Variability of alkaline phosphatase in goat milk in relation to its use as an effective index of pasteurisation

Final report on Project SO 1003 to Food Standards Agency Scotland

Variability of ALP activity in goat milk throughout lactation

Suitability of methods for the assessment of effectiveness of pasteurisation of goats milk

Residual ALP activity and microbiological quality of pasteurised goat milk retailed in Scotland

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CONTENT

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Food Standards Agency Scotland
Project SO 1003

EXECUTIVE SUMMARY

The microbiological safety of milk depends on efficient pasteurisation and prevention of recontamination of the finished product. Pasteurisation, a heat treatment equivalent to a minimum holding of milk at no less than 71.8°C for 15 seconds – inactivates almost all potential pathogens found in raw milk. Validation of the effectiveness of the pasteurisation process is based on destruction of a natural milk enzyme-alkaline phosphatase (ALP). The sensitivity of this test hinges on the initial concentration of ALP in raw milk. The higher the initial activity, the more sensitive is the test. In addition, the sensitivity depends on the method of analysis of residual enzyme activity.

The detection limit for the reference test for ALP is equivalent to contamination of properly pasteurised cows milk by 0.1% raw milk. The implications of applying the test to pasteurised goat milk were explored because goat milk was reported to have natural levels of ALP around 10% of the activity found in cows milk. In such circumstances the sensitivity of the reference method for ALP would be reduced ten fold i.e. contamination of pasteurised goat milk by raw milk could reach a level of 1% before it would be detected. Variations in the initial pool of indigenous alkaline phosphatase in milk lead to different amounts of raw milk being allowed in the pasteurised milk product at the statutory pass level.

The research undertaken in this project aimed to reduce the potential threat to public safety associated with consumption of inadequately processed goat milk.

The effectiveness of the ALP test is determined by three factors: (1) The level of ALP in milk and its variability, (2) The sensitivity of the method of measuring ALP (spectrophotmetric, fluoroescence, bioluminescence) and (3) The levels set in legislation as acceptable standards for residual ALP activity.

The work undertaken within this project encompassed: a lactational study of the variability of ALP in a herd of British Saanen goats; a comparison of the effectiveness of bioluminescence, fluorescence and spectrophotometric measurements of ALP; a study of the formation of heat stable ALP; and a limited survey of residual ALP and microbiological quality of pasteurised goat milk retailed in Scotland.

There is little information in the literature regarding the variability levels of ALP in individual goat milks or the influence of lactational effects on secretion of ALP into milk. Seventeen British Saanen goats from the Hannah Research Institute herd were therefore sampled on a weekly basis throughout a full lactation, i.e. April 2001 to early January 2002. Individual morning and evening milking samples were tested for ALP daily during the first eight weeks of lactation Thereafter the morning milk from individual goats was sampled once a week throughout the remaining lactation period. Alkaline phosphatase was determined by the Fluorophos method (IDF Standard 155A:1999). Statistical modelling of the lactational data explored relationships between ALP levels in milk and goat genotype, age, lactation history and milk composition.

ALP levels in milk were lowest in the early stages of lactation and increased as the lactation progressed and milk yields declined. ALP levels were higher in milk samples from evening milk as compared with morning milk. The lowest mean value recorded for ALP in an individual goat for morning milk in May was 5823 mU/L. 15 of the 16 animals producing milk in May had ALP levels under 32000mU/L. The lowest level of ALP in an individual goat milk sample was 3630 mU/L. Values for ALP in goat milk increased as lactation progressed. In November, the minimum mean value for ALP in an individual goat morning milk was 18658 mU/L. The minimum value for an individual goat was 10410 mU/L. Mean vales for ALP in November ranged from 18658 mU/L to 782000mU/L.

The mean value throughout lactation (11 samples) for ALP in bulk herd goat milk was 38880 mU/L. The equivalent value for cows milk (mean of 22 samples) was more than tenfold higher at 560049 mU/L.

Early lactation pasteurised bulk goat milk which was contaminated with raw milk at levels of 1.0% raw milk did not fail the Fluorophos, Bioluminescence or Sanders and Sager ALP test. In mid lactation failures for goat milk were obtained at levels ranging from 0.7 to 0.9% contamination. In cows milk failures for the ALP tests were evident at 0.08 to 0.1% contamination of pasteurised milk with raw milk.

Results indicate that all tests currently available are not suitable for ALP determination in goat milk.

Twenty nine samples of pasteurised goat milk were collected from retail outlets in Ayrshire. All samples had satisfactory residual phosphatase levels but two of the samples had unacceptably high counts for Enterobacteriaceae.

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LAYMAN'S SUMMARY

The microbiological safety of milk depends on efficient pasteurisation and prevention of recontamination of the finished product with bacteria. Pasteurisation is a heat treatment equivalent to a minimum holding of milk at no less than 71.8°C for 15 seconds which inactivates potentially harmful bacteria found in raw milk. The test to determine the effectiveness of pasteurisation in pasteurised milk is called the alkaline phosphatase (ALP) test. The test was developed in the 1930's when scientists found the enzyme alkaline phosphatase, which is present in milk from all species, was inactivated at slightly higher temperature conditions than those required to kill *Mycobacterium tuberculosis,* the organism responsible for Tuberculosis. This heat treatment was also shown to effectively destroy other milk borne bacteria which may cause human disease.

Validation of the effectiveness of the pasteurisation process is based on destruction of a natural milk enzyme-alkaline phosphatase (ALP). The effectiveness of this test hinges on the initial concentration of ALP in raw milk, determining the amount of ALP remaining active after pasteurisation. The detection limit for the reference test for ALP is equivalent to the contamination of properly pasteurised cows' milk by 0.1% raw milk. However, the amount of ALP in milk varies between species and within individual animals within a species.

In this study, the implications of applying the test to pasteurised goat milk were explored as goat milk is reported to have natural levels of ALP around 10% of that found in cows' milk. Therefore, in such circumstances the sensitivity of the test for ALP is reduced tenfold, i.e. contamination by raw milk could reach a level of 1% before a pasteurized milk would fail the current statutory ALP test.

Prior to this study detailed information regarding the ALP levels in goats' milk was not available, and consequently an investigation was undertaken to explore factors influencing changes in ALP levels in twelve British Saanen goats throughout a full lactation. ALP levels in goat milk were shown to be extremely variable in individual animals within a herd. More importantly, levels of ALP in goat milk were consistently at least tenfold lower than those found in cows' milk. Herd goat milk in early lactation contained the lowest levels of ALP and a 1% contamination of pasteurised milk with raw milk did not produce a fail in the current statutory colorimetric ALP test. It was shown however that the more sensitive tests of bioluminescence and fluorescence could be used to detect a 0.2% contamination of pasteurised goat milk with raw milk in early lactation but to use these tests effectively the current legislative limit for ALP in pasteurised goat milk would have to be reassessed and reduced considerably. Ideally, new test methods are required to assess the effectiveness of pasteurisation of goat milk.

INTRODUCTION

The microbiological safety of milk depends on efficient pasteurisation and prevention of recontamination of the finished product. Pasteurisation, a heat treatment equivalent to a minimum holding of milk at no less than 71.8°C for 15 seconds, inactivates almost all potential pathogens found in raw milk. Validation of the effectiveness of the pasteurisation process is based on destruction of a natural milk enzyme-alkaline phosphatase (ALP). The sensitivity of this test hinges on the initial concentration of ALP in raw milk. The higher the initial activity, the more sensitive is the test. The sensitivity of the test depends on the method of analysis of residual enzyme activity.

The detection limit for the reference test for ALP is equivalent to contamination of properly pasteurised cows milk by 0.1% raw milk. The implications of applying the test to pasteurised goat milk were explored because goat milk was reported to have natural levels of ALP around 10% of the activity found in bovine milk. In such circumstances the sensitivity of the reference method for ALP is reduced tenfold. i.e. contamination of pasteurised goat milk by raw milk could reach a level of 1% before it would be detected. The research undertaken aimed to reduce the potential threat to public safety associated with consumption of inadequately processed goat milk. The extent of the potential problem in goat milk was explored throughout a full lactation.

SUMMARY OBJECTIVES

Objective No.	Objective Description
01	Lactational study of the variability of alkaline phosphatase in 17 British Saanen Goats as measured by fluorescence.
02	Comparison of effectiveness of bioluminescence, fluorescence and spectrophotometric methods in determining the efficiency of pasteurisation of goat, sheep and cow milk.
03	Study of the origins and factors influencing the formation of heat stable alkaline phosphatases in goat milk
04	Survey of residual ALP activity in commercial pasteurised and unpasteurised goat and sheep milk on sale in Scotland
05	Survey of the microbiological quality of pasteurised and unpasteurised goat milk on sale in Scotland

MILESTONES

Milestone No.	Target Date	Milestone Title
01/01	31.10.01	Complete report on ALP levels in individual goat milk from early to mid lactation
01/02	1.02.02	Complete report to FSA on ALP levels in goat milk from mid to late lactation
01/03	31.06.02	Complete report on variability in ALP activity in individual goat milks and a bulk sample throughout lactation
02/01	31.10.01	Complete report on comparison of measurement of ALP in bulk goat milk in early to mid lactation and the sensitivity of detecting raw milk contamination using spectrophotometric, fluorescence and bioluminescence techniques.
02/02	1.02.02	Complete preliminary report on comparison of measurement of ALP in bulk goat milk in mid to late lactation and the sensitivity of detecting raw milk contamination using spectrophotometric, fluorescence and bioluminescence techniques.
02/03	31.06.02	Complete final report on comparison of measurement of ALP in bulk goat milk throughout lactation and the sensitivity of detecting raw milk contamination using spectrophotometric, fluorescence and bioluminescence techniques.
03/01	31.10.01	Complete preliminary report on heat stable ALP in individual goat milks in early and mid lactation
03/02	1.02.02	Complete preliminary report on heat stable ALP in individual goat milk in late lactation
03/03	31.05.02	Complete final report to FSA on the occurrence of heat stable ALP in goat milk
04/01	31.05.02	Complete preliminary report on the survey of residual ALP measurements in retail samples of pasteurised goat milk on sale in Scotland.
05/01	31.05.02	Complete report on the microbiological quality of commercially produced pasteurised goat milk retailed in Scotland.
06/01	31.06.02	Complete final report: variability of ALP activity in goat milk throughout lactation; suitability of methods for the assessment of effectiveness of pasteurisation of goats milk ; residual ALP activity and microbiological quality of pasteurised goat milk retailed in Scotland.

DELIVERABLES

Deliverable	Target Date	Deliverable Title
01/01	31.10.01	Preliminary report to FSA on ALP levels in individual goat milk from early to mid lactation
01/02	1.02.02	Preliminary report to FSA on ALP levels in goat milk from mid to late lactation
01/03	31.06.01	Final report on variability in ALP activity in individual goat milks and a bulk sample throughout lactation
02/01	31.10.01	Preliminary report to FSA on comparison of measurement of ALP in bulk goat milk in early to mid lactation and the sensitivity of detecting raw milk contamination using spectrophotometric, fluorescence and bioluminescence techniques.
02/02	1.02.02	Preliminary report to FSA on comparison of measurement of ALP in bulk goat milk in mid to late lactation and the sensitivity of detecting raw milk contamination using spectrophotometric, fluorescence and bioluminescence techniques.
02/03	31.06.02	Final report to FSA on comparison of measurement of ALP in bulk goat milk throughout lactation and the sensitivity of detecting raw milk contamination using spectrophotometric, fluorescence and bioluminescence techniques.
03/01	31.10.01	Preliminary report on heat stable ALP in individual goat milks in early and mid lactation
03/02	31.03.02	Preliminary report on heat stable ALP in individual goat milk in late lactation
03/03	31.03.02	Final report to FSA on the occurrence of heat stable ALP in goat milk
04/01	30.04.02	Preliminary report on the survey of residual ALP measurements in retail samples of pasteurised goat milk on sale in Scotland.
05/01	30.04.02	Preliminary report on the microbiological quality of commercially produced pasteurised goat milk retailed in Scotland.
06/01	31.05.02	Final report with conclusions derived from all data

OBJECTIVE 01
Lactational study of variability in alkaline phosphatase in goat milk in a Scottish herd

17 British Saanen goats from the Hannah Research Institute herd will be sampled on a weekly basis throughout a full lactation, i.e. April to early November 2001.

Individual morning and evening milking samples (800 samples) will be tested for ALP using the Fluorophos method on a daily basis (Monday to Friday) during the early stages of lactation (8 weeks). Yields from individual goats will be recorded. Thereafter the morning milk from individual goats will be sampled once a week throughout the remaining lactation period and analysed using the Fluorophos method (350 samples). Yields from individual goats will be recorded. Total solids and fat content of milk will be determined. Alkaline phosphatase will be determined by the Fluorophos method (IDF Standard 155A:1999). Individual milk samples will be tested for presence of heat stable ALP (320).

Summary of work completed

17 British Saanen goats from the Hannah Research Institute herd were sampled on a weekly basis throughout a full lactation, i.e. April 2001 to early January 2002 (note extended lactation period). Individual morning and evening milking samples were tested for ALP (>800 samples) using the Fluorophos method on a daily basis (Monday to Friday) during the first eight weeks of lactation. Thereafter the morning milk from individual goats was sampled once a week throughout the remaining lactation period and analysed using the Fluorophos method (>350 samples). Yields from individual goats were recorded. Total solids, fat and protein content of milk was determined. Alkaline phosphatase was determined by the Fluorophos method (IDF Standard 155A:1999). Individual milk samples were tested for presence of heat stable ALP (>320) and somatic cell count. Statistical modeling of the lactational data was used to explore relationships between ALP levels in milk and goat genotype, age, lactation history and milk composition.

The work described below fulfilled requirements for **Milestones 01/01; 01/02; 01/03** and since results on heat stability are included in the statistical analysis in this section the requirements for **Milestones 03/01; 013/02; 03/03** are also considered here, although data is presented separately later in the report. **Deliverables 01/01; 01/02; 01/03** and **Milestones 03/01; 013/02; 03/03** are also complete.

RESULTS AND DISCUSSION

Introduction

Alkaline phosphatase levels were monitored in raw milk from 17 British Saanen goats from early to late lactation using the Fluorophos method. In the first two months of the study, both morning and evening samples were taken from individual goats. Yields from individual goats were measured throughout the sampling period.

Goat herd

The Hannah Research Institute goat herd comprises of 17 British Saanen goats. Details of age, lactation history and genotype are shown in Table 1. The goats surveyed ranged in age from 1 to 8 years. The herd at HRI has been developed to study casein genotypes. The different protein genotypes produce milk in which the proportions of α_s genotypes differ. Large variations in both genotype and casein polymorphism are generally found in individual animals in goat herds. A and B types are associated with a high proportion of α_{s1}-casein in total casein, E and F with medium levels of α_{s1}-casein in total casein and O types are null alleles which produce no α_{s1}-casein.

Birth of kids to individual goats occurred at regular intervals throughout April and May 2001. Individual goats produced between one and four kids and twelve of the goats gave birth to twins. Goat 705 lost both kids at birth. Sampling of goat milk morning samples was initiated on the 30th April and evening milk samples were first taken during the week beginning the 7th May.

Results for alkaline phosphatase (ALP) measured by Fluorophos
Individual animals monitored daily in May, June and July 2001
(Completion of Milestones 01/01; 01/02 and Deliverables 01/01; 01/02)
Results for daily individual measurements of ALP in goats during May and June are shown in Figures 1a) to 1q) together with milk yield data. Mean values for ALP for individual morning and evening samples, calculated using Minitab statistical package are shown in Tables 2-5.

Mean values for ALP in morning milk in May ranged form 5823.9 mU ALP/L in goat 806 to 180903 mU ALP/L in goat 705. Levels of ALP in goat 705 appeared abnormally high in comparison to the rest of the herd. The majority of goats had ALP levels less than 17000 mU ALP/L. However within individual goats there was some variabilty in ALP levels throughout the month and ALP levels double that of the normal level were observed on one or two days during the month. The lowest level recorded was 3630 mU ALP/L (Goat 605) and the highest value was 369600 mU ALP/L (goat 705). As lactation progressed through May ALP levels increased. At this stage milk yield was also increasing.

Mean values for ALP in evening milk during May are shown in Table 3. ALP levels were higher in evening as compared with morning milk. Goat 605 produced the lowest mean level of ALP (7918 mU ALP/L) while the highest mean value was recorded for goat 705 (242795 mU ALP/L). ALP levels in 705 were again abnormally high and all other goats had levels approximately 10 times lower than those in 705. The minimum value observed for evening milks was 4895 mU ALP/L (Goat 605) and the maximum was 416050 (Goat 705). Milk yields in evening samples were considerably reduced as compared with morning samples.

Results for daily individual measurements for June are shown in Figures 2a) to 2r). Mean values for ALP in morning and evening milk in June are shown in tables 4 and 5. Mean values for ALP in June ranged from 7888 mU ALP/L (Goat 605) to 426229 mU ALP/L (Goat 705). It was clear that as the lacation progressed levels of ALP in milk increased. Goat 610 produced milk with the lowest level of ALP (530 mU ALP/L) while goat 705 again produced the highest recorded value (188375 mU ALP/L).

For evening milks, the lowest mean recorded for ALP was 11170 mU ALP/L (Goat 639) and the highest was 631615 mU ALP/L. The minimum value observed in evening milk was 7873 mU ALP/L while the maximum value was 1554125 mU ALP/L.

Again it was noted that reduced milk yields in evening milk samples were associated with an increase in the concentration of ALP in milk.

Individual animals monitored weekly July 2001 to January 2002
(Completion Milestones 01/01; 01/02; 01/03 and Deliverables 01/01; 01/02; 01/03)
Results for levels of ALP individual animals are shown in Figures 3a) to 3q). Data for milk yields are also graphed. Milk was sampled on a weekly basis and both morning and evening milks were monitored. Statistical analysis of data for each month is shown in Tables 6 to 18. Results from Figures 3a (goat 601) show typical trends. As the lactation progresses and milk yield is reduced, the level of ALP in milk increases. Concentrations of ALP in evening milk are increased as milk yields are low. Although concentrations of ALP increase as the lactation progresses there are occasions where a sudden rapid rise in ALP is observed. This is particularly evident in samples from goats 610, 617, 622, 713, 804, 890, 891, 809, 640, 605 and 891. Goat 705, which produced consistently high levels of ALP throughout lactation died mid August. There was considerable variation in the level of ALP produced in individual goats, but it was clear that in the majority of samples levels in milk were too low for use in the phosphatase test to validate the efficiency of pasteurisation. This is particularly evident in the early stages of lactation, when ALP levels in milk are lowest.

Milk yield data and ALP concentration data for individual milks were combined to study changes in total ALP produced in morning and evening samples throughout lactation. Results shown in Figures 4a) to r) indicate that the total quantity of ALP produced in morning samples is only slightly greater than that produced in evening samples. Additionally, the total level of ALP released into milk remains quite consistent throughout lactation in some of the goats and increases slightly in others.

Milk composition and somatic cell counts were monitored from July 2001 to January 2002. Data for individual animals for morning and evening samples for July to December are shown in Figure 5a) to q) and 6a) to q). Somatic cell count data shows that Goat 705, which produced the highest recorded level of ALP in milk also had the highest somatic cell count.

Statistical analysis of data is presented in the following section. Factors influencing variability ALP levels in goat milk such as somatic, cell count, milk composition, genotype and age of goat have been considered.

CONCLUSIONS
A lactational study on ALP levels in the milk of 17 British Saanen goats has been completed. ALP levels in goat milk are extremely variable but more importantly, levels are at least 10-fold lower than those found in cows' milk. ALP levels in goat milk are not sufficiently high to be used as an index of effective pasteurisation. Levels are particularly low in the early stages of lactation when milk yields are highest and this is the period when current standard methods for assessing the effectiveness of pasteurisation would not adequately detect contamination of pasteurised goat milk with raw milk.

