Hematology and Biochemistry Reference Values for Female Holstein Cattle

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ABSTRACT

Reference intervals are presented for 14 hematology and 32 biochemistry variables from four age groups of female Holstein cattle (n=172) selected randomly from six well managed farms. Each animal was examined by a clinician and with the history available considered to be clinically normal at the time of blood collection. The variable observations were examined for outliers and Gaussian distribution prior to parametric or where necessary, nonparametric analysis.

Many differences were noted between age groups but few between farms.

RÉSUMÉ

Cette expérience visait à colliger des valeurs de référence, relatives à 14 paramètres hématologiques et à 32 paramètres biochimiques, chez 172 femelles Holstein de quatre groupes d'âge et choisies au hasard, au sein de six troupeaux bien régis. Un clinicien procéda à l'examen de chacune de ces femelles et, selon l'anamnèse dont il disposait, les considéra cliniquement normales, lors du prélèvement d'échantillons de sang. On analysa les données relatives à chacun des paramètres choisis, en accordant une attention particulière à celles dont la distribution s'avérait équivalente à celle de Gauss, ainsi qu'aux valeurs trompeuses, avant de recourir, nécessairement ou non, à l'analyse non paramétrique. On décela plusieurs différences d'un groupe d'âge à l'autre, mais non d'un troupeau à l'autre.

INTRODUCTION

The concept and the recommendations for preparation of reference values have been recently outlined in human medicine (1, 4, 5). This change in nomenclature and the associated approach in developing reference values has been interpreted as an important step towards establishing a scientific basis for clinical interpretation of laboratory data (18). The clinician must of course weigh together the history, clinical signs, disease incidence, etc with the laboratory data (9, 12, 26).

Reference values are influenced by age, sex, nutrition, physical activity, etc (9, 10, 17, 18) and in veterinary medicine the additional species, breed and management factors greatly magnify the effort required to generate reference values for each subpopulation of interest. The accuracy and precision of the laboratory techniques as modified by reagents, temperature and instrumentation etc affect reference values from a particular population (4, 5, 9, 11, 18). The numbers of observations, the assumption regarding distribution of the observations and the statistical analysis of the data can markedly influence the resulting reference intervals, especially the lower and upper limits of 95% of the population, i.e. 2.5 and 97.5 percentiles required by the clinician (3, 4, 5, 9, 12, 23). Description of the population sampled, the laboratory procedures used and the statistical analysis of the data must be available if others are to use the reference values (5, 18).

The hematology and biochemistry reference intervals presented were determine from minimal numbers of observations obtained in a described manner from four age groups of female Holstein cattle, primarily for use by clinicians

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TABLE I. Bovine Hematology Reference Intervals[a]

		SD[b]	1 - 14 days	2wks - 6 mos	6 mos - 2 yrs	2 yrs +
B-Hemoglobin	g/L	1	57 - 158[c]	85 - 141	92 - 154	85 - 132
B-Hematocrit	%	0.6	17 - 47	23 - 42	26 - 48	24 - 36
B-Erythrocytes	x 10^{12}/L	0.09	4.9 - 10.9	6.5 - 11.9	6.1 - 10.6	5.0 - 7.7
Ery-MCV	fL	0.8	31.7 - 49.6	26.6 - 44.3	31.5 - 50.9	37.8 - 56.0
Ery-MCH	pg	0.8	10.8 - 16.5	9.1 - 15.6	10.7 - 19.1	14.2 - 20.1
Ery-MCHC	g/L	0.5	274 - 398	310 - 322	310 - 390	317 - 404
B-Platelets	x 10^9/L		190 - 940	220 - 950	130 - 710	220 - 640
B-Leukocytes	x 10^9/L	0.11	2.6 - 14.6	5.6 - 13.7	6.3 - 14.4	3.8 - 11.0
B-Neutrophils						
Segmented	x 10^9/L		0.6 - 9.4	0.6 - 6.1	1.0 - 4.6	0.7 - 4.9
	%		12 - 79	8 - 64	9 - 46	15 - 61
Band	x 10^9/L		0 - 1.5	0 - 0.1	0 - 0.3	0 - 0.2
	%		0 - 13	0 - 2	0 - 2	0 - 2
B-Lymphocytes	x 10^9/L		1.0 - 6.4	2.2 - 8.7	3.4 - 9.4	1.0 - 5.8
	%		13 - 81	31 - 84	43 - 83	26 - 68
B-Monocytes	x 10^9/L		0.0 - 1.2	0.8 - 1.2	0.0 - 1.0	0.0 - 0.9
	%		1 - 12	1 - 12	0 - 9	0 - 12
B-Eosinophils	x 10^9/L		0 - 0.1	0 - 0.3	0 - 2.4	0 - 1.9
	%		0 - 1	0 - 2	0 - 21	0 - 28
B-Basophils	x 10^9/L		0 - 0.1	0 - 0.1	0 - 0.1	0 - 0.1
	%		0 - 1	0 - 1	0 - 1	0 - 1

