7	-	-	-	-
22.	28	30	27	27	33	28	-	-	-	-
23.	25	24	24	27	23	15	-	-	-	-
24.	45	55	55	47	42	50	-	-	-	-
25.	17	15	20	23	15	28	-	-	-	-
26.	7.0	8.8	9.3	9.2	9.4	10.6	-	-	-	-
27.	6.9	6.6	7.9	5.1	6.1	3.7	-	-	-	-
28.	0.8	0.7	0.9	1.1	0.9	1.0	-	-	-	-
29.	-	-	-	-	-	15.6	-	-	-	-
30.	-	-	-	-	-	105.0	-	-	-	-

Table I (Cont'd)

Animal No. 146 Date of parturition 5/11/76

	Date of Sampling									May 5/25/77
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/24/77	March 3/30/77	April 4/26/77		
1.	31.0	31.0	33.0	35.0	35.0	34.0	34.0	38.0	-	
2.	11.0	11.4	11.7	11.9	12.0	11.4	12.4	13.2	-	
3.	5.76	6.36	7.06	7.34	7.05	6.57	6.67	7.28	-	
4.	11,200	8,200	9,800	9,200	11,900	7,800	8,600	9,900	-	
5.	112	-	-	-	-	78	-	-	-	
6.	1,680	82	196	92	-	156	86	693	-	
7.	336	82	294	-	119	78	86	-	-	
8.	1,680	1,394	1,862	1,932	3,451	1,794	2,150	3,168	-	
9.	7,056	6,314	6,958	6,808	7,735	5,226	6,106	5,742	-	
10.	336	328	490	368	595	468	172	297	-	
11.	8.0	7.9	8.4	8.0	8.6	8.4	8.8	8.8	-	
12.	4.0	3.9	4.1	3.8	3.8	3.8	3.5	3.8	-	
13.	33.5	69.5	28.7	48.7	59.6	53.8	41.1	40.3	-	
14.	-	186	170	187	113	163	196	129	-	
15.	-	276	341	277	173	237	263	166	-	

Table I (Cont'd)

Animal No. 146 Date of parturition 5/11/76

	Date of Sampling									
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/24/77	March 3/30/77	April 4/26/77	May 5/25/77	
16.	4.1	8.4	6.0	6.2	6.2	5.7	6.3	6.8	-	
17.	4.2	2.4	2.7	2.9	2.1	1.5	2.1	2.5	-	
18.	6.4	8.2	7.6	8.9	9.1	8.4	8.8	8.4	-	
19.	60	70	65	62	67	65	67	60	-	
20.	138	140	131	142	139	140	140	140	-	
21.	4.7	4.0	4.1	3.9	4.2	4.3	4.4	4.5	-	
22.	27	32	31	27	31	31	29	27	-	
23.	15	16	16	19	18	16	15	17	-	
24.	30	28	37	25	25	21	25	27	-	
25.	20	10	25	28	35	47	28	35	-	
26.	8.2	8.3	9.2	9.3	9.7	8.7	9.2	10.2	-	
27.	7.1	6.5	6.7	6.5	7.2	6.2	8.3	5.9	-	
28.	0.7	0.8	0.9	1.1	0.8	0.9	1.0	0.9	-	
29.	-	-	-	-	-	8.0	6.5	8.7	-	
30.	-	-	-	-	-	100.0	101.0	100.0	-	

Table I (Cont'd)

Animal No. 051 Date of parturition 12/16/76

	Date of Sampling									
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/24/77	March 3/30/77	April 4/26/77	May 5/25/77	
1.	36.0	37.0	36.5	35.0	31.0	28.0	29.0	28.0	26.5	
2.	12.6	13.6	12.4	12.1	12.4	9.2	11.4	9.2	8.8	
3.	5.91	6.77	6.42	6.53	7.07	4.91	5.48	5.29	4.83	
4.	6,600	8,400	8,400	14,400	6,700	6,100	7,200	6,800	6,300	
5.	-	84	-	-	-	61	-	136	-	
6.	132	252	420	144	603	305	432	544	252	
7.	-	420	-	1,296	-	122	216	-	63	
8.	2,178	2,352	3,696	8,505	2,546	1,830	2,304	1,972	3,276	
9.	3,960	5,124	4,116	4,032	3,417	3,355	3,888	3,672	2,583	
10.	330	168	168	576	134	427	360	476	63	
11.	7.7	7.6	7.5	6.2	7.6	7.8	8.0	7.7	8.5	
12.	4.0	3.8	3.9	3.5	3.8	4.0	3.8	4.0	3.4	
13.	22.8	77.6	29.2	34.6	40.2	43.6	28.6	31.1	45.8	
14.	215	194	200	182	103	87	80	99	101	
15.	293	387	325	224	163	172	186	112	133	

Table I (Cont'd)