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Table 1. Age distribution, casein genotype and kid births in the HRI goat herd

Goat ID	Age (years)	Birth of Kids	No. of Kids	Casein Genotype
601	4	10.4.01	2	EE
610	4	8.4.01	4	EO1
617	4	7.4.01	2	FF
618	4	6.4.01	2	FF
622	3	10.4.01	2	EE
639	2	12.4.01	2	EE
713	8	4.4.01	2	EE
806	7	9.4.01	1	EE
890	7	9.4.01	1	FF
891	6	11.4.01	1	FF
725	8	28.4.01	1	B2E
809	7	29.4.01	2	B2E
640	2	30.4.01	2	AbB2
605	4	30.4.01	2	B2E
637	1	17.5.01	2	B2E
705	8	11.5.01	2 (died at birth)	-
899	7	21.5.01	2	FF

Table 2. Variability of alkaline phosphatase in individual morning goat milk samples in May 2001

Goat ID	Age	N	Mean	Median	StDev	SE Mean	Minimum	Maximum	Q1	Q3
601	4	20	9734	9080	2849	451	7100	20710	7955	10326
610	4	20	6047	6170	1188	188	4300	9215	4895	6665
617	4	20	8777	9205	1506	238	5745	10825	7265	10045
618	4	20	10067	10010	2078	328	7010	13700	8155	12044
622	3	20	11045	9470	4687	741	7010	26780	8965	11114
639	2	20	8358	8195	4207	665	4135	25765	5769	9424
713	8	20	16989	14665	10463	1654	10390	60175	12385	16985
806	7	20	5823.9	5745.0	625.9	99.0	4825.0	7770.0	5400.0	6160.0
890	7	20	8868	9470	2592	410	4990	17080	6415	10005
891	6					Not in milk				
725	8	16	20149	21468	9535	1686	6595	38615	10269	23561
809	7	19	23175	13228	26575	4311	8275	110950	10113	18256
640	2	19	16644	11448	13136	2131	5840	54430	7309	20429
605	4	19	12372	7135	12793	2075	3630	54430	4645	14635
637	1	4	12506	12078	2730	965	9285	16825	10245	15084
705	8	8	180903	149058	79493	19873	92540	369600	123603	222275
899	7	7	31501	17205	23053	6161	13445	79780	14003	45299

Table 3. Variability of alkaline phosphatase in individual evening goat milk samples in May 2001

Goat ID	Age	N	Mean	Median	StDev	SE Mean	Min	Max	Q1	Q3
601	4	12	18517	18135	6321	1290	8460	29650	12874	24400
610	4	12	9475	9125	2045	417	7265	15375	8275	9845
617	4	12	13089	12885	2014	411	10390	18570	11658	14119
618	4	12	16399	15710	2446	499	12850	21490	14774	17964
622	3	12	14617	15250	2921	596	10230	20780	11899	16560
639	2	12	8323	7815	1483	303	6415	11770	7118	9356
713	8	12	24476	23445	6074	1240	17490	42340	20900	25928
806	7	12	9316	8413	2440	498	6850	16735	8018	9679
890	7	12	18814	15665	10650	2174	13515	54430	14611	17349
891	6	12	20510	19780	3159	645	16480	28225	17951	22560
725	8	12	32481	33800	13881	2833	11745	58820	17588	41355
809	7	12	16884	15838	4803	980	10895	30595	13700	18829
640	2	12	26001	14998	20770	4240	8205	69555	11403	43006
605	4	12	7918	6678	3114	636	4895	16620	5871	8940
637	1	7	17958	14998	4194	1121	13950	24410	14550	22663
705	8	7	242795	200450	99868	26691	132575	416050	168128	360688
899	7	6	37155	19650	28070	8103	16065	84930	18381	64978

Table 4. Variability of alkaline phosphatase in individual morning goat milk samples in June 2001

Goat ID	Age	N	Mean	Median	StDev	SE Mean	Minimum	Maximum	Q1	Q3
601	4	19	20142	19675	8318	1908	623	39740	16493	23918
610	4	19	10865	10620	3998	917	530	19398	8575	13525
617	4	19	13854	12905	4402	1010	10920	31340	11928	13975
618	4	19	15769	14803	2835	651	12550	21883	13918	16618
622	3	19	19607	20330	4959	1138	12643	26305	14183	24353
639	2	19	9745	9505	2265	520	6860	16090	8045	10825
713	8	19	23371	22008	7303	1675	15470	44338	17940	25593
806	7	19	8360	7643	2278	523	5975	15298	6895	8838
890	7	19	13281	12905	3402	781	9815	24755	10953	15285
891	6	19	21221	19628	3579	821	16793	29088	18653	23870
725	8	19	34092	32570	16251	3728	15515	75058	18698	45580
809	7	19	30449	37615	14522	3331	7783	48223	14893	43855
640	2	19	15057	15078	8202	1882	5700	31075	7160	21088
605	4	19	7888	6985	2518	578	5218	14595	6103	9320
637	1	19	10190	10000	2489	571	6138	17503	8573	11413
705	8	19	426229	263850	306906	70409	188375	1170050	225250	513725
899	7	19	11113	10173	3036	696	7595	17238	8758	12148

Table 5. Variability of alkaline phosphatase in individual evening goat milk samples in June 2001

Goat ID	Age	N	Mean	Median	StDev	SE Mean	Minimum	Maximum	Q1	Q3
601	4	10	26773	25903	8950	2830	15240	44383	19319	32146
610	4	10	13230	13308	2665	843	9988	19275	10685	14183
617	4	10	20252	18095	6445	2038	15388	37660	16864	21260
618	4	10	22612	22255	1624	514	20640	24835	21104	24368
622	3	10	22833	20383	5763	1822	17113	35810	18904	27049
639	2	10	11170	10406	2245	710	9553	17113	9955	11575
713	8	10	32372	27054	10471	3311	22318	53798	26689	38585
806	7	10	13173	10896	6797	2149	7918	31215	10090	13101
890	7	10	17797	17411	4935	1561	9378	28513	15858	19491
891	6	10	45277	24979	60500	19132	16618	216750	24358	32351
725	8	10	42214	39030	14490	4582	24400	74925	33621	47697
809	7	10	36228	37040	16337	5166	13883	57728	20638	52699
640	2	10	21129	15608	18201	5756	8310	71035	12291	23068
605	4	10	11669	11796	3375	1067	7873	17548	8033	13961
637	1	10	14196	13728	3386	1071	8803	22330	12853	14856
705	8	10	631615	404825	502705	158969	178925	1554125	237894	1002631
899	7	10	20262	16711	11703	3701	12998	52198	14426	19238

Table 6. Variability of alkaline phosphatase in individual morning goat milk samples in July 2001

Goat ID	Age	N	Mean	Median	StDev	SE Mean	Minimum	Maximum	Q1	Q3
601	4	13	51638	46338	18031	5001	38995	109915	44005	51528
610	4	13	31179	19513	26809	7436	14908	97593	16344	31685
617	4	13	18603	12905	15178	4210	10380	55463	11101	14629
618	4	13	24429	18525	12968	3597	13503	60118	17210	30886
622	3	13	52912	31790	50225	13930	20515	167925	25974	50659
639	2	13	19710	17675	7379	2047	12263	33810	14153	24956
713	8	13	37140	30500	15507	4301	23835	72543	28536	40326
806	7	13	14693	11273	7162	1986	8540	31145	9683	18503
890	7	13	33292	16090	34671	9616	12020	108915	12935	47775
891	6	13	27474	25283	7815	2167	18825	40878	20291	33741
725	8	13	106022	106445	36561	10140	64070	187100	73754	128360
809	7	13	65012	49118	38636	10716	30640	150643	37414	74868
640	2	13	26995	20263	22573	6261	13608	99583	16044	27744
605	4	13	17691	15295	7300	2025	8435	32685	11378	23094
637	1	13	15612	14205	4006	1111	11125	24190	12759	18394
705	8	13	426517	415225	171161	47471	117270	810225	347538	524050
899	7	13	18276	17583	5807	1611	11125	34718	14590	20215

Table 7. Variability of alkaline phosphatase in individual morning goat milk samples in August 2001

Goat ID	Age	N	Mean	Median	StDev	SE Mean	Minimum	Maximum	Q1	Q3
601	4	4	91870	89503	19528	9764	70675	117800	74790	111318
610	4	4	31215	30790	15310	7655	15610	47670	16978	45878
617	4	4	32615	15538	35401	17700	13720	85665	13778	68530
618	4	4	44556	24298	46020	23010	16800	112830	16899	92473
622	3	4	96263	42500	110678	55339	37860	262190	38135	208153
639	2	4	27818	24248	12354	6177	17125	45650	18860	40345
713	8	4	40896	38168	8307	4153	34315	52935	34930	49591
806	7	4	16048	14985	3508	1754	13170	21050	13418	19740
890	7	4	21818	20465	4588	2294	17950	28390	18375	26613
891	6	4	35150	31950	10848	5424	26085	50615	26959	46541
725	8	4	109971	110768	15691	7846	90120	128230	94725	124421
809	7	4	84446	87618	21006	10503	55990	106560	63506	102215
640	2	4	26838	27585	3947	1973	21700	30480	22740	30188
605	4	4	36001	33238	9317	4659	28065	49465	29191	45575
637	1	4	29605	29053	3495	1748	26115	34200	26556	33206
705	8	2	305345	305345	144186	101955	203390	407300	*	*
899	7	4	33988	33728	642	321	33560	34935	33576	34659

Table 8. Variability of alkaline phosphatase in individual evening goat milk samples in August 2001

Goat ID	Age	N	Mean	Median	StDev	SE Mean	Minimum	Maximum	Q1	Q3
601	4	4	121404	124008	17355	8677	100285	137315	103774	136430
610	4	4	31549	31710	5194	2597	25700	37075	26493	36444
617	4	4	29875	21400	18060	9030	19810	56890	19869	48356
618	4	4	46286	32123	33472	16736	24940	95960	25664	81073
622	3	4	101560	66485	83939	41969	46890	226380	49953	188243
639	2	4	30604	30490	5062	2531	25280	36155	25839	35483
713	8	4	56084	54293	9735	4868	46590	69160	47791	66168
806	7	4	21974	21640	2997	1499	18685	25930	19334	24948
890	7	4	29576	30350	1832	916	26850	30755	27688	30691
891	6	4	41424	41178	6488	3244	34820	48520	35406	47688
725	8	4	145156	144370	7443	3721	137540	154345	138409	152690
809	7	4	109781	110890	13329	6665	94420	122925	96586	121868
640	2	4	41949	41100	9331	4666	33305	52290	33691	51055
605	4	4	56595	56188	19280	9640	33855	80150	38280	75318
637	1	4	41826	43028	4660	2330	35280	45970	36953	45499
705	8	2	275095	275095	115478	81655	193440	356750	*	*
899	7	4	50544	42085	19779	9889	38040	79965	38534	71013

Table 9. Variability of alkaline phosphatase in individual morning goat milk samples in September 2001

Goat ID	Age	N	Mean	Median	StDev	SE Mean	Minimum	Maximum	Q1	Q3
601	4	4	196110	168838	95506	47753	120535	326230	122558	296935
610	4	4	83895	80930	78023	39011	10850	162870	13930	156825
617	4	4	36158	22425	32651	16326	15080	84700	16115	69933
618	4	4	33169	29365	21798	10899	13490	60455	14583	55559
622	3	4	156743	146958	140987	70494	28090	304965	32239	291031
639	2	4	27869	27018	5276	2638	22755	34685	23278	33311
713	8	4	59640	52753	31969	15984	29075	103980	33506	92661
806	7	4	26428	22663	9914	4957	19540	40845	19729	36891
890	7	4	95025	29365	137809	68905	19975	301395	20303	235408
891	6	4	37225	34513	10826	5413	27815	52060	28550	48613
725	8	4	143898	138118	49112	24556	92190	207165	99748	193828
809	7	4	98015	91205	24515	12257	78885	130765	79051	123789
640	2	4	49740	47523	16812	8406	32180	71735	34818	66880
605	4	4	73810	69358	33757	16878	37535	118990	44666	107406
637	1	4	31471	31650	2884	1442	27810	34775	28638	34126
705	8	Died on/about 20 August								
899	7	4	56320	46433	20546	10273	45300	87115	45399	77129

Table 10. Variability of alkaline phosphatase in individual evening goat milk samples in September 2001

Goat ID	Age	N	Mean	Median	StDev	SEMean	Minimum	Maximum	Q1	Q3
601	4	4	232858	241560	108215	54107	107410	340900	125568	331445
610	4	4	144076	132545	134836	67418	23350	287865	26265	273419
617	4	4	53604	35663	44141	22071	24690	118400	25149	100000
618	4	4	52614	47018	32978	16489	24640	91780	24980	85844
622	3	4	232518	221913	199916	99958	49095	437150	55153	420488
639	2	4	39619	42130	8890	4445	27050	47165	30274	46453
713	8	4	88331	69438	40277	20138	65760	148690	66668	128889
806	7	4	39589	32765	19380	9690	24800	68025	26346	59655
890	7	4	153743	44260	227685	113843	31350	495100	33403	383565
891	6	4	46866	47945	21302	10651	19975	71600	26100	66554
725	8	4	202491	211910	71411	35705	108830	277315	129759	265805
809	7	4	145368	122543	55552	27776	108330	228055	111409	202151
640	2	4	85763	80150	41292	20646	43855	138895	49085	128053
605	4	4	127855	99305	67122	33561	85830	226980	86168	198093
637	1	4	56060	53303	13966	6983	44085	73550	44448	70430
705	8	Died on/about 20 August								
899	7	4	108588	89975	52363	26182	71045	183355	71695	164093

Table 11. Variability of alkaline phosphatase in individual morning goat milk samples in October 2001

Goat ID	Age	N	Mean	Median	StDev	SE Mean	Minimum	Maximum	Q1	Q3
601	4	5	317830	202960	361506	161671	94170	958500	109770	583325
610	4	5	40278	28225	27262	12192	21055	85710	21068	65515
617	4	5	21873	18615	8157	3648	16330	36060	16693	28683
618	4	5	68449	16965	112096	50131	13930	268765	14765	147875
622	3	5	90180	96930	51653	23100	39625	166430	42085	134900
639	2	5	33873	29260	11897	5321	26525	54885	26910	43143
713	8	5	73722	82770	20631	9226	41305	90585	53393	89528
806	7	5	28816	26780	9686	4332	20755	44440	20985	37665
890	7	5	42568	27815	24791	11087	21925	78770	24110	68403
891	6	5	50956	41305	26566	11881	32730	97560	34650	72088
725	8	5	273105	186225	194203	86850	101225	595815	140393	449258
809	7	5	93071	101385	25434	11374	59305	117915	66588	115398
640	2	5	66513	66660	12008	5370	53190	84930	56135	76818
605	4	5	86622	93735	17676	7905	55235	97200	73335	96353
637	1	5	50795	47530	11480	5134	38610	69160	42153	61070
705	8	Died on/about 20 August								
899	7	5	94954	57235	77288	34564	30615	223450	42615	166153

Table 12. Variability in alkaline phosphatase in individual evening goat milk samples in October 2001

Goat ID	Age	N	Mean	Median	StDev	SE Mean	Minimum	Maximum	Q1	Q3
601	4	5	379061	275085	371996	166362	121290	1032950	149703	660408
610	4	5	49207	34430	43669	19529	22070	126165	22748	83055
617	4	5	30286	26870	7028	3143	24345	38845	24413	37868
618	4	5	59265	24205	73246	32756	18800	189215	20123	115938
622	3	5	130867	133750	84908	37972	47510	254055	51600	208693
639	2	5	43970	36200	18865	8436	32730	77275	32995	58830
713	8	5	94382	84905	32718	14632	58040	142510	67325	126178
806	7	5	37781	37900	10055	4497	23790	51580	29293	46210
890	7	5	65562	53945	23345	10440	49305	105250	50360	86573
891	6	5	69787	52590	43595	19496	34180	139495	36375	111798
725	8	5	346846	326400	219067	97970	106900	630050	139390	564525
809	7	5	121269	102490	59478	26600	80860	226315	86458	165470
640	2	5	95001	101640	25304	11316	60615	127430	70805	115878
605	4	5	114952	117570	26212	11722	71505	141195	94168	134428
637	1	5	71109	69090	20256	9059	48405	96170	51818	91410
705	8	Died on/about 20 August								
899	7	5	251825	83365	369205	165113	56060	910650	68565	519315

Table 13. Variability in alkaline phosphatase in individual morning goat milk samples in November 2001

Goat ID	Age	N	Mean	Median	StDev	SE Mean	Minimum	Maximum	Q1	Q3
601	4	4	782000	662650	325347	162674	540400	1262300	570563	1112788
610	4	4	18658	19710	6177	3089	10410	24800	12284	23979
617	4	4	25888	20698	13886	6943	15790	46365	16698	40268
618	4	4	19818	19330	4319	2159	15650	24960	15966	24156
622	3	4	103778	109388	40268	20134	50520	145815	62749	139196
639	2	4	43948	43568	6632	3316	36525	52130	37738	50538
713	8	4	116998	125418	26080	13040	80330	136825	89359	136216
806	7	4	36068	32178	13851	6926	24705	55210	25326	50699
890	7	4	48045	53798	17921	8961	23190	61395	29063	61275
891	6	4	23784	22733	4135	2067	19995	29675	20656	27963
725	8	4	222080	221965	100409	50204	101940	342450	125516	318759
809	7	4	161235	155655	58430	29215	95690	237940	110635	217415
640	2	4	63395	60635	16405	8202	46530	85780	49603	79948
605	4	4	73489	73068	14553	7276	59625	88195	60325	87074
637	1	4	38664	34010	10035	5017	32940	53695	33159	48823
705	8	Died on/about 20 August								
899	7	4	105308	85103	62820	31410	54340	196685	60499	170321

Table 14. Variability in alkaline phosphatase in individual evening goat milk samples in November 2001

Goat ID	Age	N	Mean	Median	StDev	SE Mean	Minimum	Maximum	Q1	Q3
601	4	4	834750	755400	175358	87679	731600	1096600	733788	1015063
610	4	4	27336	26330	13317	6658	13220	43465	15013	40666
617	4	4	34373	36325	8623	4312	22275	42565	25538	41255
618	4	4	28420	30673	5895	2948	19765	32570	22243	32345
622	3	4	128814	123475	62196	31098	58360	209945	74398	188569
639	2	4	53286	55695	14746	7373	34845	66910	38161	66003
713	8	2	171283	171283	69887	49418	121865	220700	*	*
806	7	4	33414	34373	8054	4027	22710	42200	25450	40419
890	7	4	68018	72538	19960	9980	40180	86815	47368	84148
891	6	4	45239	45083	15429	7715	27650	63140	30385	60249
725	8	2	243828	243828	142443	100723	143105	344550	*	*
809	7	2	280690	280690	214762	151860	128830	432550	*	*
640	2	4	97870	108223	28146	14073	56635	118400	68409	116979
605	4	4	105760	112143	24091	12045	71900	126855	80501	124636
637	1	4	66584	58798	25535	12767	45535	103205	47593	93361
705	8	Died on/about 20 August								
899	7	4	151850	110558	100511	50256	85550	300735	88566	256426

Table 15. Variability in alkaline phosphatase in individual morning goat milk samples in December 2001

Goat ID	Age	N	Mean	Median	StDev	SE Mean	Minimum	Maximum	Q1	Q3
601	4	3	548583	530500	79238	45748	479950	635300	479950	635300
610	4									
617	4	3	26393	23260	7830	4521	20615	35305	20615	35305
618	4	3	16158	14525	3845	2220	13400	20550	13400	20550
622	3	3	94010	71990	65254	37674	42615	167425	42615	167425
639	2	3	46177	37100	21475	12399	30730	70700	30730	70700
713	8	2	289748	289748	122969	86953	202795	376700	*	*
806	7	3	25673	22870	6301	3638	21260	32890	21260	32890
890	7	3	72068	75440	15044	8685	55625	85140	55625	85140
891	6	3	35172	33210	13713	7917	22545	49760	22545	49760
725	8	2	323380	323380	190523	134720	188660	458100	*	*
809	7	Dried off								
640	2	3	68080	76240	18240	10531	47185	80815	47185	80815
605	4	3	66772	78080	27519	15888	35400	86835	35400	86835
637	1	3	60497	53300	19972	11531	45120	83070	45120	83070
705	8	Died on/about 20 August								
899	7	3	168352	99270	162241	93670	52085	353700	52085	353700

Table 16. Variability in alkaline phosphatase in individual evening goat milk samples in December 2001

Goat ID	Age	N	Mean	Median	StDev	SE Mean	Minimum	Maximum	Q1	Q3
601	4	3	542850	508700	98757	57018	465700	654150	465700	654150
610	4	3	42605	21745	39583	22853	17815	88255	17815	88255
617	4	3	38630	29305	20707	11955	24225	62360	24225	62360
618	4	3	26677	27145	7513	4338	18940	33945	18940	33945
622	3	3	142068	74540	139997	80828	48635	303030	48635	303030
639	2	3	54543	62840	17293	9984	34665	66125	34665	66125
713	8					Dried off				
806	7	3	37633	24320	23904	13801	23350	65230	23350	65230
890	7	3	81003	91730	29411	16980	47735	103545	47735	103545
891	6	3	43895	54015	19720	11385	21170	56500	21170	56500
725	8					Dried off				
809	7					Dried off				
640	2	3	103990	76655	49452	28551	74240	161075	74240	161075
605	4	3	110588	71505	95121	54918	41235	219025	41235	219025
637	1	3	102062	76885	58291	33655	60590	168710	60590	168710
705	8					Died on/about 20 August				
899	7	3	211413	106170	194007	112010	92770	435300	92770	435300

