STUDIES ON CRITERION OF JUDGEMENT OF NUTRITIONAL DISORDER IN THE DAIRY CATTLE I. RESEARCHES ON THE NUTRITIONAL STATE OF DAIRY CATTLE UNDER PRACTICAL CONDITION

STUDIES ON CRITERION OF JUDGEMENT OF NUTRITIONAL DISORDER IN THE DAIRY CATTLE

I. RESEARCHES ON THE NUTRITIONAL STATE OF DAIRY CATTLE UNDER PRACTICAL CONDITION

By

Kaichi AMBO and Motoyoshi UMEZU

Department of Animal Husbandry, Faculty of Agriculture, Tohoku University, Sendai Japan

(Received December 23, 1957)

Dairy farming has progressed rapidly in consequence of milk production required through a change of national food phase after the Second World War in Japan. This may is shown by the increase in numbers of cows from 201,788 in 1949 to 587,000 in 1957.

On the other hand, the health conditions of cattle in farms were not good, there occur various disorders in ovary and uterus of cattle throughout the country, and about 80,000 of 260,000 cows were infertile. The rate of infertile of 30 per cent is high in comparison with from to six per cent of other countries.

The cow infertile results in a desease in cow reproduction and a bad influence upon the milk production. So far, endocrinology has progressed as a means for research of the various phenomena of reproduction. Since no attentions has been given to the fundamental and prime cause of reproductive disorders occuring in dairy cattle, the etiological treatments and prevention were not fruitful. It is well known that various functions of the reproductive organ are control led or influenced by the many sexual hormones and also by function of the animal. Similarly, harmonic control of sexual hormones to the reproductive organs are always influenced by whole the function of the animal.

The function of the animal includes blood circulation, respiration and metabolic functions in the liver and in other tissues. These functions are integrated by the term of the nutrition of animal. Therefore, their nutritional conditions may affect the reproduction and milk production of the dairy cattle in various ways, directly and indirectly.

According to Richter(1), Eckles(2), Allen(3) and Quinin(4), undernutrition tends to occur late coming in with the heat and low fertility in heifers. Riddlle(5) has reported on the absence of heats in cows a phosphorus deficient

ration. Webster(6) has stated that in phosphorus deficiency, little effect is seen until the end of gastation, when parturition may be difficult and the calves may be weak or dead. Sutton(7) has reported degeneration of germinal epithelium and epithelium of seminiferous tubuli and the absence of sperm were observed in calves kept on a low vitamin A diet until they were a year old. Harris *et al.*(8) has reported that supplementation of Vitamin A and tocopherol to herds, which showed three stillbirth, four absorption and retain placentas after birth of almost every calf, results in no stillbirth, one absorption and one retained the placenta in this herd. Although under practical conditions, in general, one rarely finds a clear case of mulnutrition due to a deficiency of one factor in the feed, nutritional disorder may be followed by a decline of vitality and resistancy against the disease in the animal. Thus, it is difficult to clear the relation between the nutritional disorder and the reproductive disorder.

In order to remove the disorder of nutrition and reproduction, the nutritional physiology may be most important. Since fundamental studies on the nutritional disorder of the dairy cattle are scanty it is difficult to explain many of the problems in concern. The nutritional physiology of ruminant can not be considered as anology of knowledge in non-ruminant such as humans, rats and etc., because there exists a stage of complicated fermentation in the rumen, previous to the nutrients absorption into the blood circulation. As mentioned, the nutrition of animals is related with the very physiological conditions. The conditions may be lead from pre-nutrition, that is food administered, to fermentation in the rumen, digestion and absorption in the gastro-intestinal tract. Practicularly, fermentation in the rumen results in the production of various volatile fatty acids, which may be the main energy source for ruminant, and the quantity proteins and vitamins, which is synthesized by the micro-organisms populating the rumen. Therefore the condition of fermentation affect with regard to the physiological conditions of the cow may be due to various factors. Therefore, whether fermentation in the rumen is adequate is a very important effective factor in the nutrition of dairy cattle.

A dairy cattle receives these effects and then exhibit it as physiological conditions.

Hence, the nutritional disorder may occur in the following two processes. One is the various phenomena occuring during pre-nutrition, as already mentioned, and the other is when the nutrients are not utilized sufficiently for organs and tissues from the physiological conditions of respiration, blood circulation, control of nerves and hormones and liver functions etc. If the nutritional disorder causes the disorder of reproduction and milkproduction the latter two may be included into the problem of post-nutrition. The systematic arrangement of the mentioned problems on the nutritional disorder are shown in Table 1.

Thus, the nutritional disorder of cows contains various important and

complicated problems. First the nutritional state of a cow under practical condition should be standard to clear these problems.

Accordingly, our series of researches were carried out for establishing a standard for judging the degree of nutritional condition and the techniques of judgement.