Animal No. 051 Date of parturition 12/16/76

		Date of Sampling								
		Sep	Oct	Nov	Dec	Jan	Feb	March	April	May
		9/27/76	10/19/76	11/23/76	12/15/76	1/20/77	2/24/77	3/30/77	4/26/77	5/25/77
16.	4.7	6.8	5.8	7.0	5.8	6.3	7.3	6.5	8.4	
17.	3.2	1.5	1.8	2.0	1.3	1.2	2.5	2.0	2.2	
18.	9.8	11.1	10.0	10.6	13.2	11.8	11.4	10.1	11.6	
19.	70	75	70	65	60	56	67	60	67	
20.	140	139	141	141	151	140	143	138	141	
21.	3.9	3.8	4.1	3.8	3.2	3.9	4.5	4.4	4.1	
22.	30	27	30	26	34	25	29	27	28	
23.	21	21	23	17	20	22	13	19	16	
24.	117	130	105	88	75	80	100	103	125	
25.	15	20	20	7	20	45	28	30	18	
26.	8.7	10.0	9.4	6.4	8.8	8.4	8.4	8.4	8.5	
27.	7.1	5.9	5.1	4.1	7.0	6.4	8.1	7.8	6.0	
28.	1.0	0.9	1.1	1.7	1.0	1.0	1.0	0.8	0.7	
29.	-	-	-	-	-	10.2	9.0	8.2	9.2	
30.	-	-	-	-	-	96.5	104.0	99.0	99.0	

Table I (Cont'd)
Animal No. 055 Date of parturition 11/12/76

	Date of Sampling									
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/24/77	March 3/30/77	April 4/26/77	May 5/25/77	
1.	32.0	32.0	28.0	27.0	31.0	32.0	29.0	32.0	34.0	
2.	12.3	11.7	9.2	9.2	11.7	11.7	11.4	11.0	11.0	
3.	5.88	6.21	5.66	5.48	6.28	6.59	6.39	6.77	6.60	
4.	7,500	7,800	4,800	6,400	8,900-	7,600	9,900	9,100	8,500	
5.	-	-	-	-	89	-	-	-	-	
6.	300	312	-	448	267	468	198	728	1,275	
7.	375	624	240	640	-	156	198	-	85	
8.	2,175	2,964	2,064	960	2,759	2,886	5,148	3,185	2,805	
9.	4,275	3,588	2,064	4,224	5,162	3,822	3,960	4,823	4,080	
10.	375	156	144	128	623	468	396	364	170	
11.	8.4	8.6	8.3	8.3	8.1	8.5	8.6	8.9	8.2	
12.	4.0	3.8	3.1	3.3	3.6	3.9	3.6	4.0	3.1	
13.	28.5	25.7	36.5	44.7	54.0	48.5	38.2	39.3	43.7	
14.	157	132	155	138	110	115	68	115	113	
15.	297	245	201	266	123	237	106	163	171	

Table I (Cont'd)

Animal No. 055 Date of parturition 11/12/76

	Date of Sampling									
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/24/77	March 3/30/77	April 4/26/77	May 5/25/77	
16.	3.7	7.2	5.4	6.5	6.8	7.2	5.1	7.6	8.6	
17.	3.0	2.0	1.9	2.4	2.2	2.1	1.4	2.7	2.9	
18.	8.6	11.0	9.5	10.1	10.5	9.8	10.7	10.9	13.1	
19.	60	70	65	63	70	70	75	62	70	
20.	141	140	135	139	147	145	142	145	141	
21.	4.2	4.3	3.6	4.0	3.9	4.6	4.4	4.5	4.8	
22.	28	31	29	27	37	26	30	30	29	
23.	23	20	16	20	16	22	15	20	19	
24.	20	25	35	18	32	28	30	30	36	
25.	20	16	5	8	22	43	25	28	30	
26.	9.0	9.9	8.3	8.1	9.4	9.0	9.1	9.2	8.7	
27.	5.0	4.7	4.1	5.0	5.3	5.8	5.5	6.7	6.2	
28.	0.9	0.8	0.9	1.0	0.9	0.9	0.9	0.9	1.0	
29.	-	-	-	-	-	9.4	6.9	8.6	8.8	
30.	-	-	-	-	-	104.5	105.0	102.0	100.0	

Table I (Cont'd)

Animal No. 020 Date of parturition 1/8/77

	Date of Sampling									
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/20/77	March 3/30/77	April 4/26/77	May 5/25/77	
1.	30.0	28.0	32.0	33.0	28.0	28.0	25.0	26.0	28.5	
2.	10.6	9.9	11.0	11.2	10.2	9.2	8.9	8.9	9.4	
3.	6.00	5.72	6.34	6.68	5.74	5.76	5.61	5.26	5.56	
4.	6,900	6,000	4,800	8,200	8,500	6,900	7,000	7,600	6,100	
5.	-	-	48	82	-	69	-	156	-	
6.	690	600	480	902	425	552	630	76	427	
7.	207	60	336	82	-	483	280	-	122	
8.	2,346	1,020	1,680	2,624	3,655	2,484	2,380	3,116	3,294	
9.	3,519	4,020	2,256	4,182	4,335	2,760	3,570	3,952	2,135	
10.	138	300	-	246	85	414	140	304	122	
11.	8.3	8.0	8.4	9.1	8.1	8.4	8.8	9.7	9.3	
12.	3.9	3.4	3.6	3.8	3.5	3.7	3.4	3.3	3.3	
13.	35.1	22.6	11.1	45.1	37.6	45.4	35.0	38.6	41.5	
14.	47	167	126	207	121	134	69	80	98	
15.	177	230	201	230	179	175	132	166	158	

Table I (Cont'd)