Table 17. Variability in alkaline phosphatase in individual morning goat milk samples in January 2002

Goat ID	Age	N	Mean	Median	StDev	SE Mean	Minimum	Maximum	Q1	Q3
601	4	2	234378	234378	24349	17217	217160	251595	*	*
610	4	3	31890	19150	22929	13238	18160	58360	18160	58360
617	4	3	38293	33995	16547	9553	24320	56565	24320	56565
618	4	3	40928	28850	25538	14744	23670	70265	23670	70265
622	3	3	110450	72355	69251	39982	68610	190385	68610	190385
639	2	3	27007	24040	5746	3318	23350	33630	23350	33630
713	8					Dried off				
806	7	3	25882	22480	6053	3495	22295	32870	22295	32870
890	7	1	191560	191560	*	*	191560	191560	*	*
891	6	3	68642	58680	27501	15878	47510	99735	47510	99735
725	8					Dried off				
809	7					Dried off				
640	2	3	62557	68630	14298	8255	46225	72815	46225	72815
605	4	3	77690	66790	24954	14407	60040	106240	60040	106240
637	1	3	75100	54590	37270	21518	52590	118120	52590	118120
705	8					Died on/about 20 August				
899	7	3	101257	63325	68872	39763	59690	180755	59690	180755

Table 18. Variability in alkaline phosphatase in individual evening goat milk samples in January 2002

Goat ID	Age	N	Mean	Median	StDev	SE Mean	Minimum	Maximum	Q1	Q3
601	4	2	262113	262113	42112	29777	232335	291890	*	*
610	4	3	32408	25145	13731	7927	23835	48245	23835	48245
617	4	3	48683	42410	21774	12571	30735	72905	30735	72905
618	4	3	61743	42405	33794	19511	42060	100765	42060	100765
622	3	3	115510	95390	35226	20338	94955	156185	94955	156185
639	2	3	29612	29375	3511	2027	26225	33235	26225	33235
713	8					Dried off				
806	7	3	29008	29675	3212	1855	25515	31835	25515	31835
890	7	1	231135	231135	*	*	231135	231135	*	*
891	6	3	85757	70540	35520	20507	60380	126350	60380	126350
725	8					Dried off				
809	7					Dried off				
640	2	3	78762	74495	11538	6661	69965	91825	69965	91825
605	4	3	93863	92535	7546	4357	87070	101985	87070	101985
637	1	3	114412	98080	44879	25911	79985	165170	79985	165170
705	8					Dried off				
899	7	3	120502	85225	75653	43678	68930	207350	68930	207350