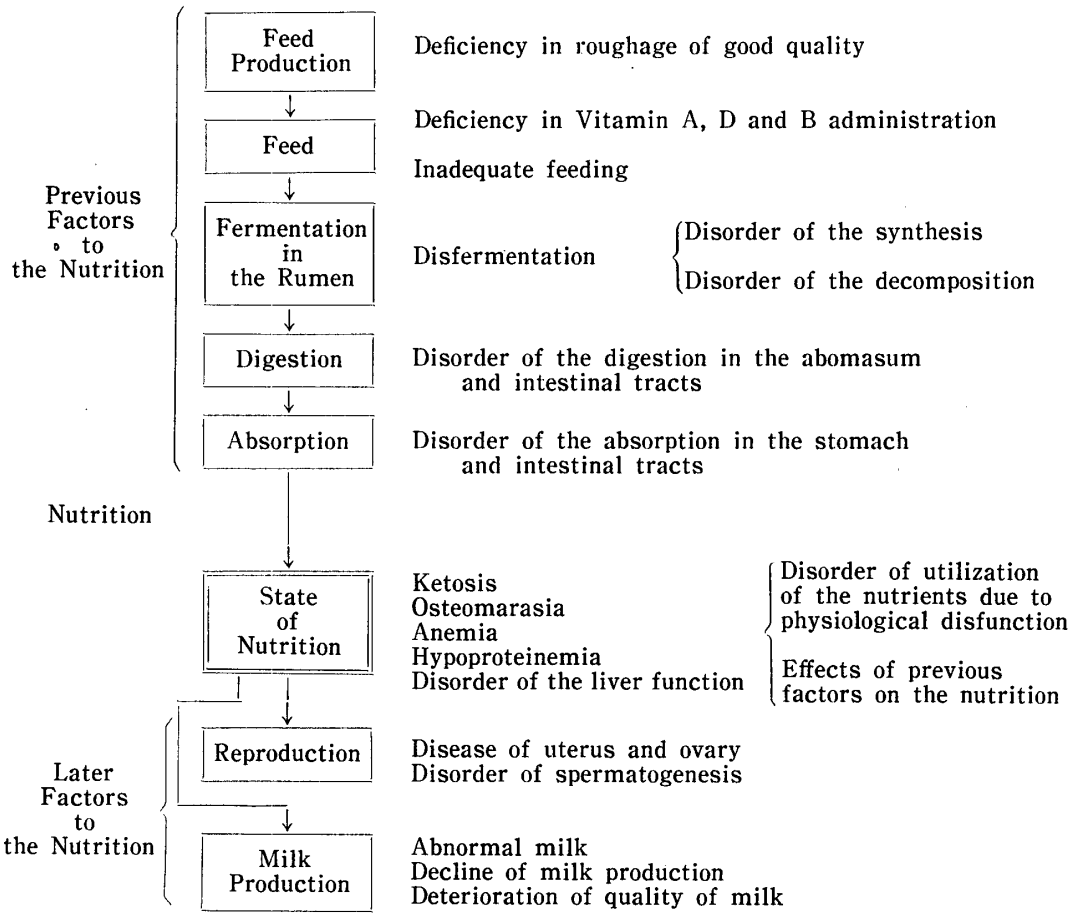

Fig. 1. The systematic arrangement of the problems concerning the Nutritional Disorder of the Dairy Cow.

I. RESEARCH ON THE STATE OF NUTRITIONAL CONDITIONS OF DAIRY COWS

At first, we researched on the nutritional conditions of a number of cows under practical conditions in order to grasp the state of nutritional disorders.

A. Preliminary researches

(1) *Researches at the Iwadeyama Region.*

Dairy cows selected for our preliminary research were a herd in the Iwadeyama region of Miyagi Prefecture. Iwadeyama is well known for its

congealed "tohu" production. Hence, cows in this region are fed with large amounts of the tohu's roughage. In the past, reproductive disorder has been reported in a number of dairy cattle. In January 1955, we observed 15 cows, which were affected from infertile in the present and or the past. And concernning them we determined the blood, milk, urine and liver functions of each cow.

Chemical Method

Various constituents in the blood, milk and urine were determined with following methods.

a. Blood

1) Total Nitrogen (T. N.): T. N. was determined by micro-Kjeldahl.
2) Total Protein Nitrogen (T. P. N.): T. N.-N. P. N.
3) Non-protein Nitrogen (N. P. N.): N. P. N. was determined by micro-Kjeldahl after protein was precipitated by 5 per cent CCl_3COOH.
4) Alubmin Nitrogen (A. N.): A. N. was estimated by micro-Kjeldahl after fractionation with 22.2 per cent Na_2SO_4 (Howe's method).
5) Globulin Nitrogen (G. N.): T. P. N.-(A. N. + F. N.).
6) Fibrinogen Nitrogen (F. N.): F. N. was established by micro-Kjeldahl after fibrin was precipitated with 0.5 per cent $CaCl_2$.
7) Urea: To permit estimation of urea, the micro-diffusion analysis was used.
8) Total Vitamin A: Total Vitamin A was determined spectrophotometically.
9) Carotene: Carotene was determined spectrophotometically.
10) Vitamin B_1: Vitamin B_1 was determined photometically by the use of Permutit-Thiocrom.
11) Blood sugar: Hargedron-Jensen's method was used.
12) Volatile Fatty Acids: Volatile Fatty Acids were determined by the Conway's diffusion method.
13) Ketone body: Estimation of ketone body was done by Thin and Robertson's method.
14) Ca: Ca was estimated by the micro-method in which Murexid's reagent was used as the indicater.
15) P: P was determeined by Nakamura's method.
16) pH: The measurements of pH were made with glass electrode.
17) Erythrocyte: The numbers of erythrocytes was counted by Thoma-Zeiss' method.

b. Milk

1) Total Nitrogen (T. N.): T. N. was determined by micro-Kjeldahl.
2) Non-Protein Nitrogen (N. P. N.): N. P. N. was determined with micro-

Kjeldahl after the proteins were precipitated with 5 per cent CCl_3COOH.

3) Urea: Microdiffusion analysis was used.

4) Protein Nitrogen: T. N.-N. P. N.

5) Total Vitamin A: Total Vitamin A was determined spectrophotometically.

6) Carotene: Carotene was determined spectrophotometrically.

7) Vitamin B_1: Vitamin B_1 was determined photometrically by the use of Permutit-Thiocrom.

8) Ketone body: Ketone body was determined by Thin and Robertson's method.

9) Per cent of Protein: Multiplying the value of protein

10) Calcium: Ca was established by the micro-method using Murexid's reagent as the indicator.

11) P: P was determined by Nakamura's method.

c. Urine

1) Ketone body: Thin and Robertson's method was used.

2) Urea: Urea was determined by the microdiffusion analysis.

3) NH_3: NH_3 was determined with the Conway's diffusion method.

4) pH: pH was measured by the use of a glass electrode.