[a]172 female Holstein animals according to four age groups
[b]Analytical bias (1 SD) observed for one level of control sample, generally high normal range (n > 30, < 50)
[c]Determined 2.5 and 97.5 percentile interval

and researchers associated with a veterinary teaching hospital.

MATERIALS AND METHODS

Blood samples were taken by jugular venipuncture, using bleeding needles, directly into vacuum tubes containing potassium ethylenediaminetetraacetic acid, ammonium heparin or sodium oxalate for whole blood and plasma or no anticoagulatant for serum collection[1]. Each week between 0730 h and 0930 h during the months June through February blood samples were collected from eight to ten animals. The advice of experienced clinicians guided the arbitrary selection of the age groups. The animals were chosen by the following procedure. The farms served by the Farm Service division of the Teaching Hospital were divided into three categories according to management practices including nutrition and general health. Six farms were chosen using random numbers of the 15 farms in the highest category and agreement obtained by the owners to allow blood samples to be collected. All animals were assigned numbers according to age group for each farm and by random numbers approximately equal numbers of animals in each age group were chosen from each farm (Table IV). Blood samples were collected from up to four age groups on two farms each week. For the youngest age group blood samples were collected as the animals became available during regular sampling at the farms, each calf having received colostrums as far as could be ascertained and being over 24 h of age. The oldest age group included animals in various stages of lactation and pregnancy and a relatively even age distribution from over two years to ten years of age.

For each animal the clinical history was reviewed with the farm manager, physical examinations were made by one of the authors (Dr. R. Rowe) or in rare cases an animal health technician and all animals were judged to be clinically healthy prior to collection of the blood samples. If clinical disease was suspected that animal was excluded and an alternate animal chosen from the list previously selected by random numbers.

The blood samples were taken directly to the laboratory. The serum, heparin and oxalate plasma were separated from the cells by double centrifugation immediately upon arrival. The laboratory tests were determined the same day or on serum or plasma stored at 4°C or –20°C as previously described (13).

[1]Vacutainer, Becton Dickinson Company Ltd., Rutherford, New Jersey.

The laboratory control program included known internal and unknown external control samples. The analytical bias inherent within most methods is presented in Tables I-III as the standard deviation (SD) observed from the known control samples ($n > 30, < 50$), generally that control sample closest to the highest range of values observed (11).

The statistical examination of the observations and calculations of the reference intervals for each variable has been fully described (11). Essentially outliers were excluded, parametric analysis was used to determine the percentiles if the observations had a Gaussian distribution before or after transformation by any of four types of transformation or, where necessary, nonparametric analysis was used.

Differences between age groups and between farms were analysed by the variance technique as discussed in detail (15).

The reference intervals are presented using the SI units expected to be adopted initially in Canadian medical laboratories.