Figure 1a) Daily sampling of ALP in raw milk from goat 601 during May

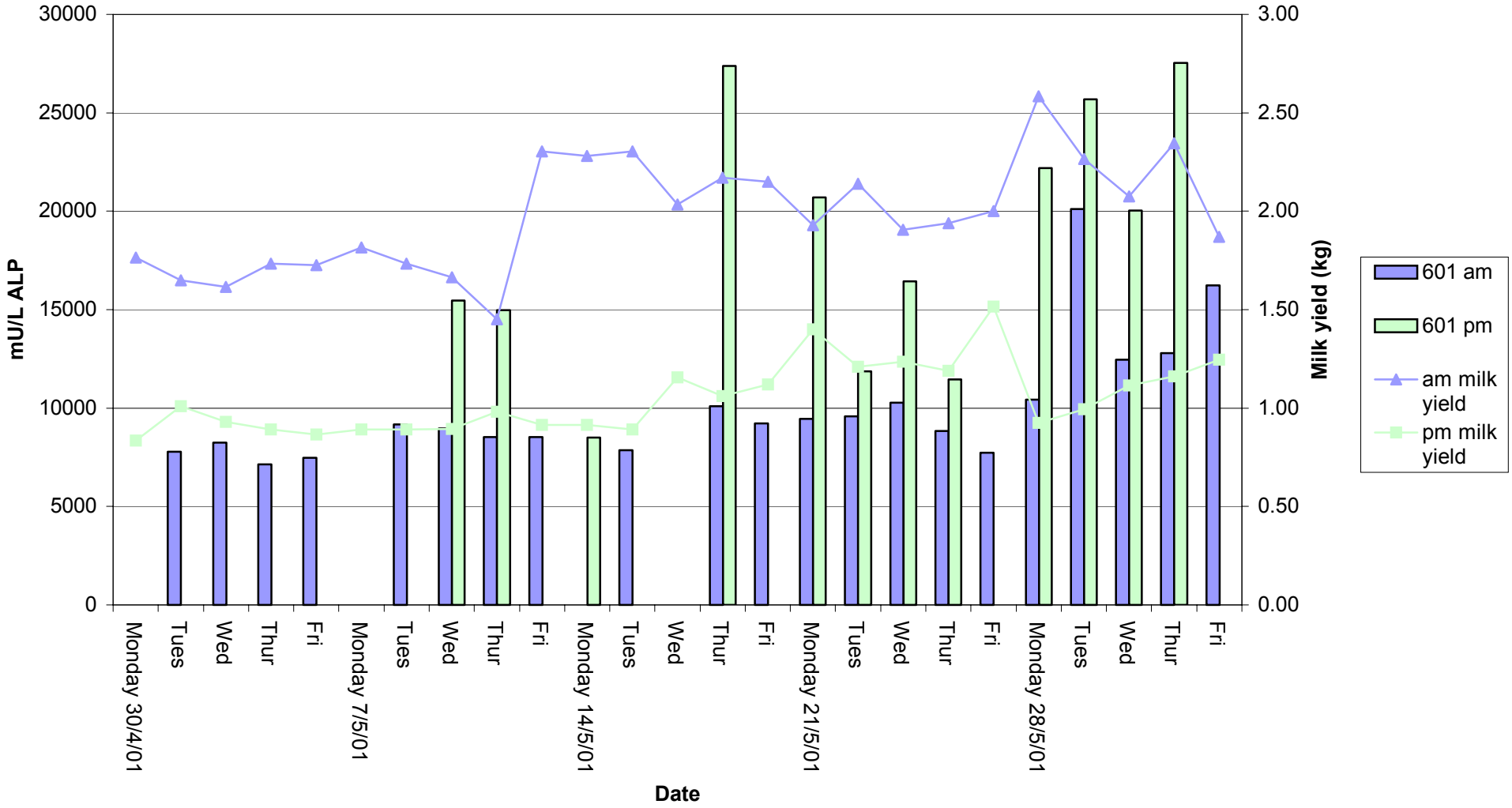


Figure 1b) Daily sampling of ALP in raw milk from goat 610 during May

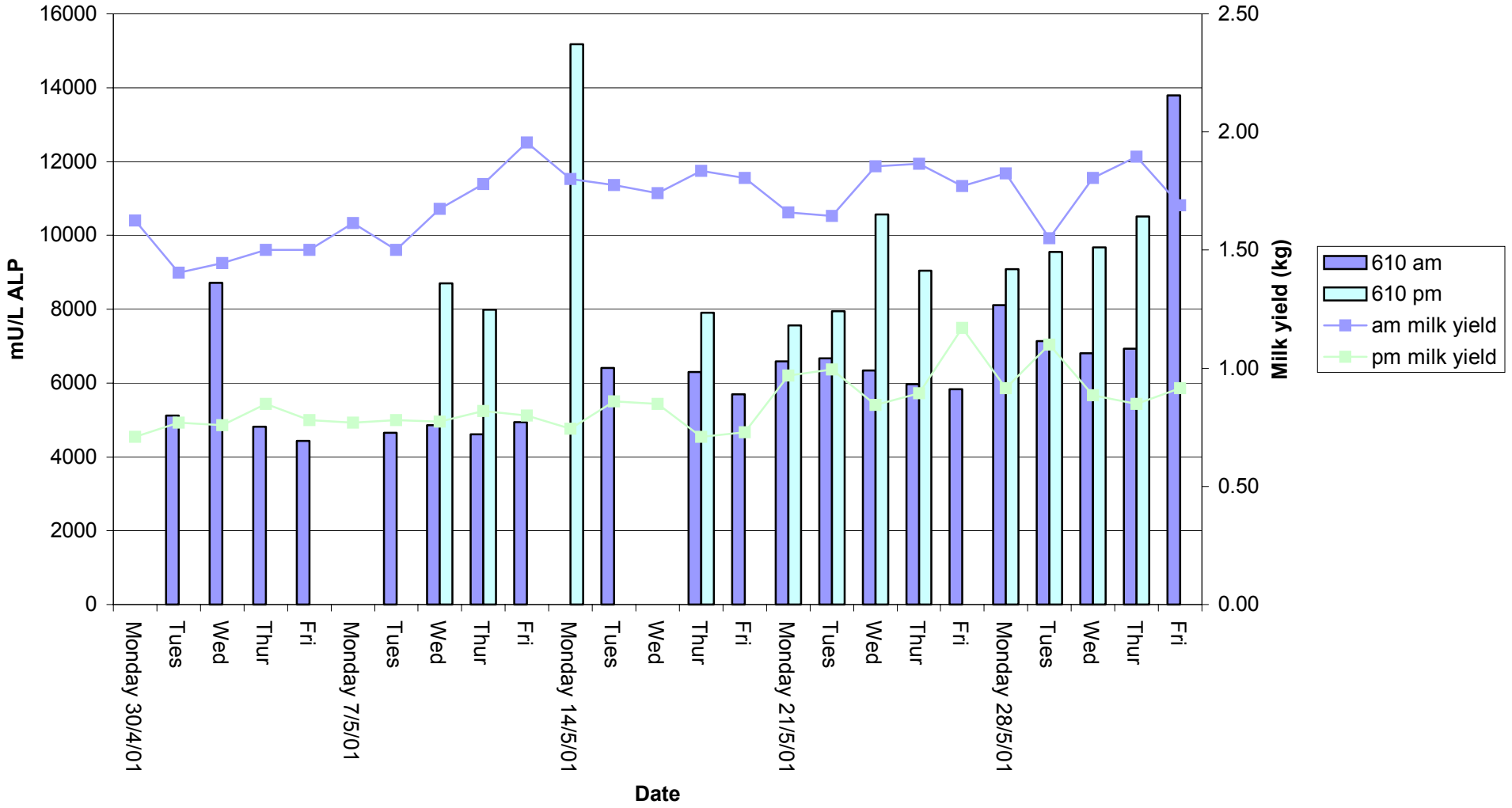


Figure 1c) Daily sampling of ALP in raw milk from goat 617 during May

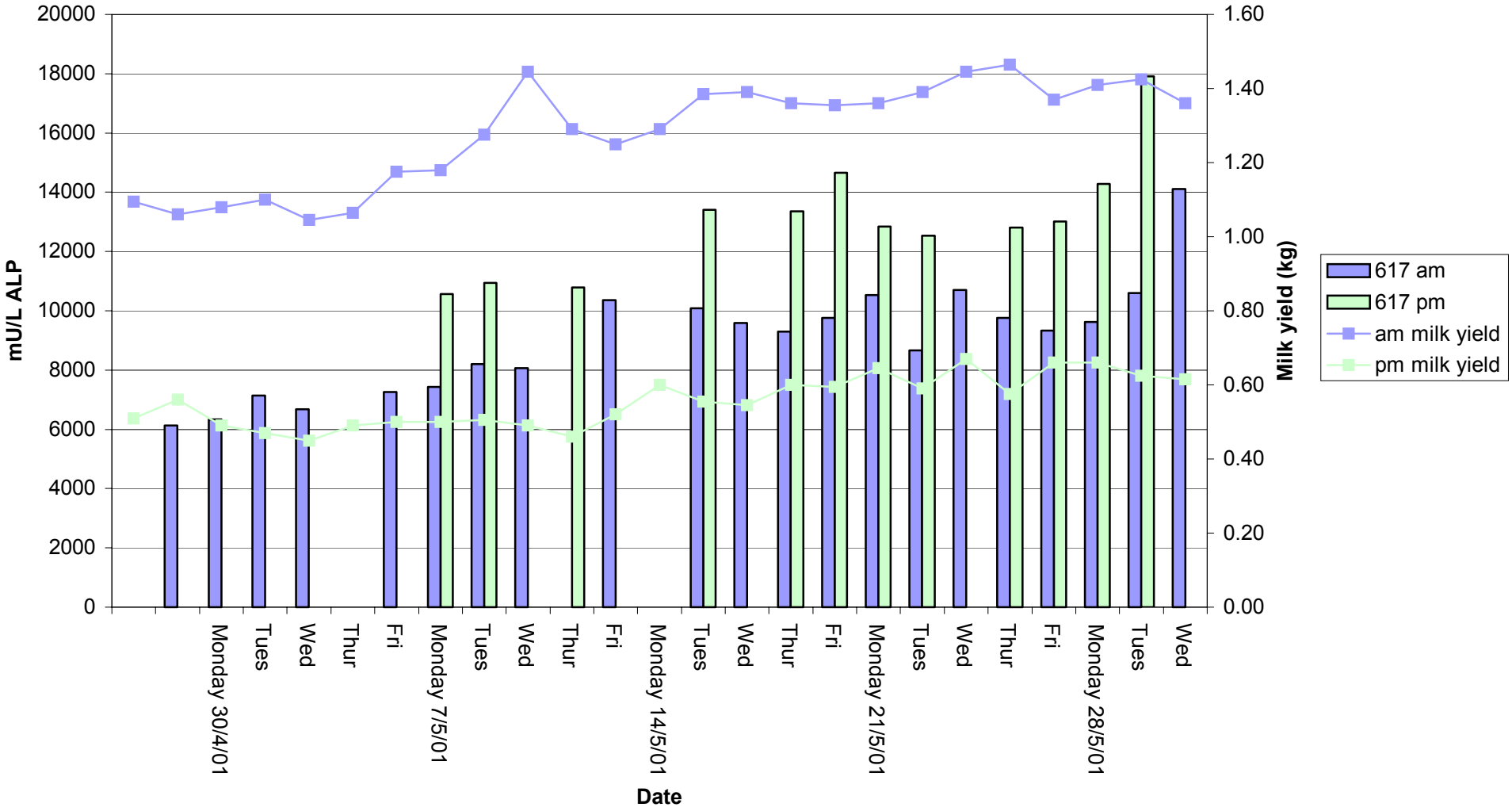


Figure 1d) Daily sampling of ALP in raw milk from goat 618 during May

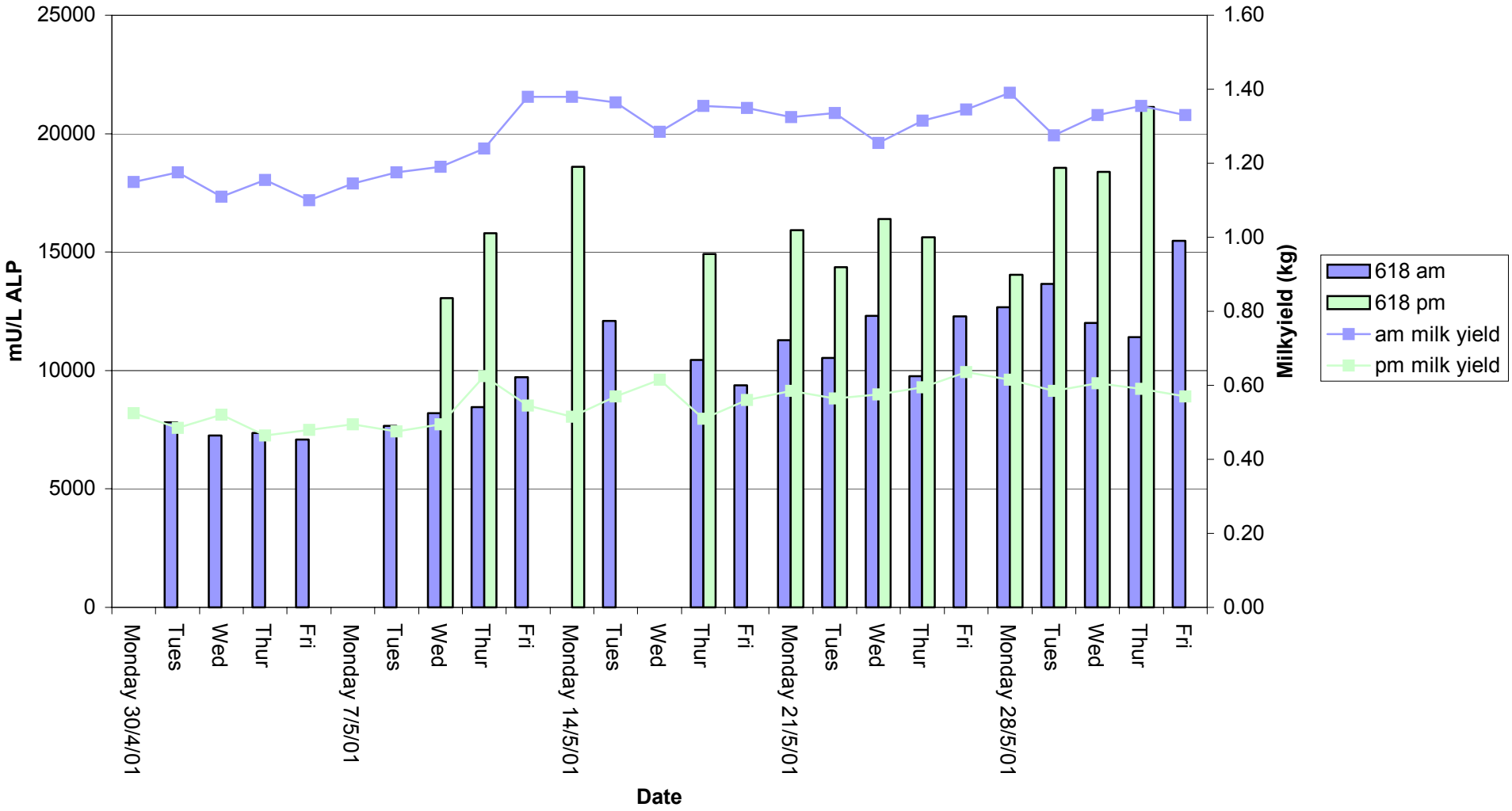


Figure 1e) Daily sampling of ALP in raw milk from goat 622 during May

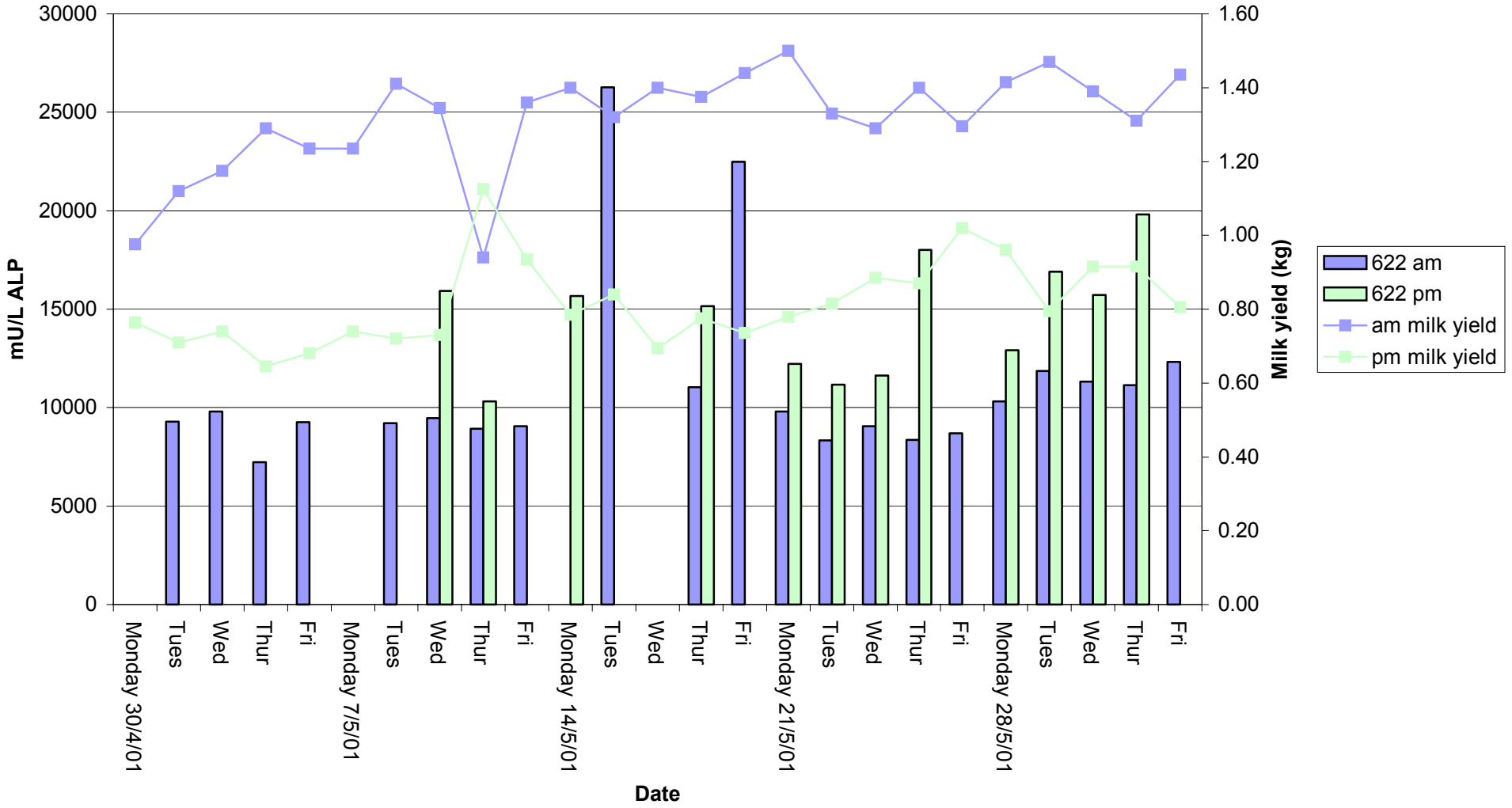


Figure 1f) Daily sampling of ALP in raw milk from goat 639 during May

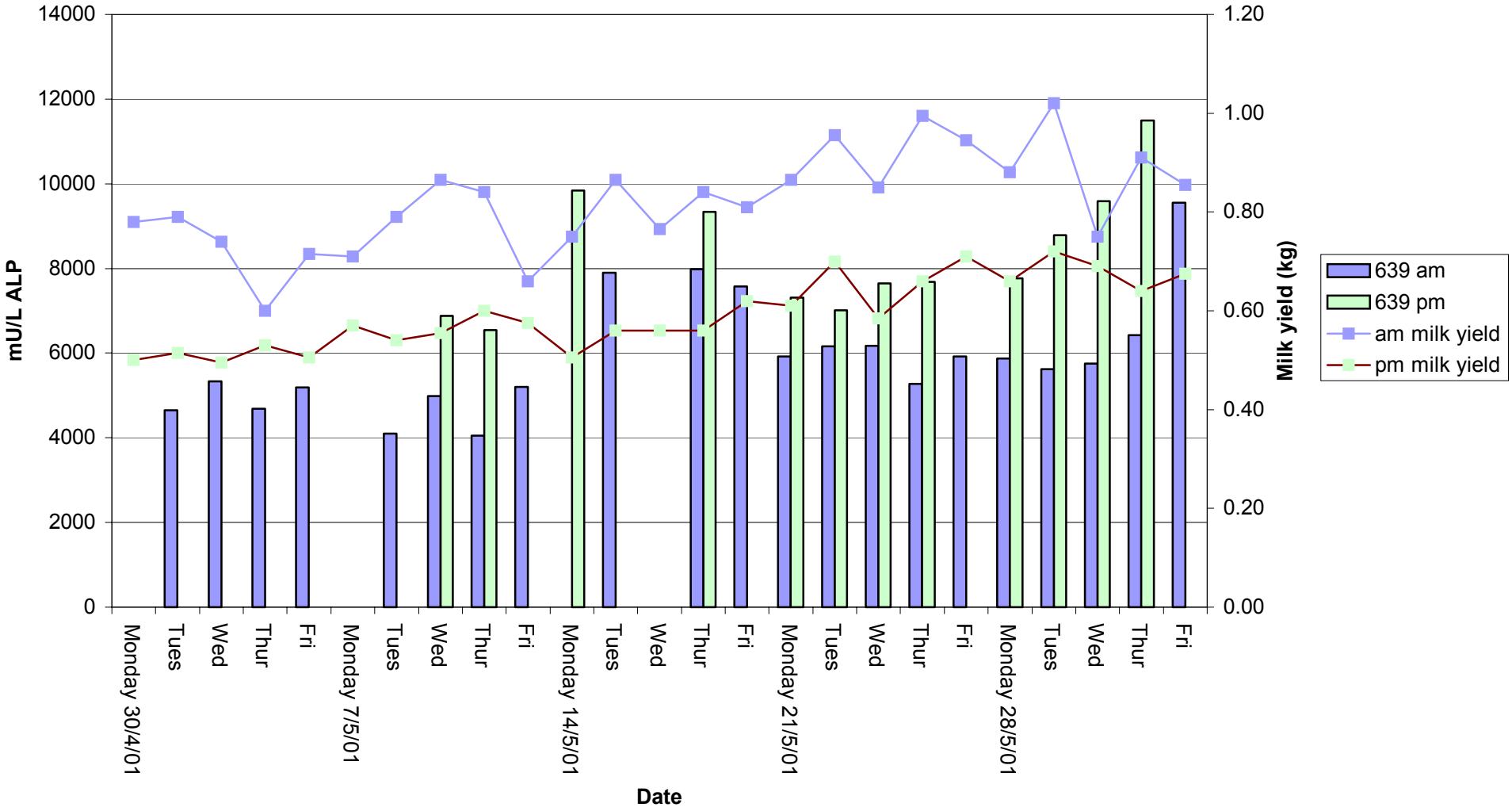


Figure 1g) Daily sampling of ALP in raw milk from goat 713 during May