5) Sugar: Sugar was qualitatively tested by Benedict's method.

6) Protein: Protein was qualitatively examined by the use of sulfosalicylic acid.

7) Urobilinogen: Urobilinogen was examined by Ehrlich's method.

8) Millon's reaction was used for the test of amino acids in urine.

d. Liver function

The following tests for liver function were carried out on the blood serum. The following four (1 to 4) were used as the test for the protein synthesis function of liver.

1) Takata's method

2) Gros' method

3) $CaCl_2$ test

4) Cadmium test

5) Serum bilirubin: Hijamans Van den Bergh's method was carried out on both types (direct and indirect) of bilirubin.

6) Tyrosine: Tyrosine was tested by Veiss's method.

Results

1) Blood.

Wide deviation in each cow are found in the respective contents of blood. The concentration of the blood plasma less than the minimum limit of standard

deviation of the mean of the all cows were Nos. 4 and 9.

No. 4 showed low content of T. P. N. and because of the low content of Al-N, the A/G ratio was reduced apparently.

Fibrinogen-N, urea and counts of erythrocyte are also low compared with the other cows. Content of urea, V. B_1 and erythrocyte counts showed a low level in No. 9.

The widest deviation of the content is shown in total V. A, from 317 to 2383 I. U./*dl.* Most of T. V. A. in blood is carotene. Nos. 3, 4 and 11 which had a high level of T. V. A., shown high content of carotene. Low content of blood sugar and apparently high level of ketone were found in No. 14 in which the V. B_1 content is less than the other cows. Low levels of erythrocyte counts are found in 14 cows with the exception of No. 1.

Both of Nos. 4 and 9 which showed the lowest level were apparently also low in serum protein contents.

2) Milk.

T. N. content in the milk was low in No. 4, in which T. N. of the blood was also low. Urea content in the milk shown elative level with that in the blood. About 80 per cent of T. V. A. in the milk is Vitamin A. This figure differs from Vitamin A in the blood having a low carotene content. Ketone contents in the milk were few. On the fat per cent of the milk, Nos. 2 and 3 showed low levels. Both of Nos. 2 and 3 showed over 3 per cent in the second research.

3) Urine.

There is no cow which with a high concentration of ketone bodies in the urine. Urea content showed wide variation. Because the urine volume of each cow differs by day critical results can not be based merely upon the content in the urine. In general, an increase in the urine follows a decrease in pH, but the reaction of urine of No. 13 in which the urea content was very high was weakly alkaline. It is interesting that sugar was positive in the urine of 10 among 15 cows.

4) Liver function.

Examination on the disorder of the liver function were carried out mainly on the protein synthetic function and for forming and excretion of the bile.

In four tests for the former function, the positive ratio of disorder is found to be low by Gros' test, high in the $CaCl_2$ and Cd test and medium by Takada's test.

Nos. 4 and 14 among 15 cows examined were of interest, especially No. 4 was positive in the reaction of four tests, hence, it is considered that there is an apparent existance of the disorder of the liver function. Nos. 6, 7, 12 and 15 were questionable or to the existence of disorder.

Table 1. Contents of the blood of dairy cows in the Iwadeyama region.

	TN mg/*dl*	TPN mg/*dl*	NPN mg/*dl*	Al-N mg/*dl*	Gl-N mg/*dl*	A/G	F-N mg/*dl*	mg/*dl*
1	1050	1023	27	494	468	1.06	61	15.4
2	1161	1123	38	548	531	1.03	44	39.6
3	1183	1162	21	556	556	1.00	50	28.5
4	790	761	29	294	467	0.59	20	18.5
5	1172	1143	29	571	531	1.08	41	37.7
6	1207	1179	28	578	559	1.03	42	25.0
7	1205	1175	30	507	569	0.89	99	33.1
8	1021	990	31	453	485	0.93	52	31.9
9	956	933	23	531	357	1.50	45	13.5
10	987	963	24	573	355	1.61	35	11.5
11	1050	1019	31	552	437	1.26	30	34.2
12	1207	1175	32	545	575	0.95	55	24.6
13	1092	1048	44	493	495	1.00	50	54.2
14	1180	1142	38	499	584	0.85	59	27.3
15	1196	1164	32	478	600	0.80	86	15.8
	1097	1067	30	510	505	1.04	51	27.4
Ave	±119	±118	±5.8	±86	±74	±0.25	±19.4	±10.1

	T. Vit. A IU/*dl*	V. A IU/*dl*	Carotene γ/*dl*	A/C	V. B_1 γ/*dl*	Sugar mg/*dl*	VFA mg/*dl*
1	774	92.8	408.7	0.23	13.2	67	9.8
2	621	111.2	305.5	0.36	10.6	71	5.5
3	2383	142.9	1342.3	0.11	12.9	77	7.1
4	2330	139.6	1312.5	0.11	16.4	66	7.5
5	758	134.6	374.5	0.36	15.9	67	7.6
6	498	92.8	243.5	0.38	10.1	82	6.0
7	670	131.5	350.2	0.37	7.8	66	5.7
8	731	108.0	374.5	0.29	5.6	66	7.7
9	618	100.0	311.0	0.32	5.6	74	5.3
10	1735	155.0	949.0	0.17	6.8	67	5.5
11	2380	215.2	1303.0	0.17	6.3	65	5.2
12	523	105.5	250.4	0.42	7.1	76	6.2
13	435	102.0	198.0	0.52	4.6	63	5.4
14	317	84.8	133.4	0.64	5.8	57	5.0
15	1171	121.4	630.5	0.20	6.8	70	8.2
	1063	122.5	565.8	0.31	9.0	69	6.5
Ave	±728	±32	±411	±0.15	±3.8	±6.4	±1.14