Animal No. 020		Date of parturition 1/8/77		Date of Sampling									
		Sep	Oct	Nov	Dec	Jan	Feb	March	April	May			
		9/27/76	10/19/76	11/23/76	12/15/76	1/20/77	2/24/77	3/30/77	4/26/77	5/25/77			
16.	7.6	7.8	5.9	6.7	6.0	7.0	7.3	7.6	7.9				
17.	3.3	1.9	1.9	2.2	1.9	1.3	1.8	1.9	2.5				
18.	8.2	8.4	7.4	8.5	8.4	7.4	8.3	7.7	8.5				
19.	65	75	70	60	57	67	71	62	65				
20.	141	139	137	141	143	141	142	141	142				
21.	4.3	4.3	3.7	4.7	4.0	4.0	4.3	4.2	4.1				
22.	29	30	29	30	35	36	34	31	28				
23.	28	25	25	23	19	24	18	24	26				
24.	35	40	55	43	40	25	41	40	43				
25.	15	12	15	10	10	20	20	18	21				
26.	9.0	8.7	8.6	9.8	8.0	8.6	8.3	9.2	8.3				
27.	6.4	6.2	3.6	4.7	4.6	5.6	6.7	7.0	6.4				
28.	0.9	0.8	1.0	1.0	1.0	0.8	0.9	0.7	1.0				
29.	-	-	-	-	-	7.0	7.7	6.4	7.8				
30.	-	-	-	-	-	95.5	101.0	101.0	96.0				

Table I (Cont'd)

Animal No. 100 Date of parturition 9/19/76

	Date of Sampling									
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/24/77	March 3/30/77	April 4/26/77	May 5/25/77	
1.	34.0	30.0	29.5	28.0	31.0	32.0	32.0	38.0	34.0	
2.	13.6	11.3	10.6	10.5	11.3	11.3	11.4	13.6	11.4	
3.	7.49	6.00	6.03	6.19	6.42	6.52	6.66	7.71	6.65	
4.	5,100	6,000	5,600	9,400	6,700	6,900	8,700	9,100	6,300	
5.	-	-	-	-	67	138	87	91	63	
6.	-	420	224	470	268	1,104	696	455	567	
7.	102	-	168	188	67	207	348	182	126	
8.	1,683	2,220	1,456	3,384	2,747	2,760	4,698	4,095	2,457	
9.	3,162	3,060	3,304	4,888	3,082	2,139	2,262	3,731	2,961	
10.	153	300	448	470	402	552	609	455	126	
11.	6.5	7.8	7.3	7.8	7.8	7.7	7.5	8.9	7.6	
12.	3.1	3.5	3.7	3.8	3.9	4.0	3.8	4.4	3.6	
13.	38.3	20.9	86.3	42.3	31.5	44.2	30.3	28.1	40.5	
14.	-	119	120	195	102	111	62	124	124	
15.	-	239	236	224	170	261	143	186	182	

Table I (Cont'd)

Animal No. 100 Date of parturition 9/19/76

	Date of Sampling									
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/24/77	March 3/30/77	April 4/26/77	May 5/25/77	
16.	4.4	5.0	3.8	5.1	4.9	6.4	5.8	5.0	4.6	
17.	2.0	1.0	0.7	1.3	1.2	1.5	1.8	1.2	1.3	
18.	8.0	9.4	7.7	8.6	7.3	6.7	8.2	7.6	8.3	
19.	70	60	72	68	70	68	77	65	70	
20.	140	141	138	141	144	141	145	146	142	
21.	3.7	4.6	4.8	4.0	4.1	4.5	4.4	5.0	4.0	
22.	23	28	26	28	29	32	31	27	31	
23.	7	12	15	15	15	19	12	16	17	
24.	35	35	30	37	47	47	50	53	39	
25.	15	5	5	5	16	36	24	25	20	
26.	7.4	8.4	8.0	8.6	8.4	8.1	8.4	8.5	8.6	
27.	3.6	6.4	7.9	7.6	7.4	7.6	6.8	7.7	7.1	
28.	0.8	0.9	0.9	1.2	1.0	0.9	1.0	0.9	1.0	
29.	-	-	-	-	-	10.0	9.7	10.3	9.2	
30.	-	-	-	-	-	98.0	103.0	96.0	97.0	

Table I (Cont'd)

Animal No. 131 Date of parturition 3/19/76

	Date of Sampling								
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/24/77	March 3/30/77	April 4/26/77	May 5/25/77
1.	31.0	31.0	31.0	34.0	33.0	32.0	34.0	35.0	34.0
2.	12.2	11.2	11.2	12.1	12.2	11.3	11.7	11.4	12.0
3.	6.47	6.48	6.82	7.77	7.76	6.28	6.55	6.01	6.85
4.	6,000	5,600	5,700	7,100	5,600	4,600	5,700	5,400	4,900
5.	-	-	57	-	56	-	-	-	49
6.	480	280	228	426	392	322	456	432	439
7.	240	168	285	-	56	46	57	54	49
8.	1,620	1,232	1,995	1,491	1,512	1,104	2,109	1,566	1,568
9.	3,480	3,752	2,850	4,757	3,136	2,576	2,793	3,024	2,548
10.	180	112	285	355	448	552	228	324	98
11.	8.0	8.1	8.0	8.5	8.6	9.2	8.6	9.3	8.3
12.	3.8	3.8	4.0	4.1	4.1	4.0	3.5	4.0	3.9
13.	35.3	43.0	45.9	49.9	42.8	53.7	22.4	23.7	40.6
14.	-	147	148	261	147	113	86	145	143
15.	-	320	185	299	206	225	103	191	201