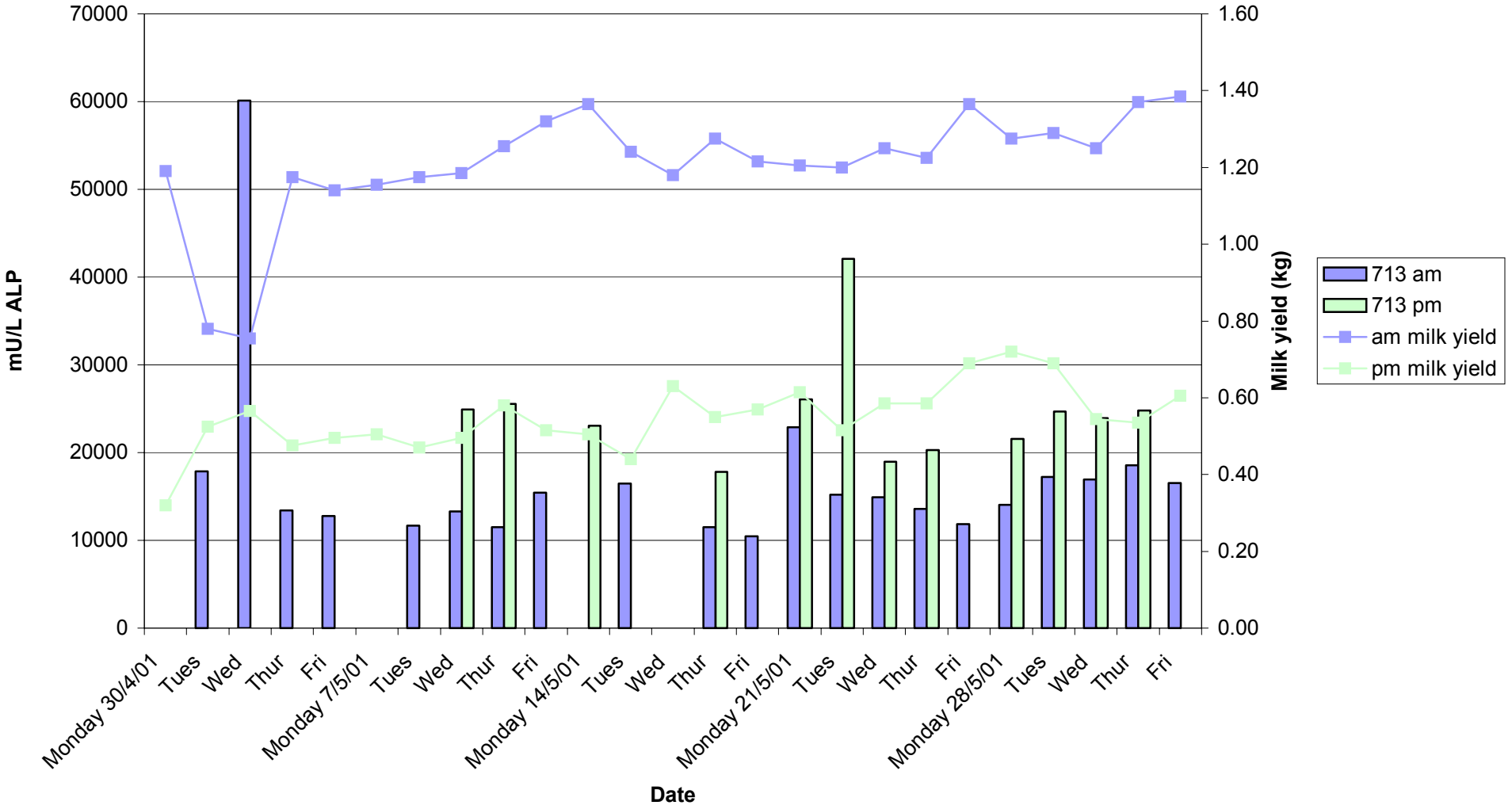


Figure 1h) Daily sampling of ALP in raw milk from goat 806 during May

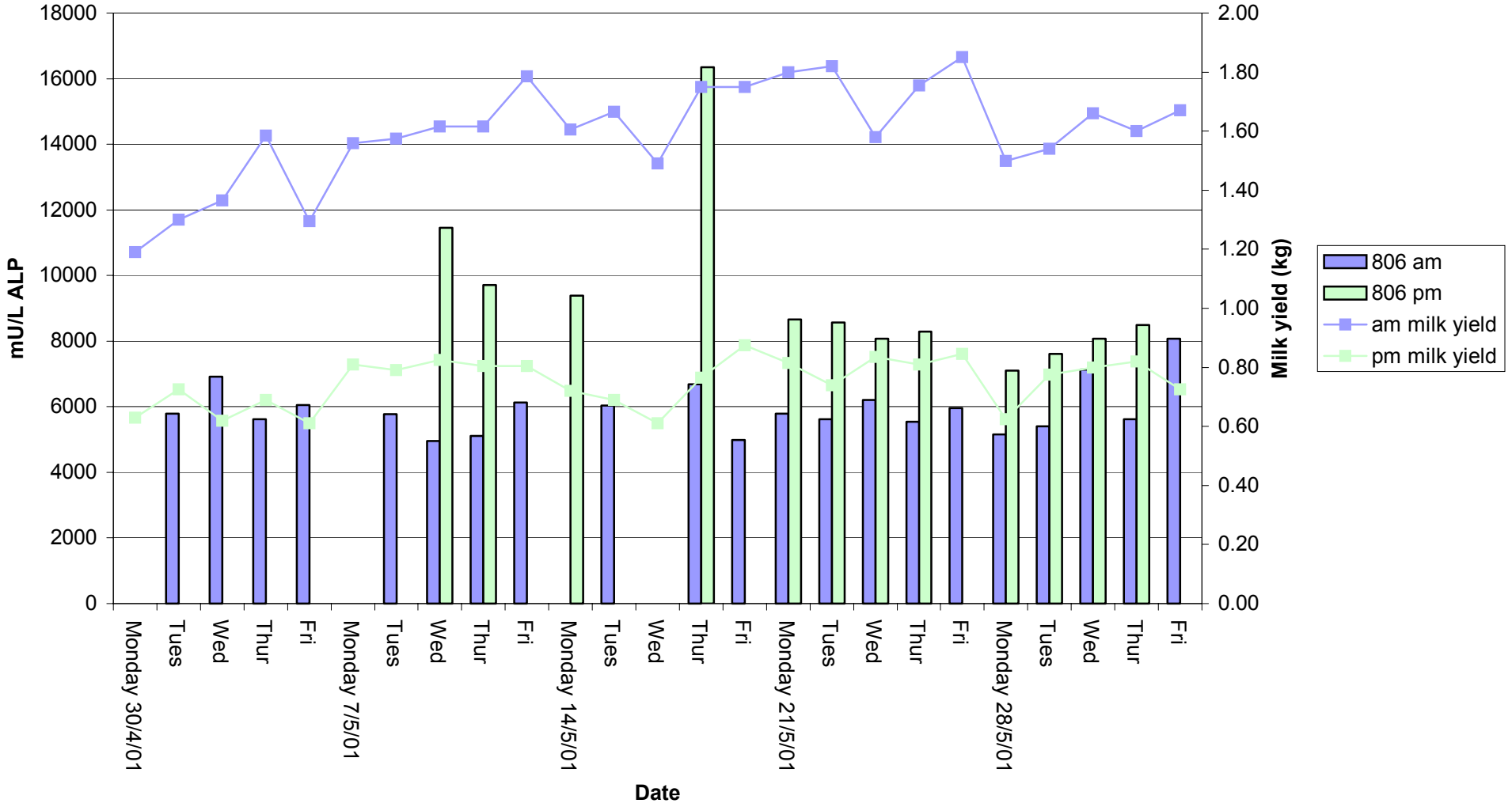


Figure 1i) Daily sampling of ALP in raw milk from goat 890 during May

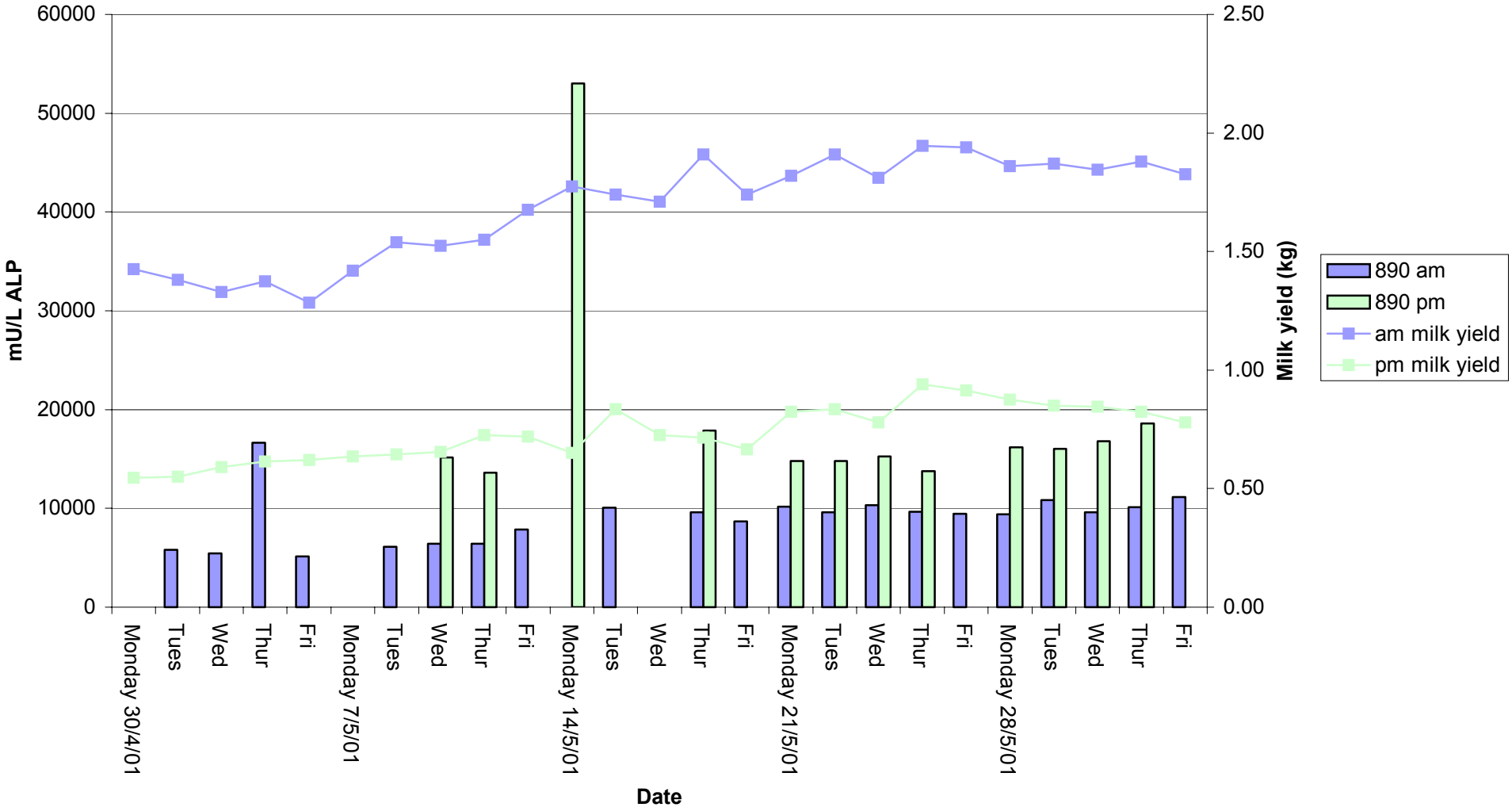


Figure 1j) Daily sampling of ALP in raw milk from goat 891 during May

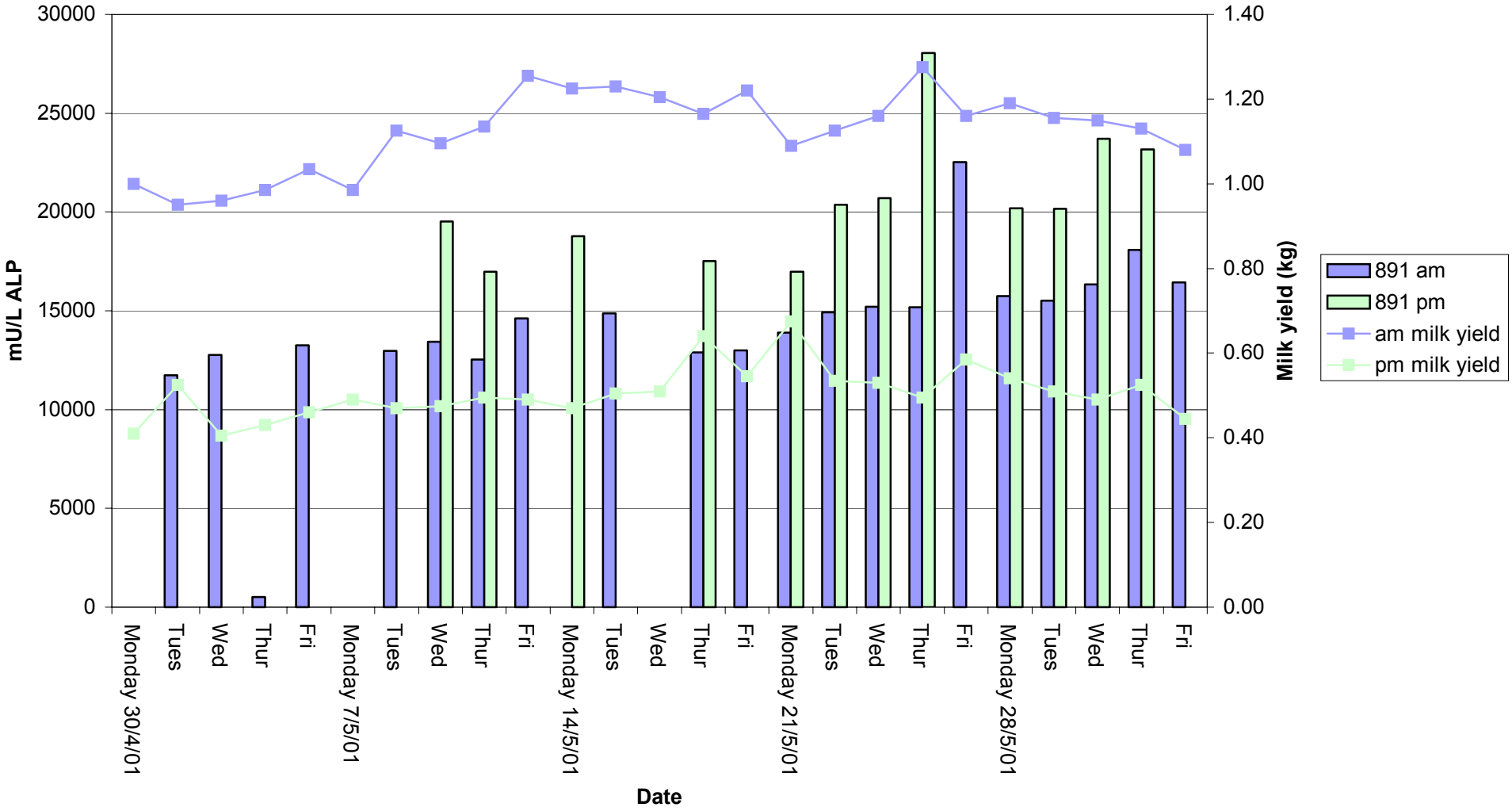


Figure 1k) Daily sampling of ALP in raw milk from goat 725 during May

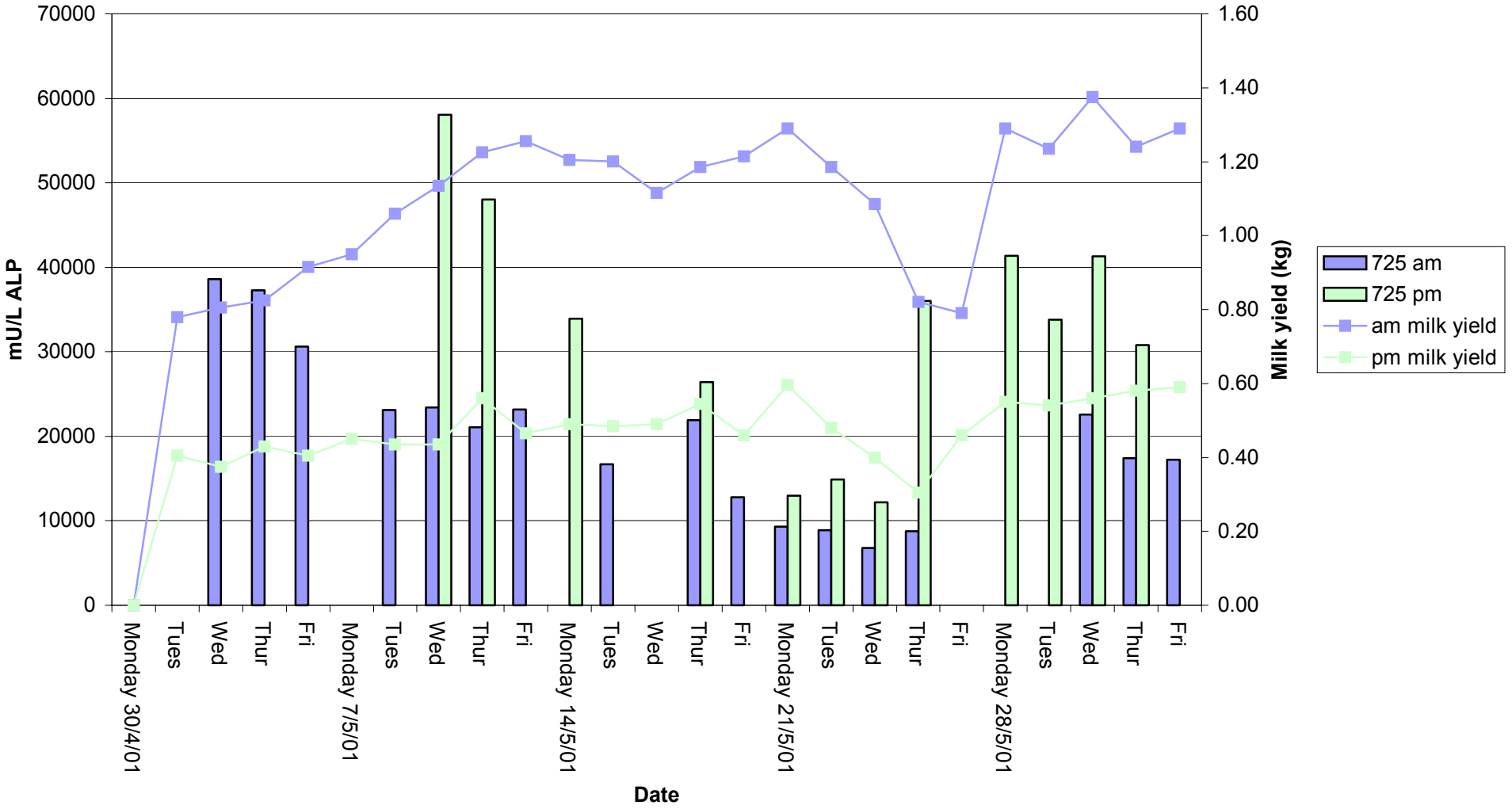


Figure 1l) Daily sampling of ALP in raw milk from goat 809 during May

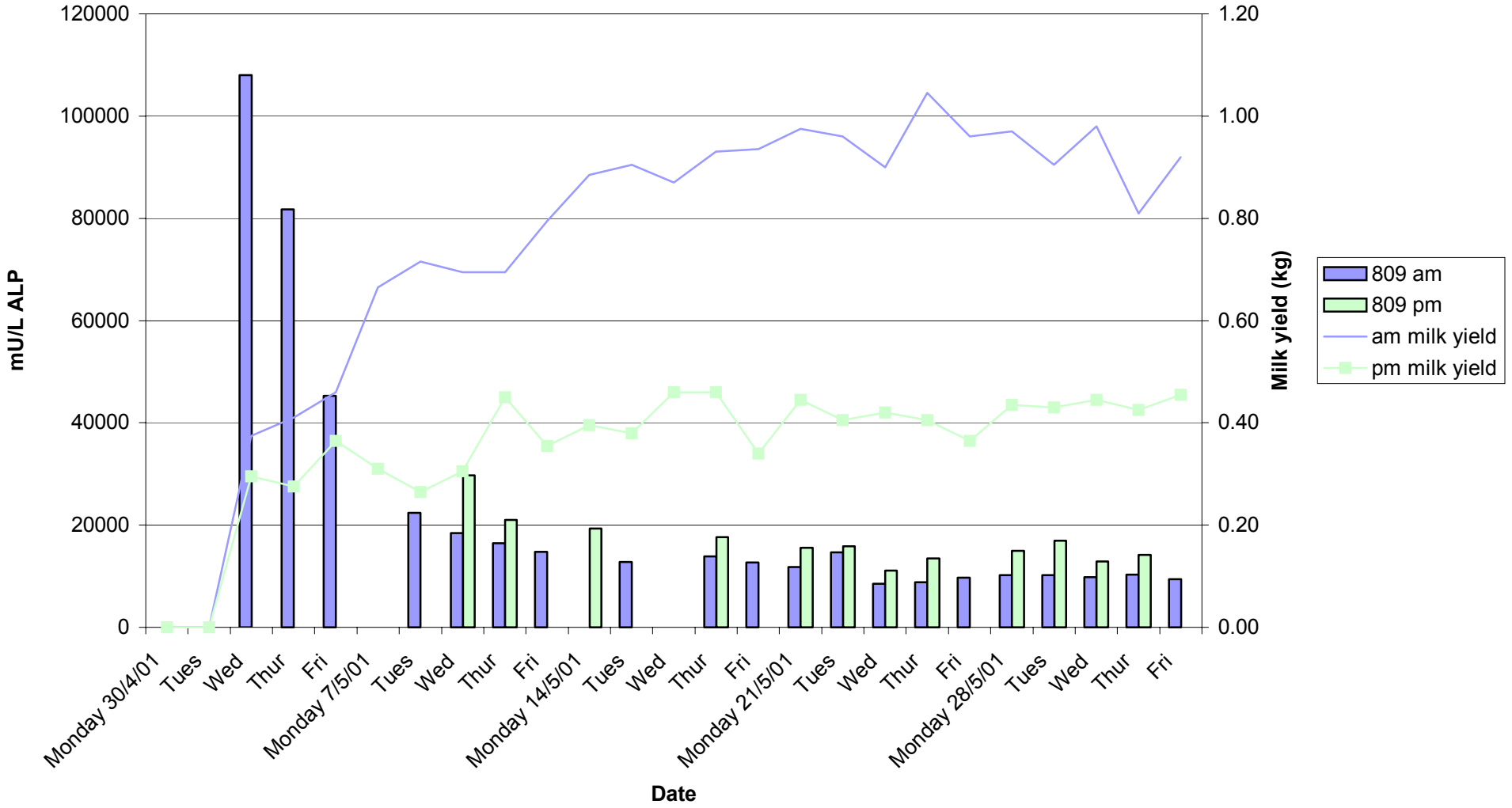


Figure 1m) Daily sampling of ALP in raw milk from goat 640 during May