	Keton		Ca mg/*dl*	P mg/*dl*	Ca/P	pH	Numbers of erythrocyte
	Total mg/*dl*	Free mg/*dl*					
1	2.9	0.7	17.6	8.7	2.0	7.60	716
2	3.7	0.9	18.0	10.1	1.8	7.60	559
3	3.1	1.3	30.7	13.0	2.4	7.70	577
4	3.3	0.5	30.2	10.0	3.0	7.50	483
5	2.3	0.9	27.3	11.9	2.3	7.60	578
6	3.1	1.1	15.6	9.3	1.6	7.70	475
7	2.0	0.2	15.6	13.4	1.2	7.70	655
8	3.3	0.9	14.1	10.0	1.4	7.65	503
9	2.2	0.4	14.6	11.7	1.3	7.65	483
10	1.7	0.8	33.2	11.4	2.9	7.60	564
11	1.8	0.9	22.9	10.7	2.1	7.65	626
12	2.5	0.9	14.1	8.3	1.7	7.70	657
13	1.7	0.7	11.2	10.4	1.1	7.58	618
14	10.2	1.7	15.1	9.9	1.5	7.60	638
15	3.7	1.3	15.1	8.0	1.9	7.72	496
	3.2	0.9	19.7	10.5	1.9	7.64	575
Ave	±2.03	±0.37	±7.0	±1.4	±0.6	±0.06	

Table 2. Contents of the milk of dairy cows in the Iwadeyama region.

No.	TN mg/*dl*	NPN mg/*dl*	Urea mg/*dl*	Protein %	T. Vit. A IU/*dl*	Vit. A IU/*dl*	Carotene γ/*dl*
1	578	36	17.3	3.4	223	195.8	15.9
2	512	48	42.3	2.9	102	89.7	6.9
3	526	36	29.6	3.1	110	95.9	14.9
4	423	37	36.5	2.4	195	160.1	20.8
5	559	39	35.0	3.2	146	120.3	16.3
6	510	39	30.8	2.9	116	101.9	8.1
7	556	40	25.0	3.2	131	111.2	12.0
8	531	47	44.6	3.0	127	110.1	10.3
9	570	24	15.4	3.4	180	167.2	7.7
10	524	28	15.4	3.1	108	90.8	10.0
11	578	39	30.8	3.4	242	204.0	23.0
12	606	38	36.5	3.6	132	114.2	11.0
13	546	46	53.8	3.1	136	126.4	5.8
14	559	43	26.9	3.2	140	131.5	6.2
15	538	38	17.3	3.1	195	170.3	15
	541	39	30.5	3.1	153	132.4	12.5
Ave	±41	±5.1	±11.0	5±0.3	±42	±12	±5.2

No.	A/C	Vit. B_1 γ/*dl*	Ketone total mg/*dl*	Fat %	Ca mg/*dl*	P mg/*dl*	Ca/P
1	12.3	25	0.3	6.8	124	75	1.66
2	13.0	18	0.3	1.9	137	72	1.83
3	6.4	27	0.2	1.5	113	72	1.57
4	7.7	20	0.1	3.7	84	60	1.40
5	7.4	9	0.3	5.7	101	51	2.15
6	12.7	27	0.6	3.1	87	68	1.28
7	9.2	19	0.3	3.2	162	56	2.90
8	11.0	25	0.4	4.0	38	73	0.52
9	21.7	13	0.3	6.4	191	81	2.37
10	9.1	27	0.0	3.6	74	63	1.17
11	9.1	19	0.7	4.9	65	63	1.15
12	10.4	19	0.4	4.7	65	70	0.93
13	21.7	11	0.3	5.7	49	66	0.71
14	21.2	48	0.4	4.7	46	77	0.60
15	11.3	12	0.1	3.2	39	70	0.56
	11.0	21	0.3	4.2	92	68	1.38
Ave	±5.5	±9.7	±0.17	±1.5	±45	±7.8	±0.69

Table 3. Contents of the urine of dairy cows in the Iwadeyama region.

No. of cows	Ketone		NH_3 mg/*dl*	pH	Sugar	Protein
	Free mg/*dl*	Urea mg/*dl*				
1	0.1	248	1.7	7.4	++	−
2	0.3	227	3.7	7.6	++	−
3		1025	3.9	7.6	++	−
4	0.4	287	1.7	7.6	++	−
5	0.4	402	1.5	7.5	++	+
6	0.2	382	2.7	7.6	−	−
7	0.1	708	3.1	7.2	++	−
8	0.6	518	1.2	7.0	−	−
9	0.2	384	0.5	7.4	−	−
10	0.1	160	0.4	7.6	−	−
11	0.4	576	0.5	7.6	++	−
12	0.1	499	1.2	7.2	−	−
13	0.2	3236	1.4	7.4	+	−
14	0.6	—	—	7.4	+	−
15	0.2	288	0.5	7.4	+	−
	0.3	639	1.6	7.5		
Ave	±0.15	±684	±1.1	±0.6		
(Positivity)					(64.1%)	(6.7%)

Table 4. Liver function of dairy cows in the Iwadeyama region.

No.	Takada's test	Gros' test	$COCl_2$	Cd.	Serum direct	Bilirubin indirect	Urine urobilinogen	Millon's test	Tyrosin mg/*dl*
1	0	−(1.95)	$\pm R_7(2)$	±	−	+	+	+	0.75
2	2	−(1.08)	$+R_8(1)$	+	−	±	−	−	0.80
3	0	−(1.30)	$+R_8(2)$	−	−	−	+	+	0.50
4	4	+(0.66)	$+R_8(1)$	++	−	−	+	−	0.60
5	1	−(1.40)	$\pm R_7(2)$	+	−	−	+	+	0.50
6	2	±(0.95)	$\pm R_7(2)$	++	−	±	+	+	0.40
7	3	−(1.23)	$++R_9(1)$	+	−	−	+	+	0.30
8	2	−(1.05)	$\pm R_7(2)$	+	−	−	+	−	0.60
9	1	−(1.97)	$+R_8(2)$	−	−	−	+	±	0.70
10	1	−(1.95)	$\pm R_7(2)$	−	−	−	+	−	0.60
11	2	−(2.30)	$-R_5(3)$	−	−	−	+	++	0.50
12	3	−(1.02)	$+R_8(2)$	+	−	±	+	−	0.75
13	2	−(1.20)	$+R_8(2)$	+	−	−	+	++	0.60
14	4	−(1.28)	$++R_{10}(0)$	+	−	−			0.40
15	3	±(0.90)	$+R_8(1)$	±	−	−	+	−	0.50
Ave.	2	(1.35)	$R_8(2)$						0.57
Positivity	33.3%	6.7%	60.0%	60.0%	0%	6.7%	92%	50%	0.14

No cow shown a high content in the blood serum. Urobilinogen in the urine was positive with the exception of one cow.