Table I (Cont'd)
Animal No. 131 Date of parturition 3/19/76

	Date of Sampling									
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/24/77	March 3/30/77	April 4/26/77	May 5/25/77	
16.	3.4	5.1	4.9	5.6	6.6	6.5	5.5.9	6.4	7.1	
17.	2.0	1.4	1.7	1.8	1.5	1.4	1.5	1.3	1.5	
18.	6.2	8.3	7.4	8.4	8.4	7.3	8.5	8.6	10.1	
19.	55	75	70	65	65	66	65	57	57	
20.	-	140	138	143	143	140	143	139	143	
21.	-	4.4	4.1	4.6	3.8	4.3	4.4	4.3	4.1	
22.	-	28	29	26	31	28	30	27	28	
23.	14	18	21	21	18	19	16	16	13	
24.	20	27	25	40	25	17	23	22	25	
25.	10	13	20	16	20	16	20	20	20	
26.	8.4	8.2	8.9	9.7	8.4	8.3	8.2	8.0	8.2	
27.	8.1	9.9	7.3	7.7	7.6	6.2	7.2	6.5	7.0	
28.	0.8	0.8	1.0	1.4	1.0	0.9	1.2	1.0	1.1	
29.	-	-	-	-	-	8.1	6.7	6.0	7.1	
30.	-	-	-	-	-	101.0	103.0	95.0	103.0	

Table I (Cont'd)

Animal No. 118 Date of parturition 10/1/76

	Date of Sampling									
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/24/77	March 3/30/77	April 4/26/77	May 5/25/77	
1.	32.0	30.0	30.0	32.0	32.0	36.0	33.0	36.0	33.0	
2.	12.0	10.6	10.0	11.4	11.6	12.0	12.5	12.2	11.0	
3.	5.40	5.38	6.29	6.39	6.59	6.96	7.00	6.47	6.85	
4.	6,100	6,100	10,300	11,300	16,400	10,500	14,300	13,100	9,900	
5.	-	61	-	226	164	105	143	262	99	
6.	61	671	515	678	2,952	630	1,573	3,013	1,485	
7.	1,224	183	412	791	164	-	143	131	-	
8.	1,939	1,647	3,399	2,825	5,904	3,675	4,290	2,489	2,574	
9.	2,142	3,294	5,459	6,441	6,560	5,460	7,722	6,812	5,247	
10.	184	244	515	339	656	630	429	393	495	
11.	9.5	10.1	5.2	7.7	9.5	9.6	9.7	9.4	9.2	
12.	3.1	2.8	1.9	3.2	3.7	3.8	3.4	3.5	3.5	
13.	34.3	30.4	76.6	43.0	37.3	48.6	37.7	38.8	42.5	
14.	-	65	169	-	123	120	76	96	114	
15.	-	104	278	-	206	221	112	138	151	

Table I (Cont'd)

Animal No. 118 Date of parturition 10/1/76

	Date of Sampling									
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/24/77	March 3/30/77	April 4/26/77	May 5/25/77	
16.	4.3	7.3	4.7	6.6	7.1	6.7	7.1	6.7	7.8	
17.	1.8	1.3	1.7	2.0	2.1	2.0	2.0	1.7	1.8	
18.	6.8	7.9	5.2	7.8	8.5	7.2	9.6	9.3	9.4	
19.	60	80	60	76	70	72	70	73	65	
20.	-	134	140	139	145	142	138	139	144	
21.	-	4.2	4.3	4.4	3.9	4.3	4.2	4.6	4.4	
22.	-	26	27	30	32	30	31	28	28	
23.	8	12	15	15	16	24	13	16	15	
24.	30	30	40	26	35	34	28	38	35	
25.	-	5	25	10	20	40	22	20	18	
26.	7.4	9.4	8.6	9.1	9.0	9.8	8.8	9.0	8.8	
27.	4.0	6.8	5.0	4.9	5.3	5.9	5.7	5.5	6.2	
28.	0.7	0.6	0.8	-	0.9	0.9	0.9	0.9	1.0	
29.	-	-	-	-	-	8.3	6.9	10.0	11.7	
30.	-	-	-	-	-	100.	103.0	107.0	102.0	

Table I (Cont'd)
Animal No. 023 (Dry throughout Research period)

	Date of Sampling									
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/24/77 ^m	March 3/30/77	April 4/26/77	May 5/25/77	
1.	39.0	40.0	40.0	40.0	35.0	39.0	45.0	42.0	39.5	
2.	13.4	14.1	13.6	13.6	13.6	13.8	15.0	14.0	13.0	
3.	7.90	7.87	7.79	8.54	8.36	7.59	8.17	7.84	6.85	
4.	7,400	7,700	7,800	7,800	7,100	6,900	8,000	9,900	6,100	
5.	-	-	156	-	71	69	-	-	-	
6.	888	539	624	702	710	414	560	4,158	976	
7.	74	462	312	702	71	138	80	-	122	
8.	2,664	2,233	3,276	2,808	2,485	2,967	3,200	2,178	2,440	
9.	3,330	4,158	2,886	3,354	3,408	2,898	3,600	3,069	2,257	
10.	444	231	546	234	284	345	560	495	305	
11.	8.5	8.5	8.3	8.9	8.4	8.3	8.9	8.8	8.3	
12.	3.9	3.9	3.9	4.0	4.0	4.1	4.0	4.2	3.8	
13.	39.2	30.4	76.7	43.4	42.4	43.5	25.4	34.0	43.2	
14.	-	102	133	195	146	125	97	162	154	
15.	-	217	207	204	174	243	186	231	233	