RESULTS

The 2.5 and 97.5 percentiles, i.e. lower and upper limits of 95% of the population samples, are given in Tables I, II and III according to age. The number of observations (n), observed mean ($\bar{x}$), standard deviation (SD) distribution of the data and significant age group differences are given in Tables IV, V and VI.

Between farm differences are present for blood urea nitrogen, blood urea nitrogen to creatinine ratios and plasma osmolality. The range of means by farm are: blood urea nitrogen 6.3-10.9 mmol/L, blood urea nitrogen to creatinine ratio 9-15 and plasma osmolality 285-290 mmol/L serum water.

DISCUSSION

Reference values have been described as observed values of a particular quantity obtained from individuals in defined states of health (1). It has been recommended that the term "normal" value be abolished because of the inherent ambiguity (6, 18, 24). If the data is subjected to statistical treatment then a term such as "reference interval" is recommended for description of the remaining values (4). Although not included the deciles and quantiles have been calculated for potential reporting of the percentile quotation with computerized patient data printout (3, 8, 23). The recommended criteria to be used for selection of subjects as source for reference values, the description of the specimen collection conditions (1) and the statistical terminology for description of these values has recently been published for man (5).

Observed values from an animal for a particular quantity are compared to previously established reference values for interpretation (1) but not necessarily for disease diagnosis (18, 26). Where established, the reference value or probability of a certain disease existing given the observed value (7) would be most useful to the clinician. Reference values are currently being established for a few laboratory tests in human medicine but have not been determined for most laboratory tests in veterinary medicine except in the mind of the experienced clinician (26). With the advent of automated laboratory equipment and routine use of series of tests, computerized data collection and analysis could hasten development of this important information.

The Coulter Model S was used to determine WBC, RBC, Hgb, hematocrit, MCV, MCH and MCHC. Evaluation of this instrument has shown it to be acceptable for analysis of blood from healthy cattle (unpublished data, JHL). A duplicate microhematocrit was examined for confirmation of the hematocrit calculated by the Coulter Model S and found to be not significantly different.

The determined 2.5 and 97.5 percentiles (Tables I, II and III) correlate very closely with the observed range of values for most tests as would be expected for this population sample size but do not correlate well with the calculated mean $\pm$ 2 SD for untransformed nonGaussian data (22).

The initial consideration was to group together the variable data for those age

TABLE II. Bovine Biochemistry Reference Intervals[a]

		SD[b]	1 - 14 days[c]	2wks - 6 mos	6 mos - 2 yrs	2 yrs +
S-Calcium	mmol/L	0.05	2.34 - 3.04	2.35 - 2.74	2.25 - 2.77	2.10 - 2.67
S-Phosphorus	mmol/L	0.10	2.07 - 3.03	1.94 - 3.26	1.58 - 3.07	1.32 - 2.65
Ca/P			1.0 - 1.7	0.9 - 1.6	0.9 - 1.8	1.1 - 2.3
S-Magnesium	mmol/L	0.02	0.70 - 1.07	0.70 - 1.11	0.78 - 1.11	0.79 - 1.19
P-Sodium	mmol/L	1.2	135 - 145	136 - 146	135 - 146	135 - 145
P-Potassium	mmol/L	0.1	3.9 - 5.6	3.4 - 4.9	3.6 - 5.1	3.6 - 5.1
Na/K			24 - 34	28 - 40	28 - 39	27 - 38
P-Chloride	mmol/L	0.9	93 - 104	94 - 104	95 - 105	96 - 105
P-Osmolality	mmol/kg	2.9	279 - 298	276 - 295	277 - 296	276 - 296
B-Urea nitrogen	mmol/L	1.0	3.6 - 15.7	2.1 - 14.3	2.1 - 13.6	1.4 - 15.7
S-Creatinine	umol/L	5.3	71 - 141	53 - 97	62 - 115	62 - 124
BUN/Creatinine			4.9 - 16.5	4.4 - 25.7	2.5 - 21.1	2.7 - 20.0
P-Glucose	mmol/L	0.2	2.8 - 7.5	2.3 - 5.8	2.9 - 4.2	2.5 - 3.8
S-Cholesterol	mmol/L	0.2	1.0 - 3.2	1.2 - 3.8	1.8 - 3.9	2.0 - 6.2
S-Bilirubin	umol/L	1.7	1.7 - 23.9	1.7 - 10.2	1.7 - 6.8	0 - 8.6
Unconj.	umol/L		0 - 22.2	0 - 6.8	0 - 5.1	0 - 5.1
Conj.	umol/L		0 - 6.8	0 - 8.6	0 - 5.1	0 - 5.1
S-Iron	umol/L	1.0	5 - 47	10 - 47	21 - 39	14 - 37
S-Iron Binding Capacity	umol/L	2.4	65 - 123	49 - 93	55 - 88	48 - 80
S-Iron Binding Saturation	%		5 - 53	7 - 63	29 - 64	25 - 58