Table 5. The state of reproduction and feeding of dairy cows in Iwadeyama region.

No. of cow	Age	Pregnancy	Data of last parturition	Body weight	Milk production	Excess or lack of untrients of feed *1	
						D. P. *2	S. V. *3
1	7	2 month		558kg	14.8kg	− 248g	+ 769g
2	7	2 month		480	22.2	− 585	− 270
3	6	Physiological infertile	Dec. '55	491	25.9	+ 668	+2415
4	6	infertile	Sept. '55	663	29.6	−1129	−2831
5	7	infertile	Jul. '53	558	7.4	− 203	+ 330
6	3	infertile	Jul. '55	397	12.9	+ 570	+3034
7	7	infertile	Aug. '55	558	12.9	− 19	− 277
8	7	Physiological infertile	Dec. '55	574	37.0	−1054	−4406
9	4	infertile	Jan. '55	409	9.2	+ 15	+ 146
10	5	2 month	Jan. '55	397	7.4	+ 521	+8198
11	7	infertile	Apr. '55	573	12.9	− 30	−1597
12	7	infertile	Aug. '54	480	12.9	+ 41	− 360
13	7	1 month	Feb. '55	480	9.2	+ 394	+ 660
14	6	Physiological infertile	Dec. '55	480	20.0	0	−1819
15	9	2 month		424	11.1	+ 210	+4980

*1 : Hirai's feeding standard was used for the feeding standard of dairy cows.
*2 : Digestible Protein.
*3 : Starch Value.

Among the 15 cows, 5 were apparently deficient in digestible protein value and 4 were excess in it.

Seven cows were deficient in the starch value level and 6 cows were excess in it. A defficiency in both digestible protein value and starch value were

found in 4 cows and a excess of both in 6. Nos. 4 and 8 were especially low in deficiency of both protein and energy. As mentioned above, cow No. 4 had a low level in the protein contents fiblinogen, and urea in the blood and red cell counts.

The protein synthetic function of the liver examined with the blood serum were positive to the reaction of disorder. After the research, it was evident that cow No. 4 was suffering from a parasitism of liver fluke.

Whether the abnormal content in some constituents in the blood is due to undernutrition extending for long periods or hepatic parasitism is a difficult problem. It is within the range of possibility that a parasitism of liver fluke is due to the undernutrition of the cow. Cow No. 14 showed a low content of blood sugar and high content of ketone in the blood and the protein synthetic function of the liver was positive to the reaction of disorder.

It seems that the abnormal figure is due to the deficient administration of starch value as an energy. It is indicated that a deficiency in the nutrients administered is as a high volume of lactation, as is shown in Table 5.

A high production of milk yielded by dairy cattle maintainning under-nutrition may result is various evil influences.

(2) *Researches at the Kawatabi Farm.*

The nutritional condition of cows fed on the Kawatabi Farm, Faculty of Agriculture, Tohoku University were studied.

The records of 6 cows examined is shown in Table 6.

Table 6. The state of reproduction of dairy cows in the Kawatabi Farm.

No. of cows	Species of cows	Age	Pregnancy	Data of last parturition
1	Holstein	12	2 month	Feb., '55
2	Ayrshire ×Holstein	7	2 month	Oct., '54
3	Holstein	6	infertile	Jul., '55
4	Holstein	2	infertile	Aug., '55
5	Japanese cattle ×Holstein	4	Physiological infertile	Nov., ,55
6	Japanese cattle ×Holstein	4	Physiolosical infertile	Dec., '55

These cows were fed with an adequate starch value and a digestible protein value of milk production, respectively. The method and items the examinations are same as in the case of the cows in the Iwadeyama region.

1) Blood.

High content of T. N. is shown in cow No. 1. Cow No. 1 showed also high concentration of T. P. N. in the blood, although Al-N is very high, and its A/G ratio was extreamely low.

Both Nos. 3 and 4 which showed considerably high concentration of T. N. had the usual content of albumin and a high content of globlin, and a low A/G ratio is shown.

The erythrocyte content was less than 6,500,000 with the exception of No. 6, and that at the 400,000 level was observed in two cases.

The results of investigation on the cows of the Kawatabi Farm as compared with those of the Iwadeyama region, generally show a high content of T.P.N., urea, V. A and carotene in the blood.

Table 7. Contents of the blood of dairy cows in the Kawatabi Farm.