Table I (Cont'd)

Animal No. 023 (Dry throughout Research period)

	Date of Sampling									
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/24/77	March 3/30/77	April 4/26/77	May 5/25/77	
16.	3.5	6.1	4.8	6.4	5.6	6.0	6.5	6.6	7.5	
17.	2.8	1.8	1.4	2.1	1.5	2.2	2.2	2.0	2.0	
18.	7.6	8.8	8.1	9.2	9.1	7.6	9.8	8.7	9.8	
19.	75	75	70	60	70	71	70	60	75	
20.	140	136	126	143	139	141	141	141	144	
21.	4.0	4.2	4.2	5.1	4.1	4.0	4.4	4.0	3.9	
22.	34	33	29	29	33	31	27	27	25	
23.	17	18	20	17	15	16	15	12	10	
24.	35	40	45	29	37	35	36	38	35	
25.	15	25	30	23	30	40	22	24	20	
26.	8.5	9.3	9.3	9.9	9.6	8.6	8.3	9.6	9.7	
27.	6.8	4.8	6.6	4.8	5.4	7.1	6.7	5.0	5.2	
28.	1.0	0.8	1.0	1.0	0.9	1.0	1.1	1.0	1.1	
29.	-	-	-	-	-	11.4	7.7	8.9	8.6	
30.	-	-	-	-	-	103.0	106.0	107.0	108.0	

Table I (Cont'd)
Animal No. 177- Date of parturition 2/16/76

	Date of Sampling								
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/24/77	March 3/30/77	April 4/26/77	May 5/25/77
1.	31.0	32.0	32.0	33.0	-	-	-	-	-
2.	11.0	10.8	10.8	11.0	-	-	-	-	-
3.	5.76	6.22	6.05	6.47	-	-	-	-	-
4.	8,200	12,400	11,300	18,700	-	-	-	-	-
5.	-	248	-	-	-	-	-	-	-
6.	328	124	226	-	-	-	-	-	-
7.	328	372	-	748	-	-	-	-	-
8.	2,708	4,464	4,068	9,911	-	-	-	-	-
9.	4,674	6,448	6,102	6,919	-	-	-	-	-
10.	164	744	904	935	-	-	-	-	-
11.	8.3	8.6	7.6	7.1	-	-	-	-	-
12.	3.7	3.7	3.8	3.6	-	-	-	-	-
13.	64.1	30.6	44.9	37.7	-	-	-	-	-
14.	179	139	165	103	-	-	-	-	-
15.	304	273	239	197	-	-	-	-	-

Table I (Cont'd)

Animal No. 177 Date of parturition 2/16/76

	Date of Sampling											
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/24/77	March 3/30/77	April 4/26/77	May 5/25/77			
16.	3.9	4.8	4.5	6.3	-	-	-	-	-	-	-	-
17.	2.7	1.4	1.9	2.7	-	-	-	-	-	-	-	-
18.	8.0	10.2	7.7	9.4	-	-	-	-	-	-	-	-
19.	65	75	70	55	-	-	-	-	-	-	-	-
20.	135	138	136	146	-	-	-	-	-	-	-	-
21.	4.1	4.3	3.5	4.3	-	-	-	-	-	-	-	-
22.	27	30	28	30	-	-	-	-	-	-	-	-
23.	18	24	24	23	-	-	-	-	-	-	-	-
24.	30	33	35	64	-	-	-	-	-	-	-	-
25.	15	23	20	5	-	-	-	-	-	-	-	-
26.	9.2	9.7	9.6	7.9	-	-	-	-	-	-	-	-
27.	7.5	4.9	3.8	6.0	-	-	-	-	-	-	-	-
28.	0.9	0.9	1.1	1.7	-	-	-	-	-	-	-	-
29.	-	-	-	-	-	-	-	-	-	-	-	-
30.	-	-	-	-	-	-	-	-	-	-	-	-

Table I (Cont'd)

	Animal No. 165		Date of parturition 1/11/77		Date of Sampling						
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/24/77	March 3/30/77	April 4/26/77	May 5/25/77		
1.	29.0	29.0	32.5	28.0	33.0	30.0	27.0	30.0	30.5		
2.	10.4	11.2	11.3	9.9	11.0	9.9	9.5	9.8	10.0		
3.	5.88	6.04	6.49	6.35	6.69	5.93	5.64	5.97	6.12		
4.	9,000	7,900	8,400	7,800	6,500	6,000	6,300	6,700	5,300		
5.	-	-	-	78	-	-	63	-	-		
6.	720	395	252	312	-	-	126	603	212		
7.	180	158	336	156	-	120	126	134	159		
8.	2,790	1,738	2,352	3,198	585	1,440	2,205	2,412	1,378		
9.	4,950	5,372	5,124	3,978	5,265	3,960	3,654	3,015	3,286		
10.	360	237	336	-	650	420	126	469	265		
11.	7.1	7.4	7.9	7.7	7.7	8.4	7.8	8.4	8.2		
12.	3.6	3.7	3.5	3.5	3.7	4.1	3.8	3.8	3.9		
13.	32.6	50.8	42.3	45.3	36.3	43.6	27.4	30.4	46.5		
14.	-	209	169	177	94	78	89	94	96		
15.	-	327	215	281	188	215	206	369	235		