[a]172 female Holstein animals according to four age groups
[b]Analytical bias (1 SD) observed for one level of control sample, generally high normal range (n > 30, < 50)
[c]Determined 2.5 and 97.5 percentile interval

TABLE III. Bovine Biochemistry Reference Intervals[a]

		1 - 14 days	2wks - 6 mos	6 mos - 2 yrs	2 yrs +
S-Transaminases					
Aspartate amino (AST)	units/L (30°C)	12 - 48[b]	18 - 50	26 - 48	24 - 45
Alanine amino (ALT)	units/L (30°C)	2 - 11	3 - 18	6 - 19	5 - 18
S-Alkaline Phosphatase	units/L (37°C)	29 - 187	16 - 129	22 - 82	3 - 46
S-Creatine Phosphokinase	units/L (30°C)	11 - 125	26 - 112	9 - 110	17 - 59
S-Lactate dehydrogenase	units/L (30°C)	151 - 412	264 - 551	311 - 668	284 - 511
S-Amylase	Caraway units/L (37°C)	0 - 1000	100 - 770	20 - 770	100 - 800
S-Protein	g/L	39 - 70	48 - 73	55 - 74	59 - 81
S-Albumin	g/L	22 - 33	25 - 38	29 - 38	29 - 39
S-Globulin	g/L	15 - 41	19 - 40	25 - 37	25 - 47
A/G		0.6 - 1.4	0.7 - 1.6	0.8 - 1.3	0.6 - 1.3
S-α globulin	g/L	7 - 13	7 - 13	8 - 13	5 - 8
S-$\beta_1$globulin	g/L	6 - 12	6 - 10	6 - 9	4 - 10
S-$\beta_2$globulin	g/L	1 - 9	3 - 8	4 - 8	4 - 10
S-γ globulin	g/L	0.5 - 12	1 - 144	4 - 13	5 - 18
P-Fibrinogen	g/L	3.3 - 9.3	2.7 - 8.2	1.9 - 7.6	2.4 - 7.4

[a]172 female Holstein animals according to four age groups
[b]Determined 2.5 and 97.5 percentile interval

groups which were not significantly different in order to obtain a larger sample on which to base the reference intervals. Since age differences were detected for some variables but not for others (10, 20, 21, 22, 25) we decided to indicate all differences in spite of the considerable overlap for many variables between age groups. Between farm differences are present for blood urea nitrogen, blood urea nitrogen to creatinine ratio and osmolality (Table V), the highest mean value originating in each case from the same farm. The blood urea and blood urea to creatinine ratios may be related to dietary protein intake (14). That other farm to farm differences were not present may be due to the intentional selection of farms with better management and nutritional balance (17). The influence of the stage of lactation and season were not examined in this study (2, 10, 16).

The low serum iron and low percent saturation of iron binding capacity observed in the youngest age group (Tables II and V) is likely of prenatal origin and has been shown to correlate directly with the hematocrit in newborn calves (19).