No.	TN mg/*dl*	TPN mg/*dl*	NPN mg/*dl*	Al-N mg/*dl*	Gl-N mg/*dl*	A/G	FN mg/*dl*	Urea mg/*dl*
1	1331	1312	19	388	850	0.46	74	9.0
2	1104	1087	17	450	453	1.00	94	9.0
3	1238	1219	19	469	694	0.68	56	9.9
4	1220	1196	24	447	703	0.64	45	11.7
5	1090	1069	21	542	467	1.16	60	16.1
6	1146	1130	16	552	514	1.07	64	10.5
	1188	1153	19	465	630	0.98	66	11.0
Ave	±84	±85	±3	±58	±165	±0.25	±15	±2.4

No.	T. Vit. A IU/*dl*	V. A IU/*dl*	Carotene γ/*dl*	A/C	V. B_1 γ/*dl*	Sugar mg/*dl*	VFA mg/*dl*
1	349	37	187	0.20	8.7	67	7.5
2	1057	92	578	0.16	12.0	74	7.7
3	665	82	350	0.23	12.0	60	5.6
4	668	77	355	0.22	14.6	61	6.0
5	358	97	157	0.59	17.1	55	5.0
6	613	113	300	0.36	19.8	70	5.8
	617	83	321	0.29	14.1	64.5	6.3
Ave	±237	±23	±137	±1.4	±3.9	±5.2	±0.9

No.	Keton		Ca mg/*dl*	P mg/*dl*	pH	Numbers of erythrocyte
	Total mg/*dl*	Free mg/*dl*				
1	1.4	0.6	16.6	6.2	7.70	550
2	0.9	0.9	16.1	7.2	7.85	400
3	1.4	0.6	13.7	7.3	7.70	460
4	1.4	0.6	12.2	7.6	7.65	510
5	1.0	0.6	15.5	8.8	7.85	650
6	2.5	0.9	13.2	9.0	7.70	705
	0.4	0.7	14.4	7.7	7.76	546
Ave	±0.7	±0.1	±1.7	±1.0		±103

2) Milk.

Milk of cow No. 5 showed the lowest concentration of T. N. protein ratio, V. A and carotene among 6 cows examined.

In the cows of the Kawatabi Farm, there was a high concent of T.N. and protein ratio and a low content of urea, V. B_1 and pottasium compared with the cows of the Iwadeyama region, on the whole.

Table 8. Contents of the milk of dairy cows in the Kawatabi Farm.

No.	TN mg/*dl*	NPN mg/*dl*	Urea mg/*dl*	Protein %	T. Vit. A IU/*dl*	Vit. A IU/*dl*	Carotene γ/*dl*
1	700	35	10.8	4.2	167	134	20
2	666	38	10.8	3.9	222	169	32
3	539	31	9.6	3.2	194	164	18
4	504	26	11.4	3.0	194	164	18
5	448	37	10.2	2.6	87	77	6
6	660	37	13.2	2.9	131	113	11
	586	34	11.0	3.5	165	137	17
Ave	±98	±0.6	±1.1	±0.6	±44	±33	±8

No.	A/C	Vit. B_1 γ/*dl*	VFA mg/*dl*	Keton free mg/*dl*	Fat %	Ca mg/*dl*	P mg/*dl*	
1	6.2	23.1	3.00	0.00	4.2	134	46.5	2.2
2	5.3	28.1	2.80	0.40	3.4	122	44.5	3.8
3	9.1	28.2	2.50	0.30	3.8	226	44.4	7.0
4	9.1	28.2	2.20	0.30	2.3	84	44.0	4.5
5	12.8	37.0	2.70	0.00	4.1	100	45.8	8.3
6	10.3	40.9	2.00	0.00	4.1	134	48.0	4.5
	8.8	31.0	2.5	0.2	3.7	133	45.7	5.1
Ave	±2.5	±10.0	±0.35	±0.2	±0.7	±49	±1.4	±2.9

The content of V. A and carotene in the blood was low in cows of the Kawatabi Farm, but these in the milk were not different between Kawatabi and Iwadeyama.

3) Urine.

Table 9. Contents of the urine of dairy cows in the Kawatabi Farm.

No.	Urea mg/*dl*	NH_3 mg/*dl*	V.F.A. mg/*dl*	Ketone		pH	Sugar	Protein
				Total mg/*dl*	Free mg/*dl*			
1	363	2.6	21	4.8	1.0	7.6	++	−
2	298	1.9	19	4.8	1.0	7.3	++	−
3	374	1.4	24	6.8	1.0	7.5	+	−
4	455	1.4	17	8.2	1.5	7.5	+	−
5	363	2.4	24	6.8	1.5	7.6	++	−
6	359	0.9	17	6.2	1.0	7.6	++	−
	369	1.7	20.5	6.3	1.2	7.5		
Ave.	±112	±0.3	±2.9	±1.2	±0.3	±0.15		

No constituents in the urine showed a common concentration. There is no difference between the cows in the Kawatabi and Iwadeyama region. Existence of sugar in the urine was found in all of the cows.

4) Liver functions.

Cow No. 1 was positive to all four tests for functional disorder in No. 3 was probable. Tests of urobilinogen and Millon's reaction was positive for all of the cows.

Table 10. Liver function of dairy cows in the Kawatabi Farm.

No.	Takada's test	Gros' test	$CoCl_2$	Cd.	Serum direct	Bilirubin indirect	Urine urobilinogen	Millon's test	Tyrosin mg/*dl*
1	###6	##(0.45)	$R_{10}(0)$	##	−	−	+	+	0.65
2	##4	−(1.15)	R_7 (2)	±	−	−	+	+	0.50
3	##4	##(0.61)	R_8 (1)	+	−	−	+	+	0.60
4	##4	±(0.78)	R_8 (1)	+	−	−	+	+	0.45
5	+3	±(0.93)	R_8 (1)	±	−	−	+	+	0.45
6	−1	−(1.65)	R_8 (2)	−	−	−	+	+	0.45

Tables 11 and 12 show the maximum, minimum and average of the respective contents in the blood and milk of the dairy cows in the Iwadeyama region and Kawatabi Farm.

Table 11. Average of the blood content of dairy cows in the Iwadeyama region and Kawatabi Farm.