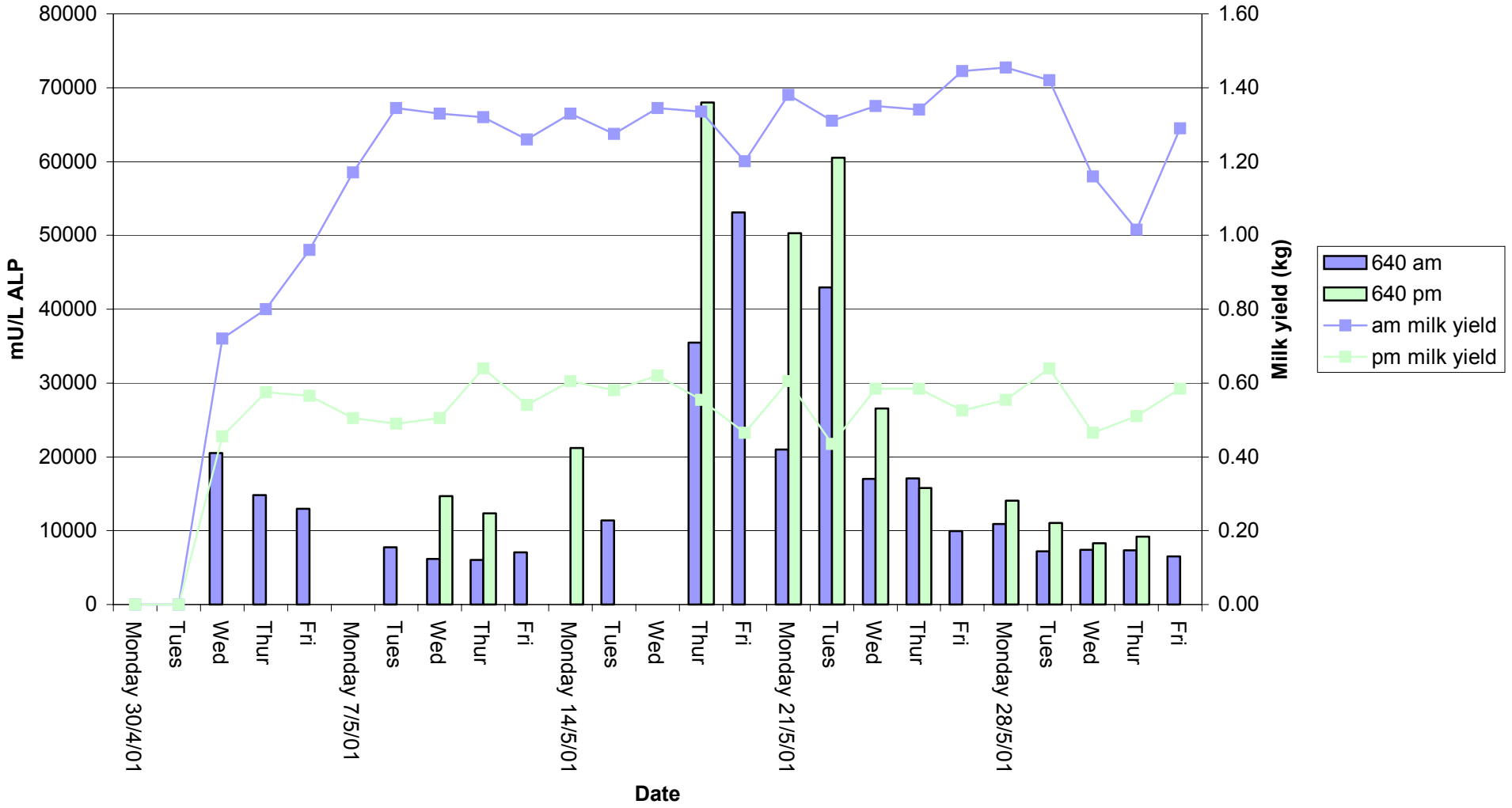


Figure 1n) Daily sampling of ALP in raw milk from goat 605 during May

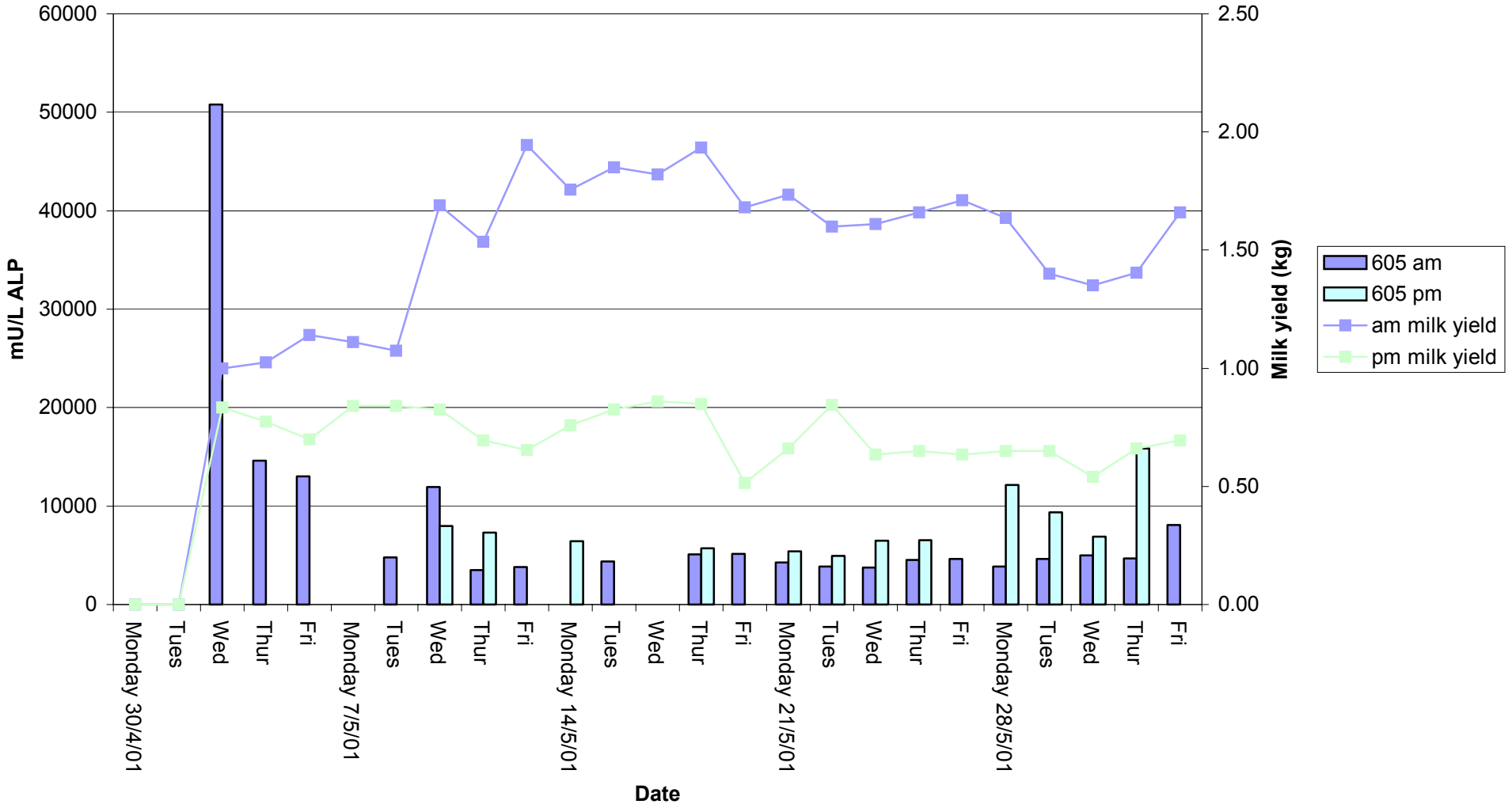


Figure 1p) Daily sampling of ALP in raw milk from goat 637 during May

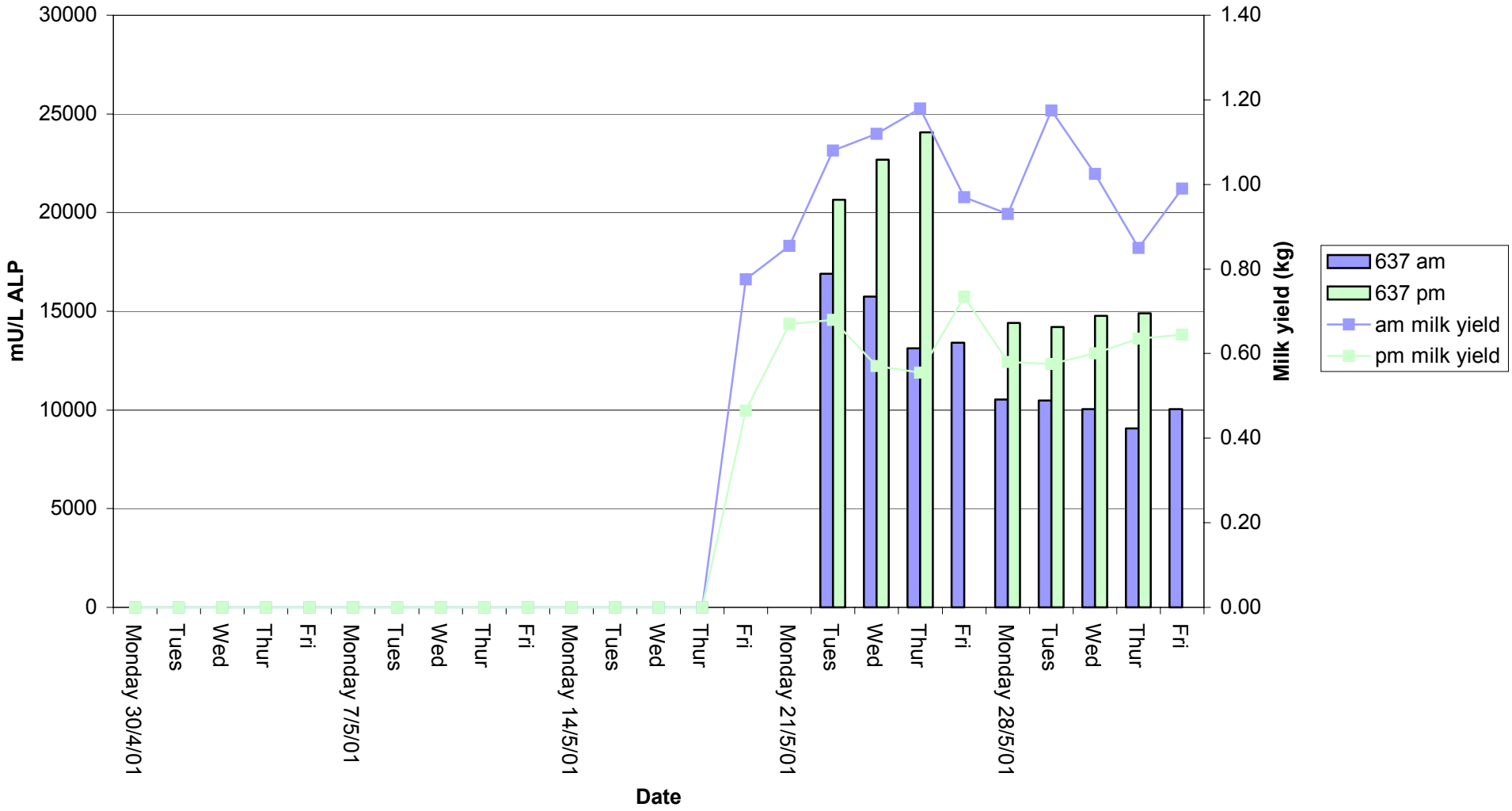


Figure 1q) Daily sampling of ALP in raw milk from goat 705 during May

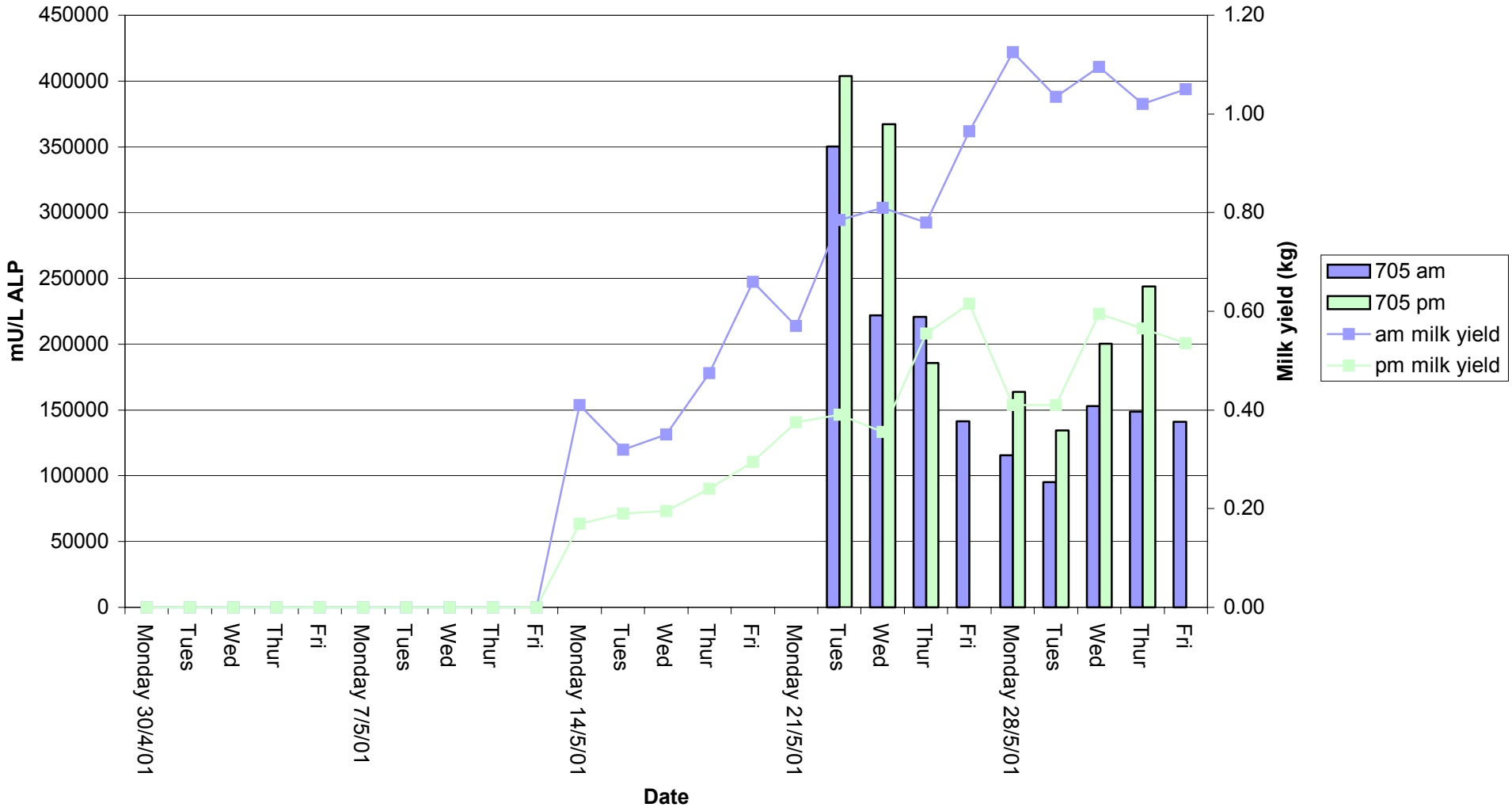


Figure 1r) Daily sampling of ALP in raw milk from goat 899 during May

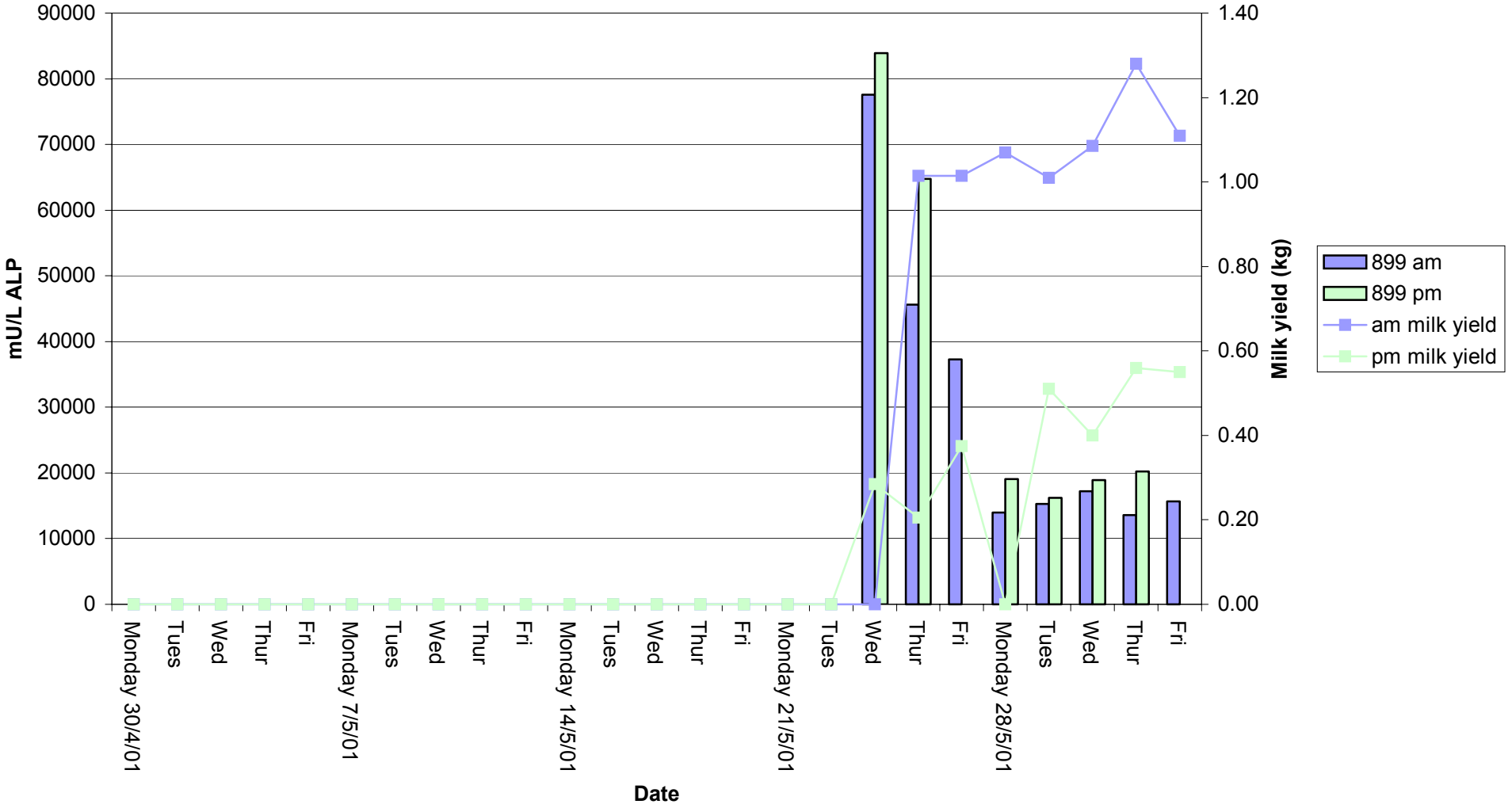


Figure 2a) Daily sampling of ALP in raw milk from goat 601 during June

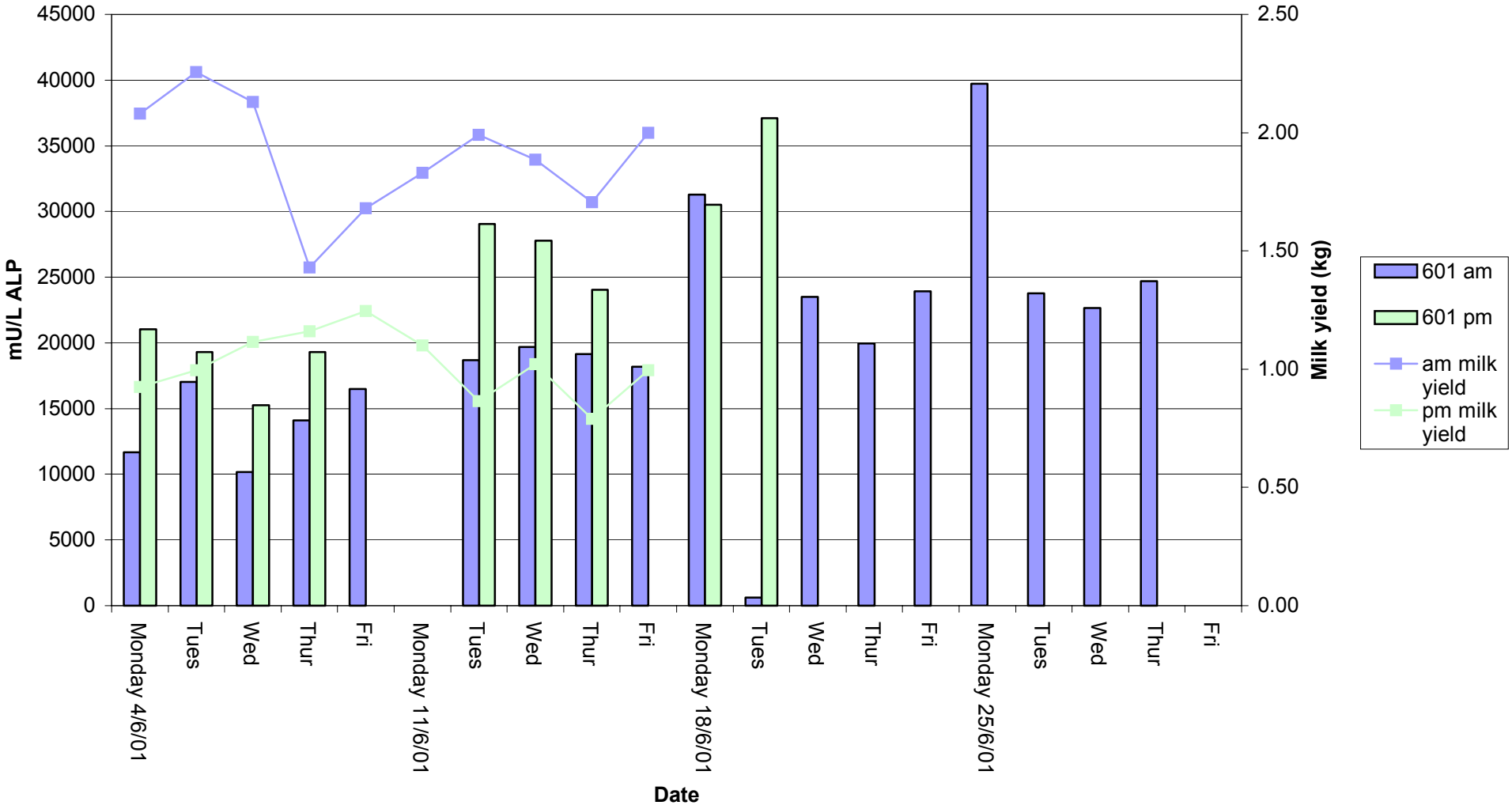


Figure 2b) Daily sampling of ALP in raw milk from goat 610 during June

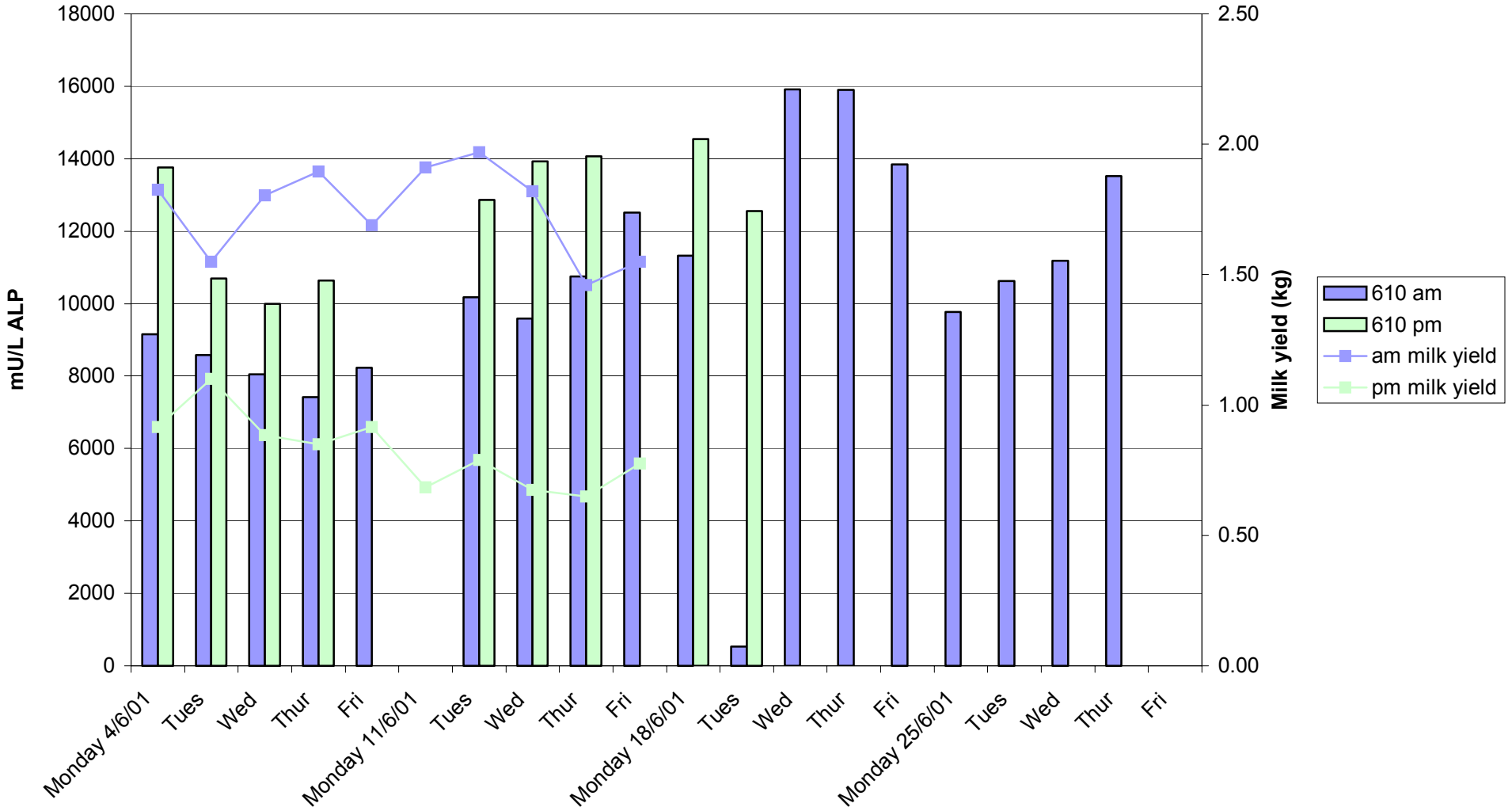