Examination of these reference values emphasizes the need to understand the

TABLE IV. Bovine Hematology Reference Values[1]

		1 - 14 days				2 wks - 6 mos				6 mos - 2 yrs				2 yrs +			
		n	d[2]	$\bar{x}^3$	SD	n	d	$\bar{x}$	SD	n	d	$\bar{x}$	SD	n	d	$\bar{x}$	SD
B-Hemoglobin	g/L	41	G	108^{a}	26	41	G	$113^{a,b}$	14	43	G	123^{b}	16	41	G	108^{a}	12
B-Hematocrit	%	41	G	$32^{a,b}$	7.5	41	G	$33^{a,b}$	4.6	43	G	34^{b}	4.1	41	G	30^{a}	3.2
B-Erythrocytes	x 10^{12}/L	41	G	7.9^{a}	1.5	41	G	9.2^{b}	1.4	43	G	8.3^{a}	1.1	41	G	6.4^{c}	0.7
Ery-MCV	fL	41	G	40.7^{a}	4.5	41	G	35.4^{b}	4.5	42	G	41.2^{a}	4.9	41	G	47.4^{c}	4.8
Ery-MCH	pg	41	G	13.7^{a}	1.4	41	G	12.4^{b}	1.7	43	G	14.9^{c}	2.1	41	G	17.2^{d}	1.5
Ery-MCHC	g/L	40	G	336^{a}	31	41	G	$351^{a,b}$	21	42	NP	360^{b}	21	41	G	361^{b}	22
B-Platelets	x 10^{9}/L	41	G	560^{a}	190	40	G	590^{a}	190	42	G	420^{b}	150	39	G	430^{b}	110
B-Leukocytes	x 10^{9}/L	40	G	$8.6^{a,b}$	3.0	41	G	9.7^{b}	2.1	42	G	10.3^{b}	2.1	41	G	7.4^{a}	1.8
B-Neutrophils																	
Segmented	x 10^{9}/L	41	$\sqrt{x}$	3.7	2.1	39	G	3.4	1.4	42	G	2.8	0.9	41	G	2.8	1.1
	%	41	G	46^{a}	17	40	G	36^{b}	14	43	G	28^{c}	10	41	G	$38^{a,b}$	12
Band	x 10^{9}/L	41	NP	0.2	0.3	38	NP	0.02	0.04	42	NP	0.02	0.06	40	NP	0.02	0.05
	%	41	NP	1.6^{a}	2.7	39	NP	0.2^{b}	0.05	42	NP	0.2^{b}	0.5	39	NP	$0.2^{a,b}$	0.5
B-Lymphocytes	x 10^{9}/L	41	G	3.7	1.7	40	G	5.5	1.7	42	G	6.4	1.5	40	G	3.4	1.2
	%	41	G	46.8^{b}	17.3	40	G	57.5^{b}	13.5	43	G	62.9^{b}	10.2	41	G	46.9^{a}	10.6
B-Monocytes	x 10^{9}/L	41	$\sqrt{x}$	0.3	0.3	40	$\sqrt{x}$	0.5	0.3	43	$\sqrt{x}$	0.2	0.2	41	$\sqrt{x}$	0.3	0.2
	%	40	$\sqrt{x}$	4.4	2.6	39	$\sqrt{x}$	4.9^{a}	0.3	43	$\sqrt{x}$	2.2^{b}	2.1	41	$\sqrt{x}$	4.1^{a}	2.5
B-Eosinophils	x 10^{9}/L	39	NP	0.01	0.03	39	NP	0.04	0.07	43	$\sqrt{x}$	0.5	0.2	41	$\sqrt{x}$	0.6	0.5
	%	39	NP	0.2	0.4	39	NP	0.5^{a}	0.7	43	$\sqrt{x}$	4.6^{b}	6.5	41	$\sqrt{x}$	7.9^{c}	6.7
B-Basophils	x 10^{9}/L	39	NP	0.0	0.0	40	NP	0.01	0.03	43	NP	0.0	0.2	39	NP	0.03	0.2
	%	41	NP	0.05	0.2	40	NP	0.1	0.3	43	NP	0.05	0.2	39	NP	0.05	0.2