Table I (Cont'd)
Animal No. 165 Date of parturition 1/11/77

	Date of Sampling									
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/24/77	March 3/30/77	April 4/26/77	May 5/25/77	
16.	2.1	4.2	4.0	4.9	4.5	4.4	4.8	5.9	6.8	
17.	2.4	1.3	1.4	1.8	1.4	1.2	0.9	1.9	1.7	
18.	8.5	9.8	7.0	8.5	6.3	8.9	8.8	7.5	9.2	
19.	65	70	65	62	50	52	70	50	60	
20.	131	136	137	140	139	139	137	133	140	
21.	3.8	4.2	4.4	4.6	4.6	4.5	4.3	4.6	4.1	
22.	22	27	30	24	28	28	27	23	23	
23.	22	23	21	23	21	28	15	22	25	
24.	30	40	42	53	35	32	40	40	38	
25.	17	16	15	7	14	27	25	23	27	
26.	8.9	8.8	10.0	9.8	9.5	9.2	8.2	8.7	9.0	
27.	6.9	7.0	5.8	4.4	5.2	6.3	5.5	5.3	6.3	
28.	0.9	0.9	1.0	1.4	1.1	1.1	1.0	0.9	0.9	
29.	-	-	-	-	-	14.1	11.6	10.8	25.8	
30.	-	-	-	-	-	100.0	104.0	102.0	104.0	

Table I (Cont'd)
Animal No. 117 Date of parturition 8/18/76

	Date of Sampling									
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/24/77	March 3/30/77	April 4/26/77	May 5/25/77	
1.	28.0	30.0	33.0	33.0	33.0	34.0	33.0	32.0	-	-
2.	9.9	10.6	11.0	11.9	11.4	11.0	12.4	-	-	-
3.	5.18	5.80	7.50	7.02	6.73	6.59	6.44	6.18	-	-
4.	7,900	7,600	7,200	7,800	9,400	6,700	7,600	6,100	-	-
5.	79	76	-	-	94	-	76	-	-	-
6.	395	608	216	390	1,786	469	760	305	-	-
7.	158	152	72	-	-	67	76	61	-	-
8.	2,449	1,748	1,944	2,262	2,538	2,010	2,888	2,135	-	-
9.	4,661	4,636	4,608	4,914	4,418	3,417	3,572	3,355	-	-
10.	158	380	360	234	564	737	228	183	-	-
11.	8.8	8.7	8.7	9.2	8.6	8.3	8.0	8.7	-	-
12.	3.0	3.5	3.9	3.9	4.0	4.1	4.0	4.1	-	-
13.	46.3	55.9	49.5	58.7	47.4	48.3	32.2	31.1	-	-
14.	120	108	153	280	102	113	104	126	-	-
15.	212	283	191	313	248	304	289	212	-	-

Table I (Cont'd)

Animal No. 117 Date of parturition 8/18/76

	Date of Sampling							April 4/26/77	May 5/25/77
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/24/77	March 3/30/77		
16.	2.8	4.7	4.4	5.6	6.0	5.2	4.7	5.8	-
17.	1.1	0.8	0.9	1.2	1.0	0.7	1.8	1.0	-
18.	6.3	7.6	7.2	7.3	8.2	7.3	9.2	8.7	-
19.	65	80	70	69	70	70	75	70	-
20.	-	138	133	139	142	139	140	142	-
21.	-	3.7	3.7	4.3	4.3	4.0	4.2	3.8	-
22.	-	29	32	24	29	31	33	27	-
23.	17	18	25	27	17	23	17	20	-
24.	40	55	45	41	32	43	40	38	-
25.	10	20	20	20	22	37	21	27	-
26.	8.6	9.0	9.2	10.8	7.5	9.5	10.0	10.1	-
27.	6.2	5.5	4.6	4.7	5.5	5.6	5.6	4.0	-
28.	0.5	0.6	0.8	1.4	0.7	0.9	0.9	0.9	-
29.	-	-	-	-	-	10.2	10.5	10.0	-
30.	-	-	-	-	-	101.0	104.0	102.0	-