Figure 2c) Daily sampling of ALP in raw milk from goat 617 during June

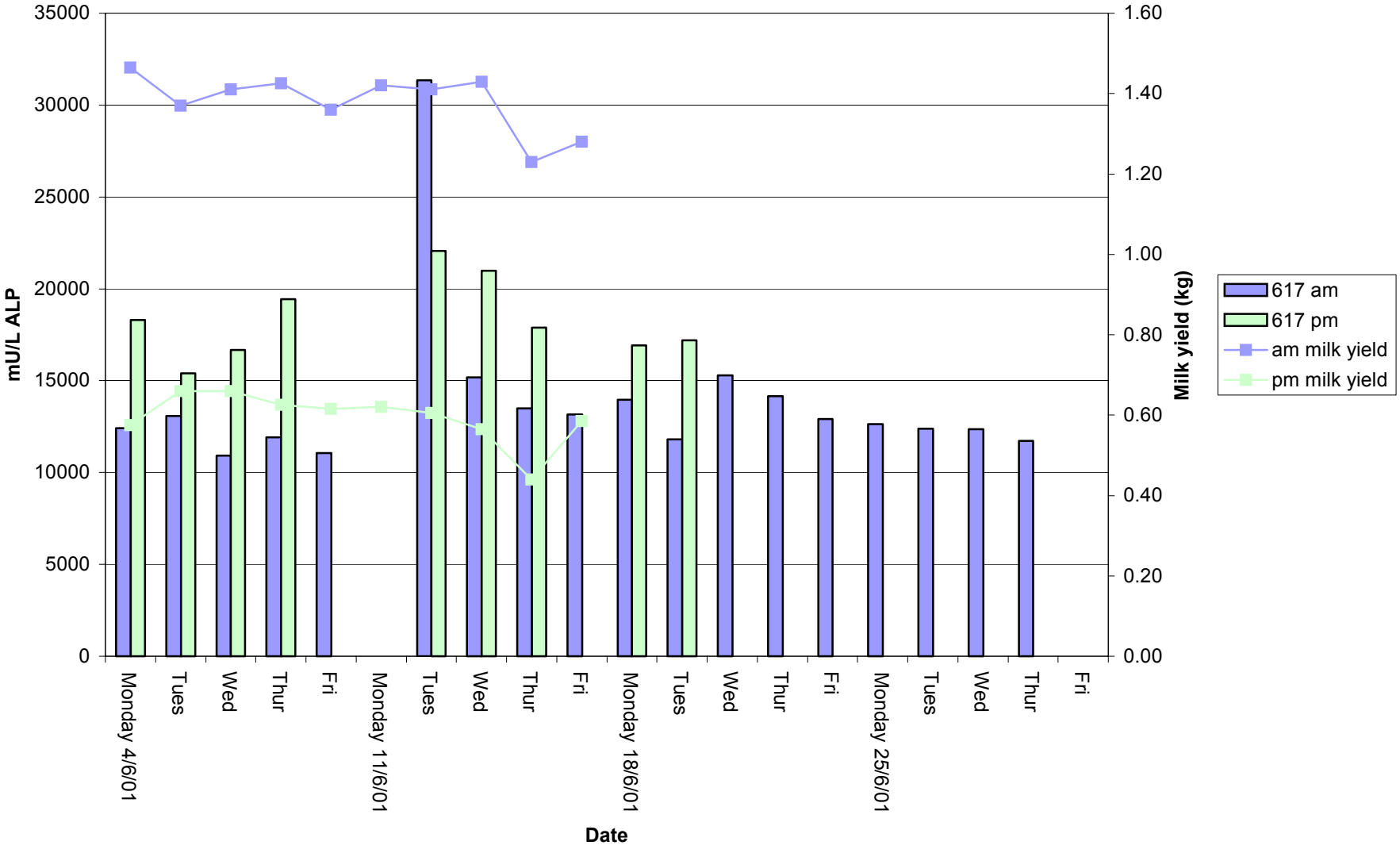


Figure 2d) Daily sampling of ALP in raw milk from goat 618 during June

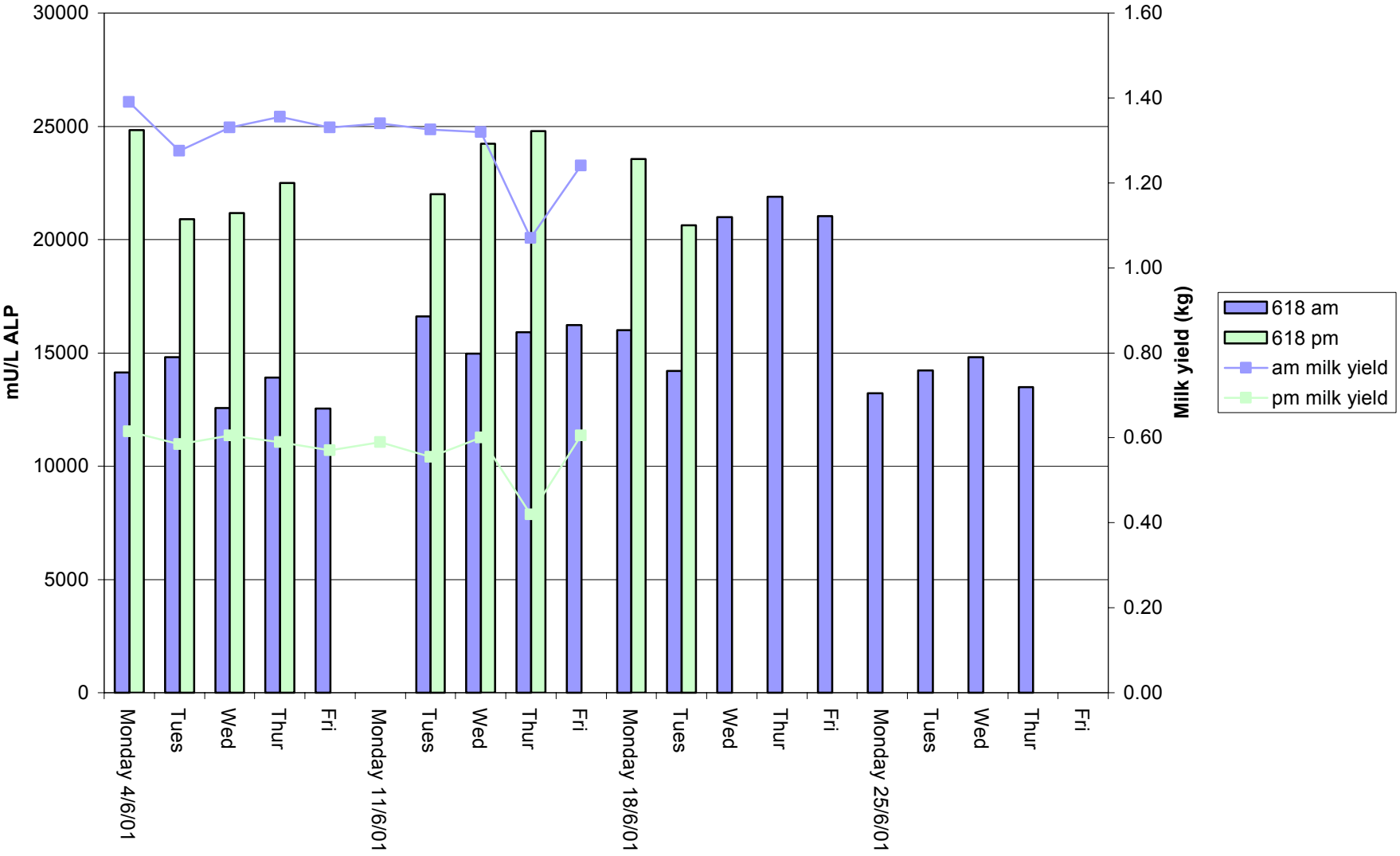


Figure 2e) Daily sampling of ALP in raw milk from goat 601 during June

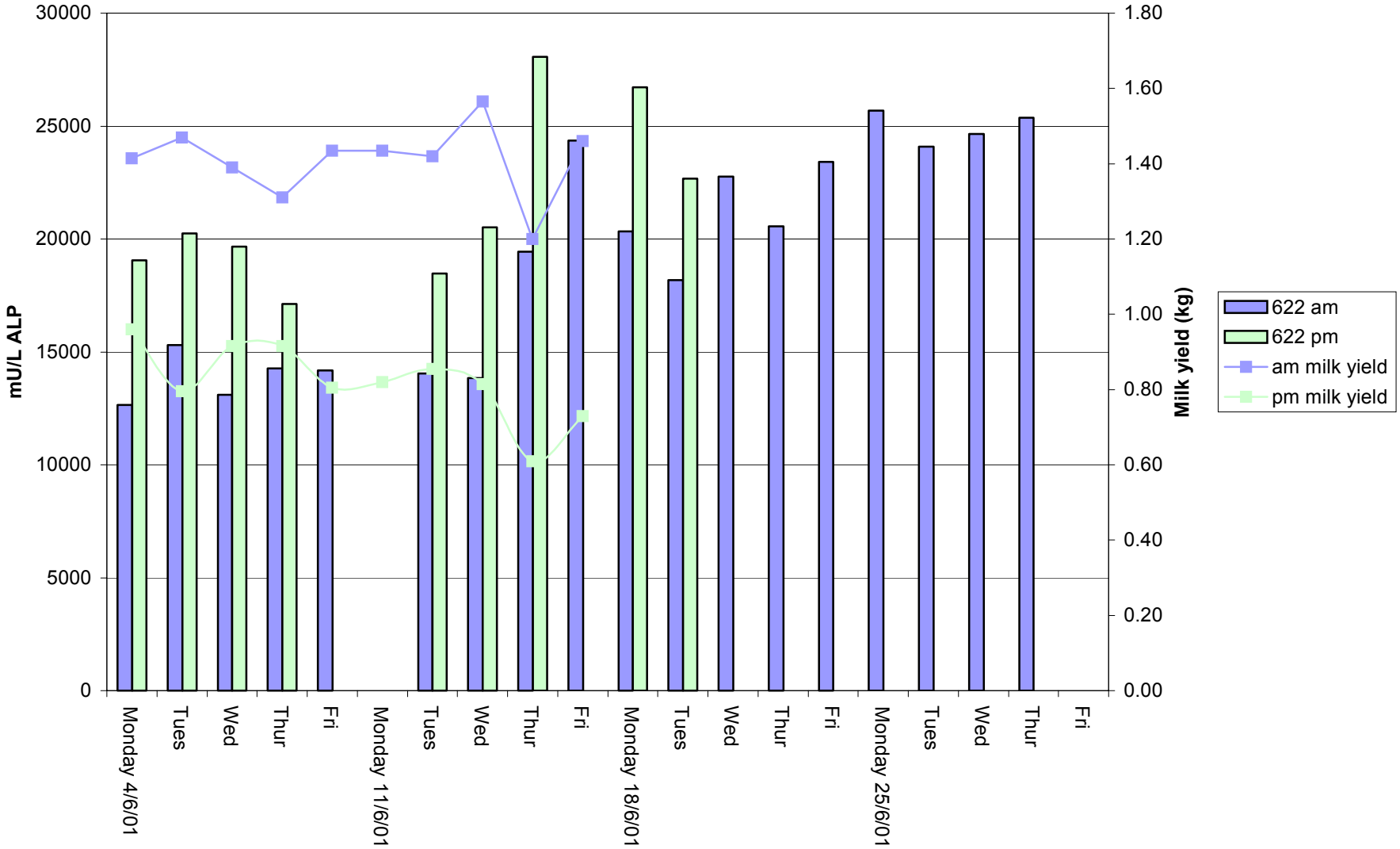


Figure 2f) Daily sampling of ALP in raw milk from goat 639 during June

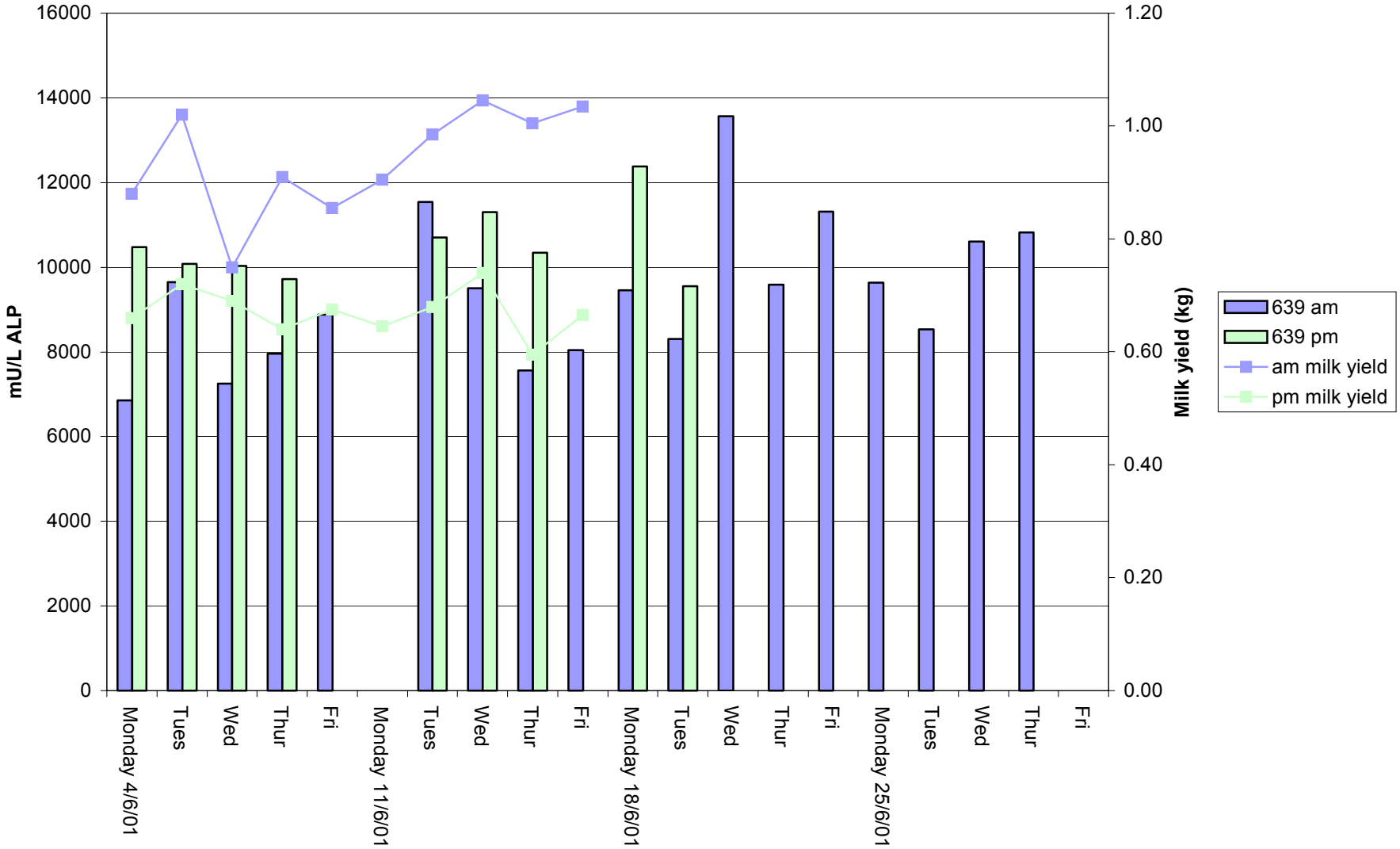


Figure 2g) Daily sampling of ALP in raw milk from goat 713 during June

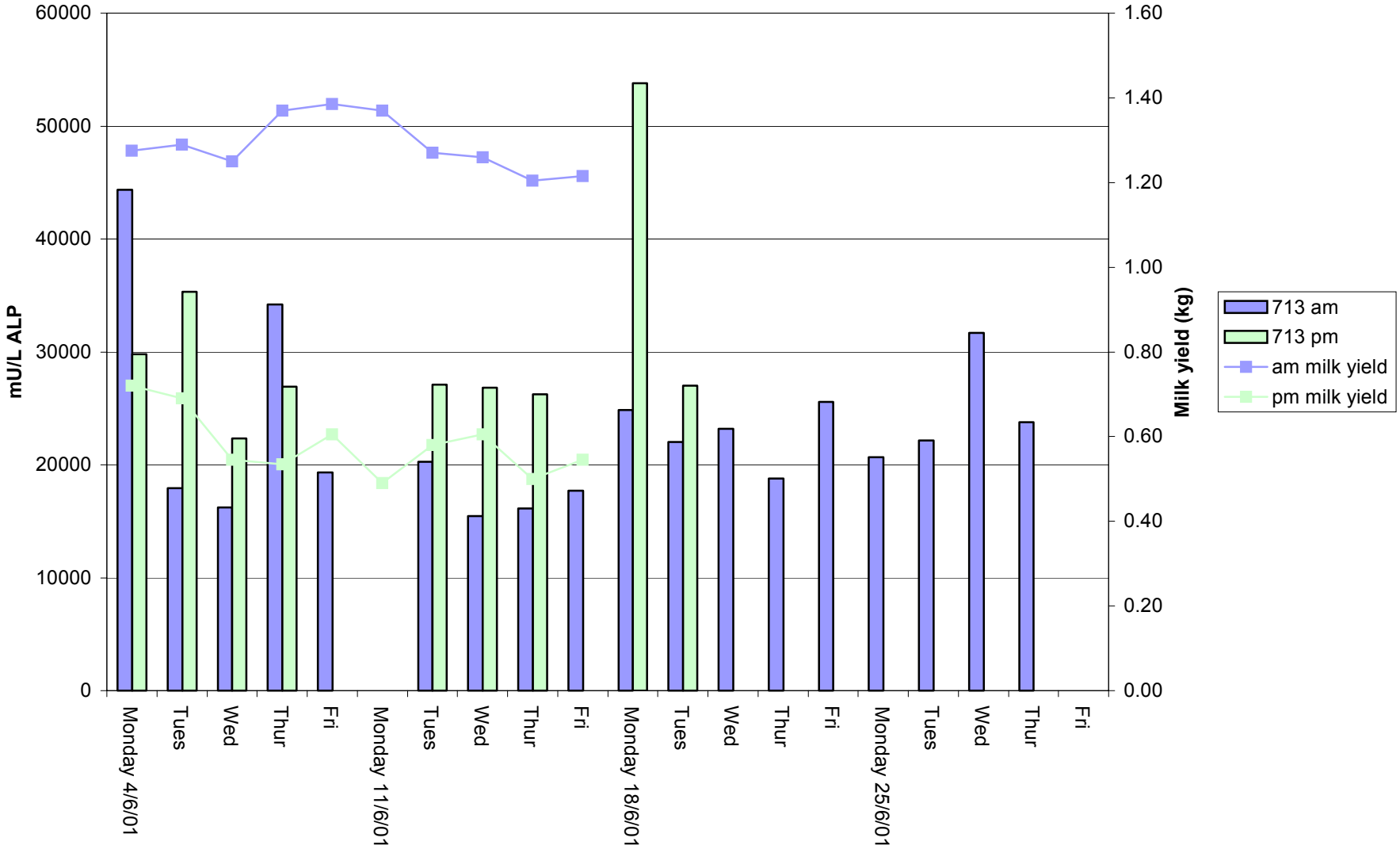


Figure 2h) Daily sampling of ALP in raw milk from goat 601 during June

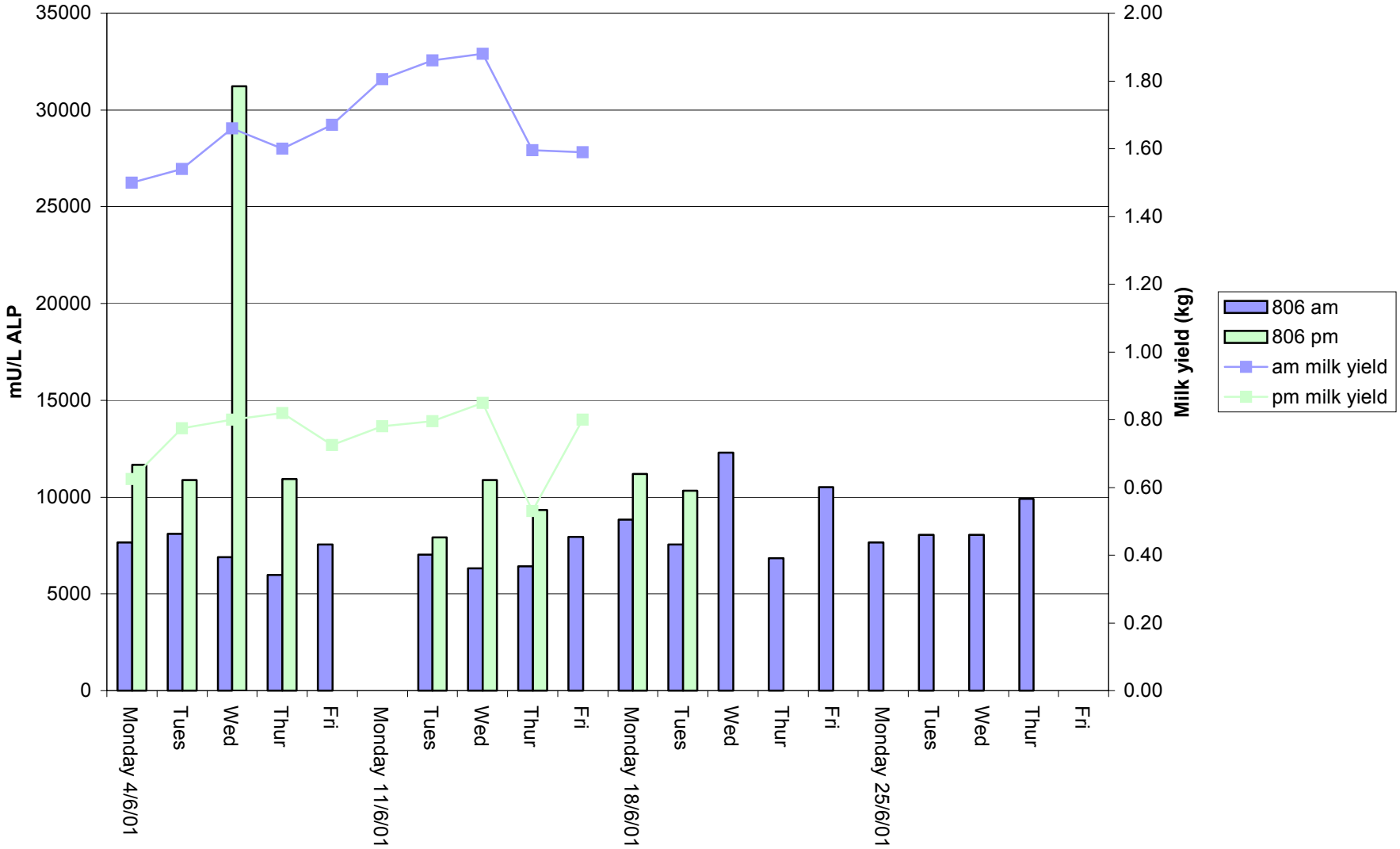


Figure 2i) Daily sampling of ALP in raw milk from goat 890 during June