[1]172 female Holstein animals according to four age groups
[2]Distribution of data, transformation or method of analysis: G = Gaussian distribution, $\sqrt{x}$ = square root transformation, NP = nonparametric analysis
[3]Means with different superscripts are significantly different at the $\alpha = 0.05$ level (Tukey's test)

TABLE V. Bovine Biochemistry Reference Values[1]

		1 - 14 days				2 wks - 6 mos				6 mos - 2 yrs				2 yrs +			
		n	d[2]	$\bar{x}$[3]	SD	n	d	$\bar{x}$	SD	n	d	$\bar{x}$	SD	n	d	$\bar{x}$	SD
S-Calcium	mmol/L	43	G	2.70[a]	0.17	42	G	2.54[b]	0.10	43	G	2.50[b]	0.12	43	G	2.37[c]	0.15
S-Phosphorus	mmol/L	44	G	2.55[a]	0.29	42	G	2.61[a]	0.32	43	G	2.32[b]	0.34	43	G	1.97[c]	0.36
Ca/P		43	G	1.4	0.2	41	G	1.3	0.01	42	G	1.4	0.2	43	G	1.6	0.3
S-Magnesium	mmol/L	44	G	0.90[a]	0.08	42	G	0.90[a]	0.08	43	G	0.95[a,b]	0.90	43	G	0.97[c]	0.08
P-Sodium	mmol/L	43	G	140[a]	2.6	42	G	141[a]	2.3	43	G	141[a]	2.8	43	G	140[a]	2.6
P-Potassium	mmol/L	44	G	4.7[a]	0.4	42	G	4.2[b]	0.4	42	G	4.3[b]	0.4	43	G	4.3[b]	0.4
Na/K		43	G	29.9	2.6	42	G	34.0	3.0	42	G	32.7	3.0	43	G	32.6	3.0
P-Chloride	mmol/L	43	G	98[a]	2.8	42	G	99[a,b]	2.6	43	G	100[b]	2.4	42	G	100[b]	2.4
P-Osmolality	mmol/kg	43	G	289[a]	4.8	42	G	286[a]	5.0	43	G	286[a]	4.8	42	G	286[a]	5.0
B-Urea																	
Nitrogen	mmol/L	43	G	9.3[a]	3.1	42	G	8.6[a,b]	3.0	43	G	7.8[a,b]	3.0	43	$\sqrt{x}$	6.9[b]	3.8
S-Creatinine	μmol/L	43	G	100[a]	18	41	G	71[b]	9	42	G	88[c]	18	43	G	97[c]	18
BUN-Creatinine		42	G	10.7	2.9	41	G	14.1	5.7	43	G	11.3	5.6	43	$\sqrt{x}$	9.2	5.6
P-Glucose	mmol/L	44	G	5.2[a]	1.2	42	G	4.1[b]	0.9	42	G	3.6[c]	0.3	43	G	3.2[c]	0.3
S-Cholesterol	mmol/L	44	G	2.1[a]	0.6	42	G	2.5[a,b]	0.6	41	G	2.8[b,c]	0.5	43	G	4.1[c]	1.1
S-Bilirubin																	
Total	μmol/L	43	$\sqrt{x}$	10.3[a]	1.7	42	NP	5.1[b]	1.7	42	NP	3.4[b]	1.7	43	NP	3.4[b]	1.7
Unconj.	μmol/L	43	$\sqrt{x}$	6.8[a]	1.7	42	NP	3.4[b]	1.7	43	NP	1.7[b]	1.7	43	NP	1.7[b]	1.7
Conj.	μmol/L	42	NP	3.4[a]	1.7	42	NP	1.7[a,b]	1.7	43	NP	1.7[a,b]	1.7	43	NP	1.7[b]	1.7
S-Iron	μmol/L	43	log e	15[a]	11	42	G	29[b]	9	43	G	30[b]	5	43	G	26[b]	5.9
S-Iron Binding Capacity	μmol/L	44	G	94[a]	15	42	G	71[b]	11	43	G	71[b]	8	43	G	64[c]	8
S-Iron Binding Saturation	%	44	NP	21[a]	17	42	NP	40[b]	12	43	NP	42[c]	7	43	NP	40[c]	8