Table I (Cont'd)
Animal No. 015 Date of parturition 5/3/77

	Date of Sampling									
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/24/77	March 3/30/77	April 4/26/77	May 5/25/77	
1.	37.0	37.0	38.0	37.0	35.0	34.0	36.0	38.0	30.0	
2.	13.2	13.3	13.6	12.8	13.6	11.3	13.5	13.6	10.2	
3.	8.30	8.36	7.93	7.77	6.93	7.05	7.52	7.44	5.54	
4.	10,600	9,600	8,900	8,900	18,600	10,300	11,800	13,500	7,400	
5.	-	96	89	178	186	-	-	-	-	
6.	848	-	89	534	186	824	354	675	888	
7.	106	480	267	178	186	206	236	-	-	
8.	3,286	4,320	2,670	3,382	9,300	4,635	6,254	7,560	3,182	
9.	6,042	4,416	5,607	4,450	8,556	4,120	4,366	4,725	3,108	
10.	318	288	178	178	186	412	590	540	222	
11.	9.1	8.9	9.1	9.4	9.3	9.1	8.5	8.9	9.4	
12.	3.8	3.5	3.7	3.7	3.4	3.5	3.6	3.7	3.2	
13.	47.1	33.7	82.6	49.4	47.9	46.2	29.6	28.7	36.6	
14.	-	124	181	179	110	128	123	130	135	
15.	-	210	296	281	230	231	232	189	236	

Table I (Cont'd)
Animal No. 015 Date of parturition 5/3/77

	Date of Sampling									
	Sep 9/21/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/24/77	March 3/30/77	April 4/26/77	May 5/25/77	
16.	3.8	4.4	3.7	5.8	5.1	5.7	5.7	6.3	6.9	
17.	2.6	1.2	1.1	1.9	1.3	1.0	1.6	1.5	1.0	
18.	7.3	8.3	7.2	7.5	8.1	6.5	7.3	7.5	7.2	
19.	72	70	67	69	80	70	67	60	35	
20.	139	136	141	142	142	140	144	143	143	
21.	4.6	4.1	4.3	4.3	4.1	4.0	4.4	4.5	2.8	
22.	32	33	33	29	32	30	30	31	22	
23.	24	24	23	22	16	21	19	16	18	
24.	40	42	45	34	30	25	28	37	33	
25.	16	22	25	25	18x	35	18	15	12	
26.	8.6	9.8	9.0	9.3	9.4	8.8	9.0	9.8	7.5	
27.	7.4	6.6	7.5	6.8	5.2	7.8	6.9	6.1	6.7	
28.	1.1	0.9	1.1	1.2	1.0	1.3	1.5	1.4	1.1	
29.	-	-	-	-	-	9.8	6.9	5.8	11.4	
30.	-	-	-	-	-	102.0	109.0	105.0	100.0	

Table I (Cont'd)
Animal No. 095 Date of parturition 1/5/77

	Date of Sampling									
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/24/77	March 3/30/77	April 4/26/77	May 5/25/77	
1.	36.0	35.0	36.5	35.0	30.0	30.0	27.0	24.0	28.0	
2.	12.8	12.5	12.0	12.3	11.9	9.9	9.4	8.9	9.2	
3.	7.36	7.30	7.58	6.95	6.27	5.78	5.59	5.13	5.27	
4.	8,800	7,900	8,100	9,000	12,400	8,000	6,400	11,000	6,200	
5.	-	79	-	-	124	-	-	-	-	
6.	704	237	324	180	248	160	448	-	248	
7.	176	395	162	180	124	240	64	220	-	
8.	2,728	1,738	3,240	3,510	7,564	3,680	2,112	6,930	2,728	
9.	4,928	5,056	3,888	4,590	3,224	3,280	3,328	2,640	2,852	
10.	264	316	486	450	1,116	640	448	1,100	372	
11.	8.0	8.5	8.1	8.1	7.5	8.0	8.1	8.8	9.0	
12.	3.4	3.5	3.6	3.7	3.4	3.5	3.5	3.1	3.0	
13.	41.4	47.6	79.8	45.4	40.0	47.0	32.9	39.9	43.6	
14.	22	115	148	202	42	128	37	143	63	
15.	35	147	185	272	81	154	103	270	106	

Table I (Cont'd)

Animal No. 095 Date of parturition 1/5/77

Date of Sampling									
Sec	Oct	Nov	Dec	Jan	Feb	March	April	May	
9/27/76	10/19/76	11/23/76	12/15/76	1/20/77	2/24/77	3/30/77	4/26/77	5/25/77	
16.	2.8	4.7	4.6	6.6	5.9	5.0	5.1	6.3	8.1
17.	1.5	1.1	1.5	1.6	1.3	1.2	1.2	1.4	1.9
18.	10.2	11.2	9.7	9.8	10.9	8.7	9.6	9.4	9.8
19.	65	80	65	63	50	45	74	62	67
20.	144	139	141	139	145	140	137	142	139
21.	4.4	4.1	4.2	3.9	3.4	3.6	3.9	4.4	3.9
22.	27	26	30	30	36	33	29	33	28
23.	15	18	20	16	14	18	17	12	20
24.	20	20	25	20	43	20	25	50	48
25.	15	20	25	15	15	26	25	10	21
26.	8.8	10.3	9.2	9.5	9.0	9.0	8.7	8.5	8.2
27.	6.2	4.0	5.4	4.3	4.9	6.1	3.5	5.7	5.9
28.	0.9	0.8	1.1	1.2	0.9	0.9	0.9	0.7	0.8
29.	-	-	-	-	-	7.5	9.4	41.0	10.3
30	-	-	-	-	-	94.5	102.0	95.0	101.0

Table I (Cont'd)