	Max.	Min.	Ave.	Duke *et al.*
T. N.	1331	790	1123	
T. P. N.	1312	761	1096	1189
N. P. N.	44	17	37	20-40
Al-N.	573	294	501	580.8
Gl	50	355	531	636.8
A/G	1.61	0.46	0.93	0.99
F. N.	90	20	55	72
Urea	54	9	22.7	6-27
T. V. A.	2383	317	936	
V. A	143	37	111	
Carotene	1342	133	496	
V. B_1	19.8	5.6	10.5	
Sugar	82	55	68	40-70
V. F. A.	5.0	9.8	6.4	
Ketone	10.2	0.9	2.7	
Ca	33.2	11.2	18.7	9-12
P	13.4	6.2	9.7	2.3-9.6
Erythrocyte count	716	400	567	630
pH	7.85	7.50	7.70	7.60

Table 12. Average of milk contents of dairy cows in the Iwadeyama region and Kawatabi Farm.

	Max.	Min.	Ave.	
T. N.	700	423	533	
N. P. N.	47	26	38	24
Urea	53.8	9.6	24.3	21-23
Per cent of prot.	4.2	2.4	3.2	3.13
T. V. A.	242	87	156	2000
V. A	204	77	133	1670
Carotene	32	6	14	866
V. B_1	48	9	24	20-35
Per cent of fat	6.8	1.5	4.1	3.6
V. F. A.	3.0	2.0	6	
Ketone	0.7	0.0		
Ca	226	38	104	70-75
P	77	44	66	88-90

From the results of researches on the nutritional state of the dairy cows in the Iwadeyama region and Kawatabi Farm, we recognized the following facts.

1. Many cows were fed inadequate volumes of both protein and or calories.
2. As a rule, the numbers of erythrocytes were in the low level, and numerous cows considered to be mainfested with anemia were found.
3. Low concentration of the protein contents in the blood plasma were seen in many cows.
4. The number of cows which showed positive reaction for test of functional disorder of the liver. These cows showed, in general, abnormal concentration of protein in the blood plasma, that is the content exceeded the upper limit of the standard deviation of the average of 21 cows.

B. The second research

From the preliminary research, we have found that low protein concentration in the blood, abnormally low counts of erythrocyte, and the existance of functional disorder of the liver occurs in many cows. Therefore, as the second research we studied on these three constituents for a larger number of dairy cows.

The dairy cows used in this research consisted of 51 cows of the Akiu and Osawa regions in Miyagi Prefecture.

Chemical Method

Protein concentration in the blood serum was determined with the Hitachi-protein Refluct Meter. Erythrocytes counts were measured by microscopical counting using the Thoma-Zeiss' Method. The functional disorder of the liver were carried out with Gros' test with use of Häym's solution.

In the protein concentrat ion in the blood serum, 4 cows showed 5 per cent level of protein concentration and 2 cows showed 8 per cent level. The former were considered to be apparently hypoproteinemia and the later were determined as hyperproteinemia.

Numbers of erythrocytes for the cows studied range between 29,600 and 74,700. 70,000 level of erythrocyte counts were found in only 2 cows and 60,000 level was found in 3 cows out of 51 cows, indicating that these cows all fall into anemia. 3 cows showed 30,000 level and 1 cow showed 20,000 level, thus it may be safely said that these 4 cows were apparently anemia.

In examination of the liver function disorder by Gros' test, the existance of disorder of the liver function was suggested in 9 cows.

From the results of the preliminary and the second researches, the distribution of the frequency of the protein concentration in the blood serum, numbers of erythrocyte and Gros' test of 63 cows are shown in Figs. 2, 3 and 4.

Table 13. Contents of the blood and liver function of dairy cows in the Akiu and Osawa region.

Number of cow	Serum protein	Contents of erythrocytes	Liver function (Gros' test)
	%	million	cc
1	6.1	534	1.47
2	5.8	541	2.00
3	7.6	455	1.25
4	6.6	499	1.63
5	6.8	540	1.27
6	6.3	372	2.00
7	6.0	500	1.65
8	7.1	435	1.12
9	6.0	485	1.70
10	7.7	474	1.48
11	6.8	587	1.55
12	7.7	561	1.36
13	8.4	453	0.93
14	6.8	512	1.15
15	7.6	428	1.20
16	7.3	561	1.83
17	7.6	539	1.68
18	6.2	600	1.70
19	7.1	422	0.93
20	7.3	296	1.00
21	5.7	636	1.86
22	6.4	476	1.05
23	7.7	465	0.94
24	7.2	468	1.32
25	6.6	318	1.20
26	6.2	670	2.00
27	6.0	555	2.00
28	5.6	511	2.00
29	5.8	598	2.00
30	7.1	509	1.67
31	7.2	532	0.90
32	6.2	473	1.49
33	6.9	717	1.32
34	6.2	473	1.49
35	6.2	523	1.74
36	7.0	431	1.02
37	7.2	551	0.92
38	8.1	419	0.81
39	7.1	550	1.00
40	6.7	512	1.30
41	6.9	692	1.32
42	6.6	345	0.89
43	7.6	436	1.10
44	6.4	508	1.50
45	6.8	534	1.60
46	6.7	522	2.00
47	7.7	502	2.00
48	6.9	524	0.68
49	6.6	535	0.89
50	6.7	747	0.73

In Fig. 1, the average of protein concentration in the blood serum of 63 cows was 6.7 ± 0.69 per cent. Serum protein concentration of the dairy cow reported by Dukes is 6.71 per cent and this value is similar to our results.

However, there are 12 cows (19.04 per cent) under 6 per cent of protein concentration, which are considered as hypoproteinemia, and there are 3 cows

(4.76 per cent) about 8 per cent of protein concentration, which are considered as hyperproteinemia. Therefore, 15 of the 63 cows about 20 per cent, showed abnormal protein concentration in the blood serum.