[1]172 female Holstein animals according to four age groups

[2]Distribution of data, transformation or method of analysis: G = Gaussian distribution, $\sqrt{x}$ = square root transformation, log e = log base e transformation, NP = nonparametric analysis

[3]Means with different superscripts are significantly different at the $\alpha = 0.05$ level (Tukey's test)

TABLE VI. Bovine Biochemistry Reference Values[1]

	1 - 14 days				2 wks - 6 mos				6 mos - 2 yrs				2 yrs +			
	n	d[2]	$\bar{x}$[3]	SD	n	d	$\bar{x}$	SD	n	d	$\bar{x}$	SD	n	d	x	SD
S-Transaminases																
Aspartate amino (AST) units/L (30°C)	44	$\sqrt{x}$	19[a]	13	41	G	34[a]	8	43	G	37[a]	5	41	G	34[a]	5
Alanine amino (ALT) units/L (30°C)	40	$\sqrt{x}$	5[a]	0.3	41	G	10[b]	4	43	G	12[b]	3	41	G	12[b]	3
S-Alkaline Phosphase units/L (37°C)	40	log e	73[a]	52	42	G	72[a]	28	43	G	52[b]	15	42	G	24[c]	11
S-Creatine Phosphokinase units/L (30°C)	44	log e	38[a,b]	53	41	G	69[b]	21	42	G	60[a,b]	26	39	G	38[a]	11
S-Lactate Dehydrogenase units/L (30°C)	44	G	281[a]	66	41	G	408[b]	73	43	G	490[c]	91	42	G	397[b]	58
S-Amylase Caraway units/L (37°C)	44	$\sqrt{x}$	240[a]	220	42	G	400[b]	160	43	G	360[a,b]	170	39	G	450[b]	180
S-Protein g/L	44	G	54[a]	8	42	G	60[b]	6	43	G	64[c]	5	42	G	70[d]	6
S-Albumin g/L	44	NP	26[a]	2	42	G	31[b]	3	43	G	33[c]	2	43	G	34[c]	2
S-Globulin g/L	44	G	28[a]	7	42	G	29[a]	5	42	G	31[a]	3	43	G	36[b]	5
A/G	44	G	1.0[a]	0.2	42	G	1.1[b]	0.2	43	G	1.1[a,b]	0.1	43	G	1.0[a]	0.2
S-α globulin g/L	44	G	10[a]	2	42	NP	10[a]	2	43	NP	10[a]	1	42	G	10[a]	1
S-$β_1$globulin g/L	44	G	9[a]	2	42	NP	7[b]	1	43	NP	7[b]	1	42	NP	7[b]	1
S-$β_2$globulin g/L	43	$\sqrt{x}$	4[a]	1	42	G	5[a,b]	1	43	G	6[b,c]	1	43	NP	7[c]	1
S-γglobulin g/L	44	$\sqrt{x}$	4[a]	1	42	G	7[b]	3	43	G	8[b]	2	43	G	11[c]	3
P-Fibrinogen g/L	44	G	6.3[a]	1.5	42	G	5.4[b]	1.4	43	G	4.7[b]	1.4	42	G	4.9[b]	1.3

[1]172 female Holstein animals according to four age groups
[2]Distribution of data, transformation or method of analysis: G = Gaussian distribution, $\sqrt{x}$ = square root transformation, log e = log base e transformation, NP = nonparametric analysis
[3]Means with different superscripts are significantly different at the $\alpha = 0.05$ level (Tukey's test)

preparation and limitations of published reference values as used to assist the rule in or rule out of clinical diseases (7, 12, 24, 26).

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