Animal No. 139 Date of parturition 3/30/76, 4/16/77

	Date of Sampling									
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/24/77	March 3/30/77	April 4/26/77	May 5/25/77	
1.	36.0	37.0	34.0	34.0	33.0	35.0	36.0	34.0	31.0	
2.	13.0	13.3	12.1	12.5	11.3	11.1	12.0	11.0	10.2	
3.	6.82	7.39	7.19	7.25	6.98	6.04	6.61	6.06	5.66	
4.	8,300	8,000	9,200	8,700	6,900	6,100	8,400	4,200	5,700	
5.	-	80	-	-	-	-	-	42	-	
6.	249	160	368	609	207	732	1,176	168	513	
7.	249	80	644	174	69	122	168	-	-	
8.	3,486	2,400	3,496	3,132	2,691	2,196	3,528	924	2,280	
9.	4,232	4,800	4,324	4,611	3,588	2,562	2,940	2,310	2,679	
10.	83	480	368	-	345	488	588	756	228	
11.	7.2	8.3	8.2	8.4	8.1	8.3	7.8	8.2	9.4	
12.	3.5	4.0	4.0	3.9	3.8	3.7	3.6	3.6	3.5	
13.	39.2	24.9	56.5	54.2	51.3	42.1	31.5	34.8	37.7	
14.	152	115	102	213	164	113	109	108	113	
15.	166	273	197	381	260	206	197	235	198	

Table I (Cont'd)

Animal No. 139 Date of parturition 3/30/76, 4/16/77

	Date of Sampling								
	Sep 9/27/76	Oct 10/19/76	Nov 11/23/76	Dec 12/15/76	Jan 1/20/77	Feb 2/24/77	March 3/30/77	April 4/26/77	May 5/25/77
16.	3.5	6.0	4.7	6.4	6.5	6.5	5.9	6.5	7.4
17.	3.1	1.4	1.4	2.0	1.6	1.4	1.5	1.1	2.0
18.	6.5	7.9	7.7	8.1	8.4	6.4	7.9	8.1	9.1
19.	45	90	70	65	75	63	65	60	57
20.	132	139	140	145	142	137	143	141	138
21.	3.6	3.7	3.9	4.6	4.3	3.5	4.0	4.1	4.3
22.	26	28	32	28	31	32	29	29	29
23.	20	20	22	20	15	21	20	16	20
24.	35	40	37	40	40	30	35	52	40
25.	25	30	30	20	33	40	20	12	20
26.	8.0	9.2	9.6	10.1	9.4	8.9	9.4	9.0	8.9
27.	5.3	7.2	4.6	4.5	4.9	5.7	4.8	5.6	7.4
28.	0.6	0.8	1.0	1.2	1.0	0.9	1.0	1.8	0.9
29.	-	-	-	-	-	7.6	6.1	7.2	8.6
30.	-	-	-	-	-	100.5	107.0	97.0	95.0

VARIATIONS IN HEMATOLOGIC PARAMETERS AMONG HIGH-PRODUCING
DAIRY CATTLE ON HIGH AND LOW PROTEIN DIETS
I. EFFECTS OF PROTEIN INTAKE AND STAGE OF LACTATION
ON HEMATOLOGICAL CONSTITUENTS AND ACTIVITIES OF
RED BLOOD CELL ENZYMES OF HIGH-PRODUCING DAIRY CATTLE
II. HEMOGLOBIN-BINDING PROTEINS (HAPTOGLOBIN-Hp)
IN NORMAL HEALTHY DAIRY CATTLE SERA

by

KING A. N. ESIEVO

D.V.M., Ahmadu Bello University, Zaria, Nigeria, 1974

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the
requirements for the degree

MASTER OF SCIENCE

Department of Infectious Diseases

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1977

ABSTRACT

I. Blood samples were collected on nine occasions during a 9-month period from 19 high-producing Holstein-Friesian dairy cows for the determination of packed cell volume, erythrocyte count, hemoglobin, serum total protein, albumin, globulin and iron concentrations and iron-binding capacity. Erythrocyte glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase activities and reduced glutathione concentration were also determined.

Dry and lactating cows on the lowest protein ration (13%) had the highest mean values for packed cell volume, hemoglobin concentration and erythrocyte count. Concentrations of other constituents were not affected significantly by levels of dietary protein.

Packed cell volume, red blood cell count, hemoglobin, serum albumin, serum iron concentrations and iron-binding capacity decreased early in lactation and rose to pre-lactation levels by mid-lactation.

Packed cell volume and hemoglobin concentration remained low for periods up to 4 months. Red blood cell count was lowest in the second month, and serum albumin concentration was lowest in the first month and remained low up to the second month.

Iron-binding capacity was lowest in the first month of lactation while serum iron concentration was lowest in the third month of lactation.

Thirteen percent protein ration had no anemia-producing effect on the cows.

II. Nineteen clinically healthy high-producing dairy cattle sera were assayed for haptoglobin (Hp) monthly for a 9-month period, using the cellulose acetate strip electrophoretic method based on the peroxidase-like activity of hemoglobin-haptoglobin complex. The haptoglobin was densitometrically quantitated and a range of 11-86 mg Hp/100 ml serum (as hemoglobin binding capacity) was found. There were no significant differences among dry cows on 13% protein ration and lactating cows on 13%, 15% and 17% protein rations. There was no evidence of stress clinically.