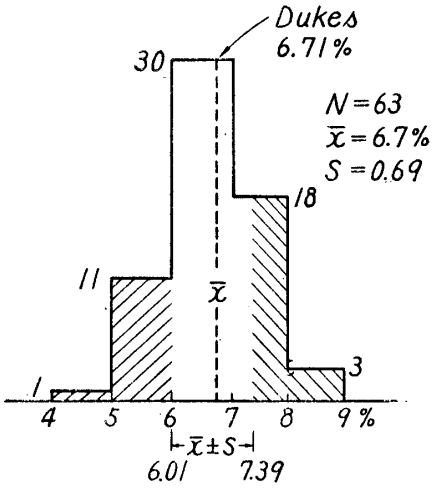

Fig. 2. Distribution of the frequency on the protein concentration in the blood serum of 63 cows.

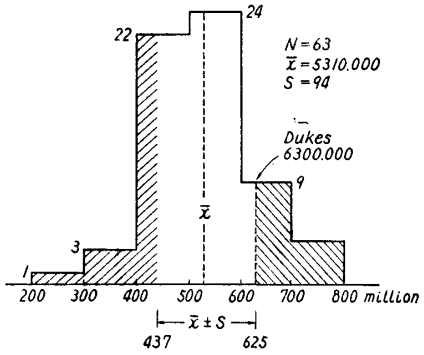

Fig. 3. Distribution of the frequency on the numbers of erythrocytes of 63 cows.

In Fig. 2, the numbers of erythrocytes in the blood of 63 cows range between 2,960,000 to 7,510,000, the average being 5,310,000 ± 910,000. According to Dukes, the numbers of erythrocytes of dairy cows is 6,300,000. This number exceeds 6,250,000 which is the upper limit of the standard deviation of the average in cows we studied. This fact apparently suggests that these dairy cows show anemia as a whole.

Table 14 shows the relation between protein concentration in the blood and numbers of erythrocytes in 62 cows. The numbers of erythrocytes reached the highest level in the group in which serum protein concentration was 6.2 to 7.0 per cent.

Neither serum protein concentration were higher nor lower than that group, the numbers of erythrocytes those groups tend to decrease. This relation, particularly the numbers of the erythrocyte apparently decrease in proportion as serum protein concentration increases.

Fig. 3 shows the results of Gros' test for the examination of the functional disorder of the liver.

Table 14. Relationship between the protein concentration in the blood serum and the numbers of erythrocyte.

Protein concentration in blood serum	Numbers of erythrocytes	Number of cow
4.6—5.0	483	1
5.1—5.5	—	0
5.6—6.0	554	11
6.1—6.5	554	11
6.6—7.0	561	19
7.1—7.5	486	12
7.6—8.0	487	6
8.1—	452	3

According to the standard of diagnosis in medicine, the disorder of the liver is diagnosed as negative when the volumes of consumed Häym's solution is above 1.00 cc.

If the volumes of consumed Häym's solution is 0.99~0.75 cc, it is diagnosed as a doubt-positive, as positive for volumes of 0.74~0.63 cc and as strong-positive for volumes under 0.62 cc.

Taking this standard, a positive for liver disorder is shown in 7.04 per cent of 63 cows researched and a doubt-positive is shown in 18.30 per cent.

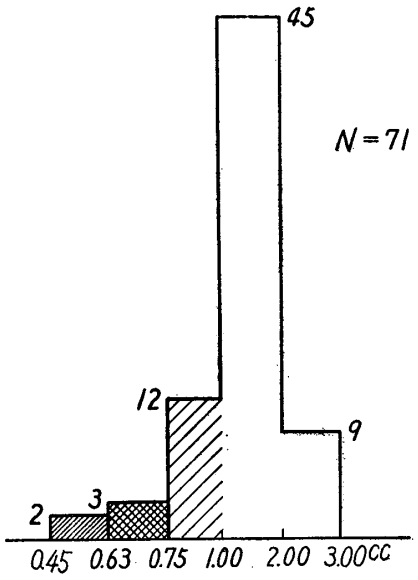

Fig. 4. Distribution of the Gros' test of 71 cows.

Summary

After the Second World War, dairy farming has progressed rapidly and the numbers of dairy cows has increased in Japan. With such a tendency, the nutritional and reproductive disorders were gradually increasing. These disorders caused damage to dairy farming.

Accordingly, as the first step, we mentioned a concept of dairy cow's nutrition and pointed out some problems in their nutrition. Next, the first stage in a series of our studies, we researched on the various constituents of blood, milk and urine of the cow under the practical conditions in Miyagi Prefecture, so that we may find an ample means for nutrition of the dairy cow in the field.

Consequently, we found that there are many cows, 1) which showed a low count of erythrocyte, 2) which showed a low concentration of protein in the blood plasma, 3) which showed a positive for the tests of functional disorder of the liver and, which were not provided with adequate feed.

Acknowledgements

The authers wish to thank Messrs. K. Shimizu, Y. Takeyasu, A. Shibata, K. Kimura, H. Oikawa, Y. Sasaki, Y. Watanabe, J. Suzuki, K. Seto and Miss H. Itabashi, Laboratory of Animal Physiology, Tohoku University, and Mrs. S. Wada and Miss H. Takahashi, Laboratory of Chemistry of Nutrition, Tohoku University for their technical assistance in these researches.

Reference

1) Richter, J. (1926). Die Sterilitat des Rinds. Berlin.
2) Eckles, C. H., R. B. Becker, and L. S. Palmer. (1932). Minn. Agr. Expt. Sta. Tech. Bull., **91**,
3) Allen, G. S. (1943). Vet. Record., **55**, 168—169.
4) Quinin, J. (1929). 15th Ann. Rept. Director of Vet. Services, Union of S. Africa, 833—1055.
5) Riddle, W. H., J. S. Hughes, and J. B. Fitch. (1934). Kansas Agr. Expt. Sta. Tech. Bull., **36**,
6) Webster, W. M. (1932). Australian Vet. J., **8**, 199—223.
7) Sutton, T. S., W. E. Krauss, and S. L. Hansard (1940). J. Dairy Sci., **23**, 574.
8) Harris, P. L., W. J. Swanson, and K. C. D. Hickman (1947). J. Nutrition, **33**, 411—428.