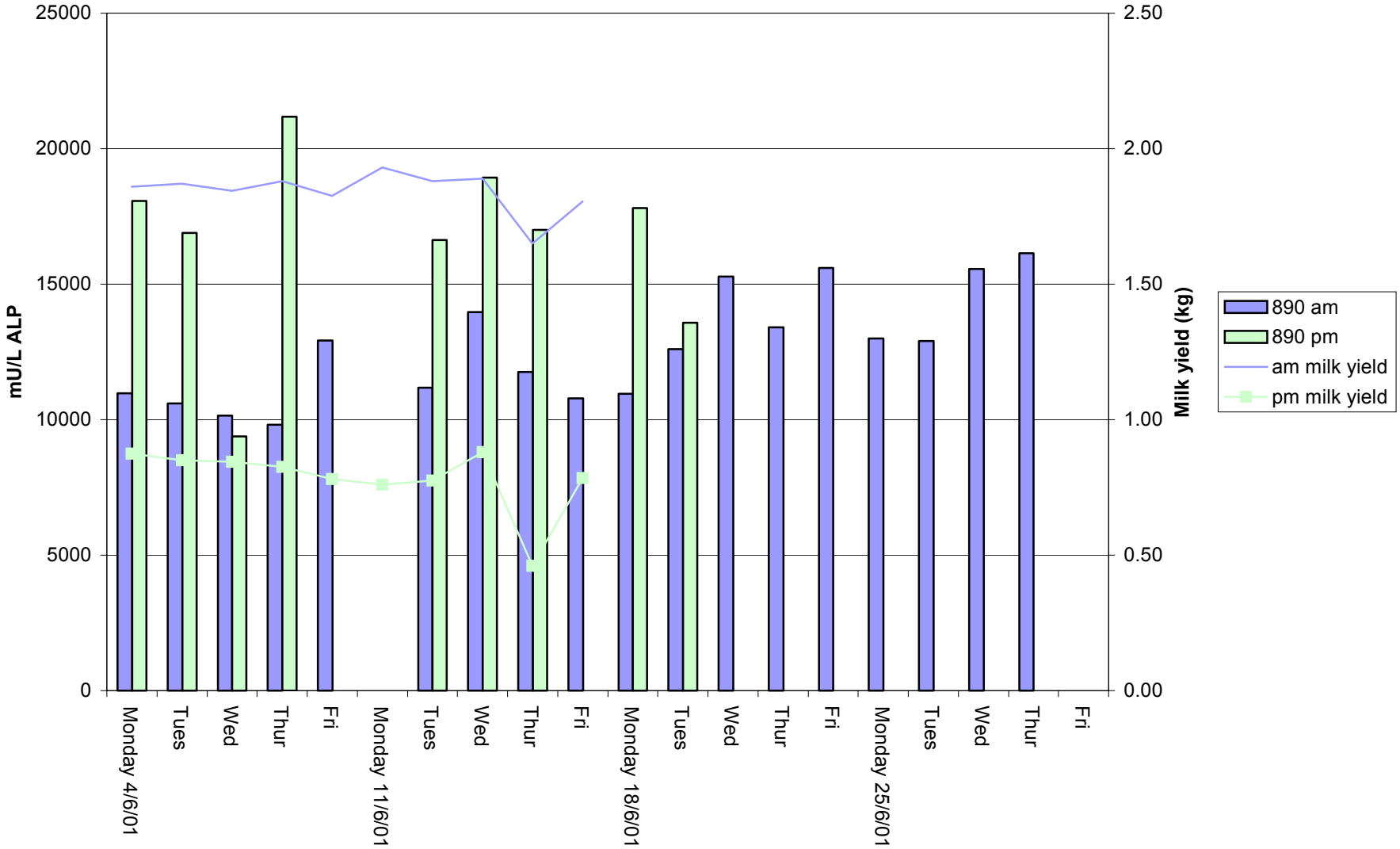


Figure 2j) Daily sampling of ALP in raw milk from goat 891 during June

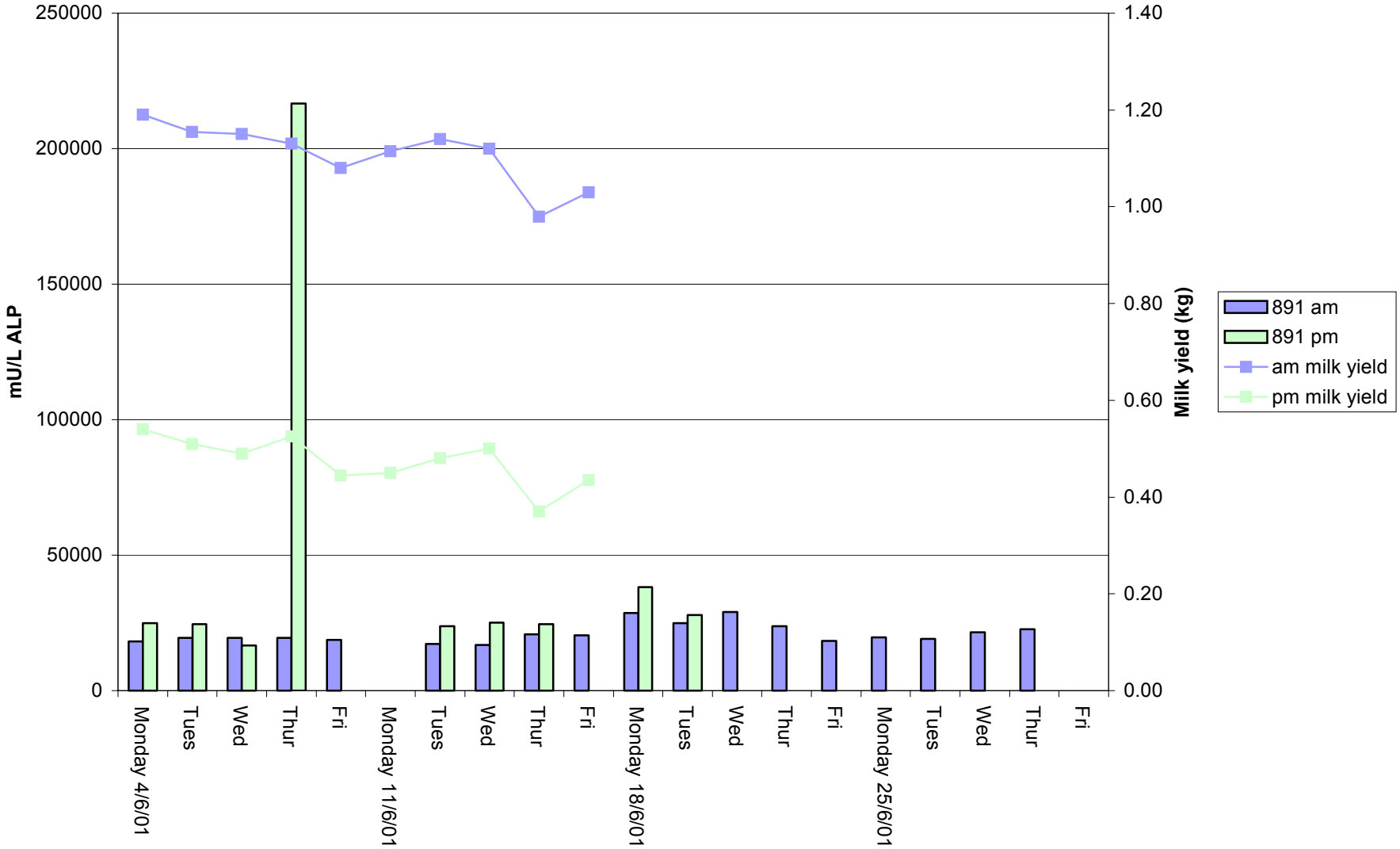


Figure 2k) Daily sampling of ALP in raw milk from goat 725 during June

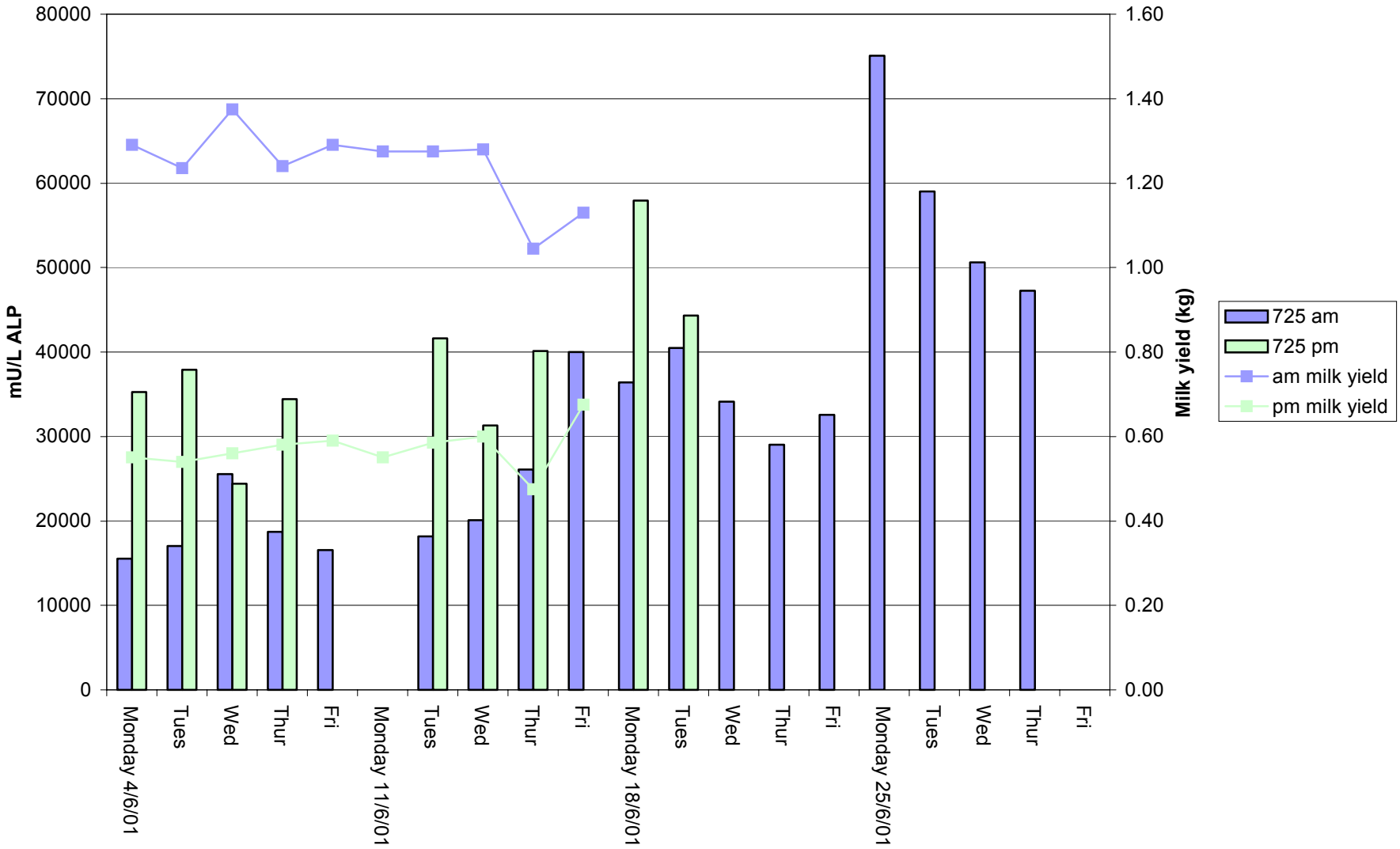


Figure 2l) Daily sampling of ALP in raw milk from goat 809 during June

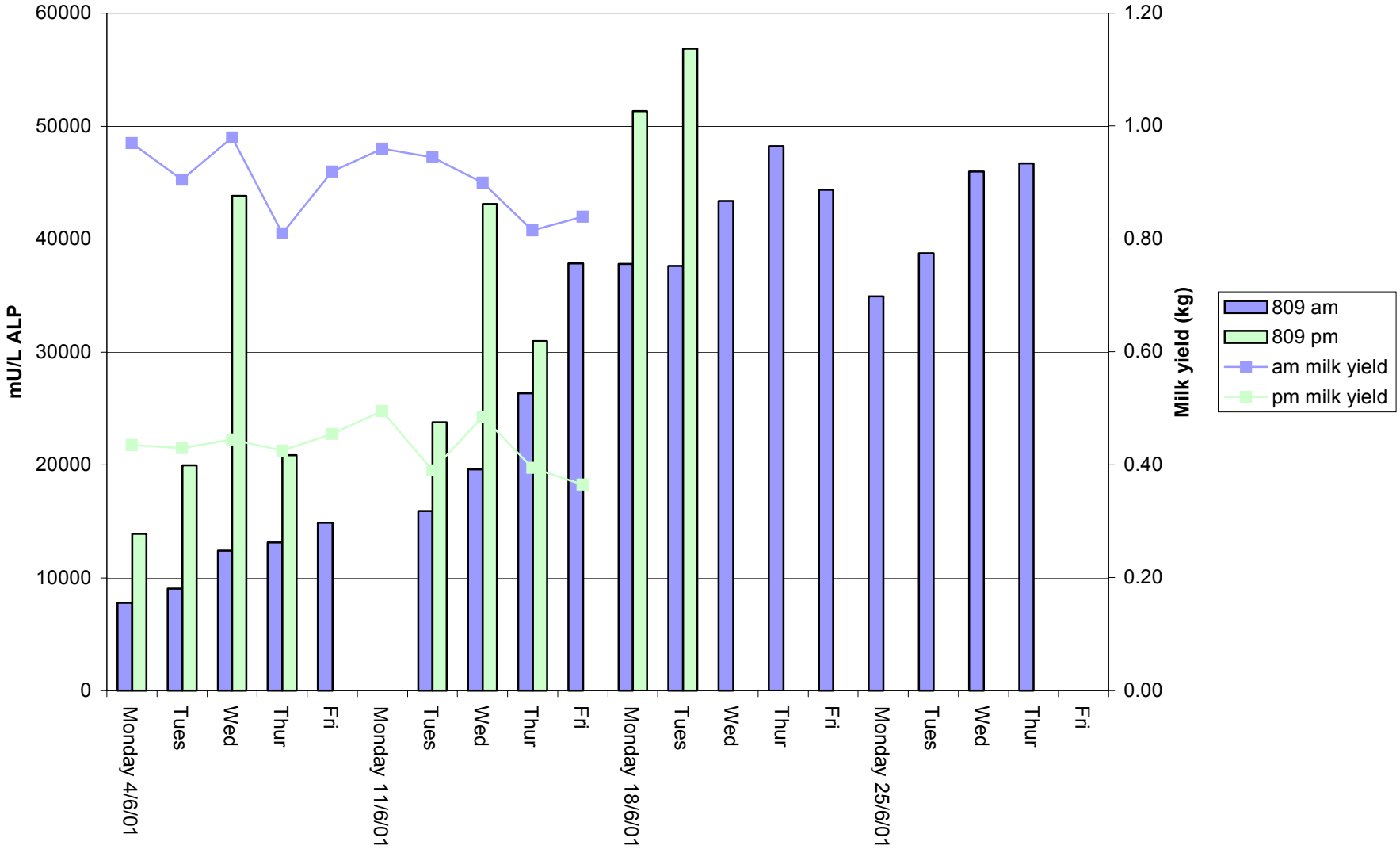


Figure 2m) Daily sampling of ALP in raw milk from goat 640 during June

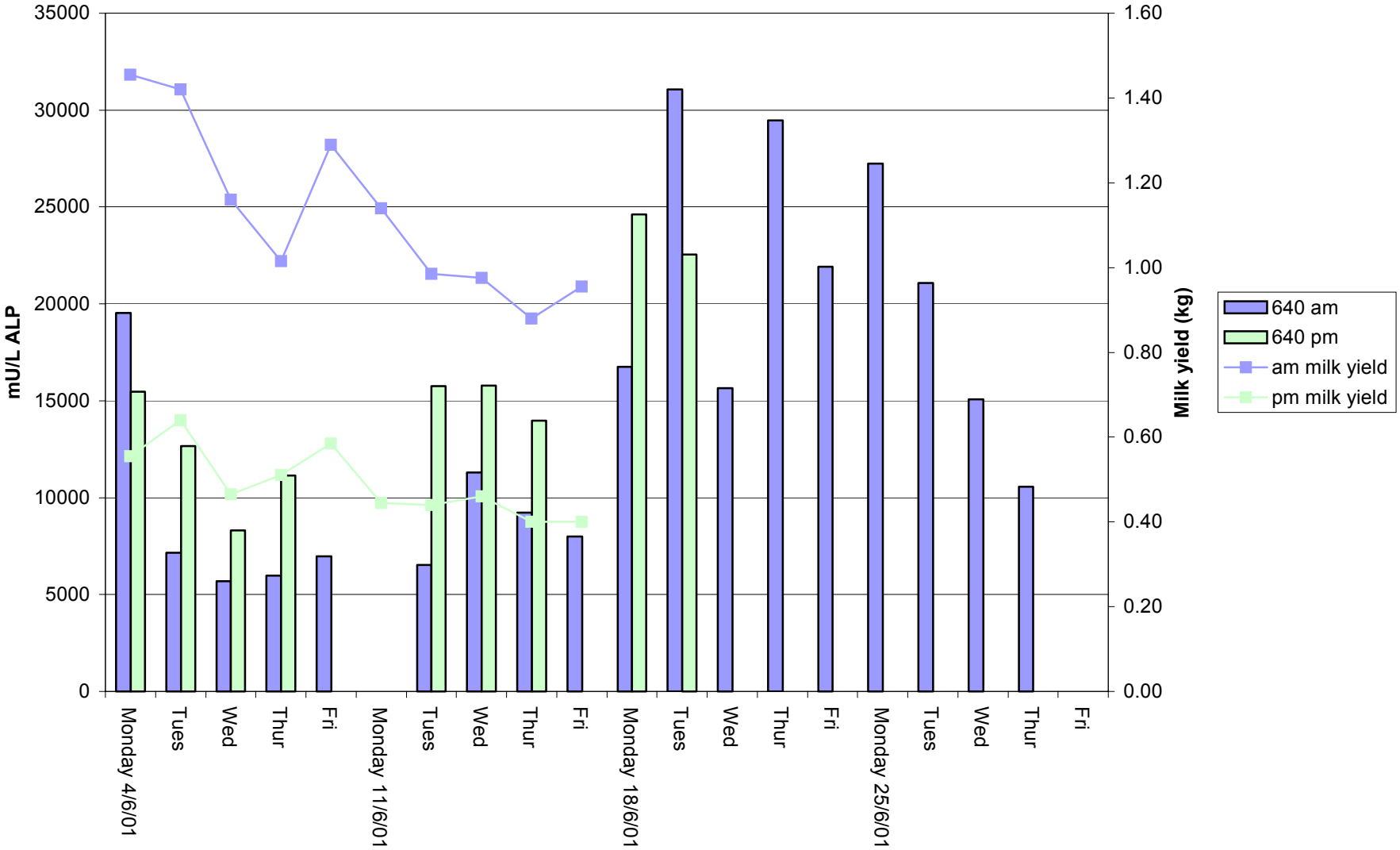


Figure 2n) Daily sampling of ALP in raw milk from goat 605 during June

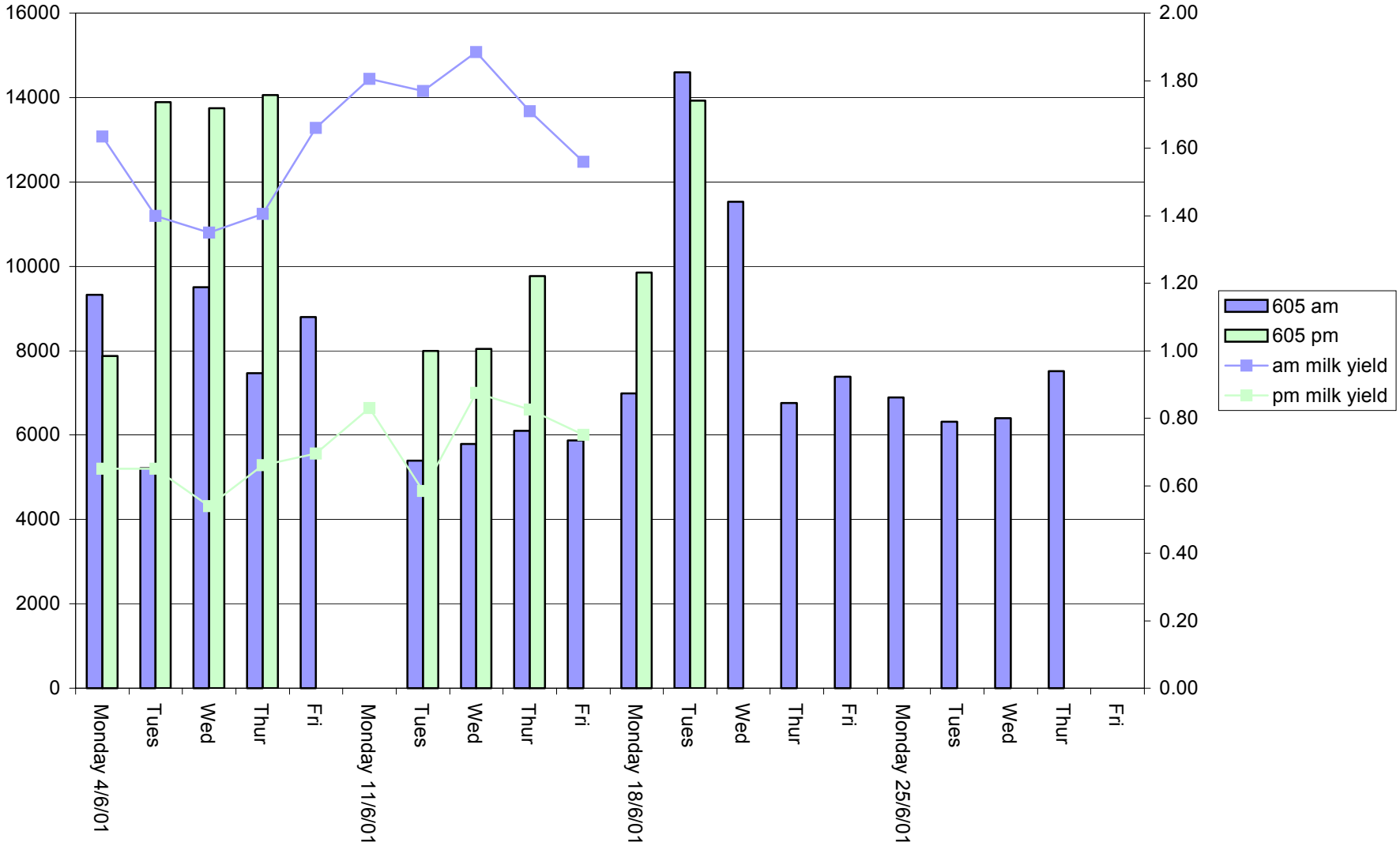


Figure 2o) Daily sampling of ALP in raw milk from goat 637 during June

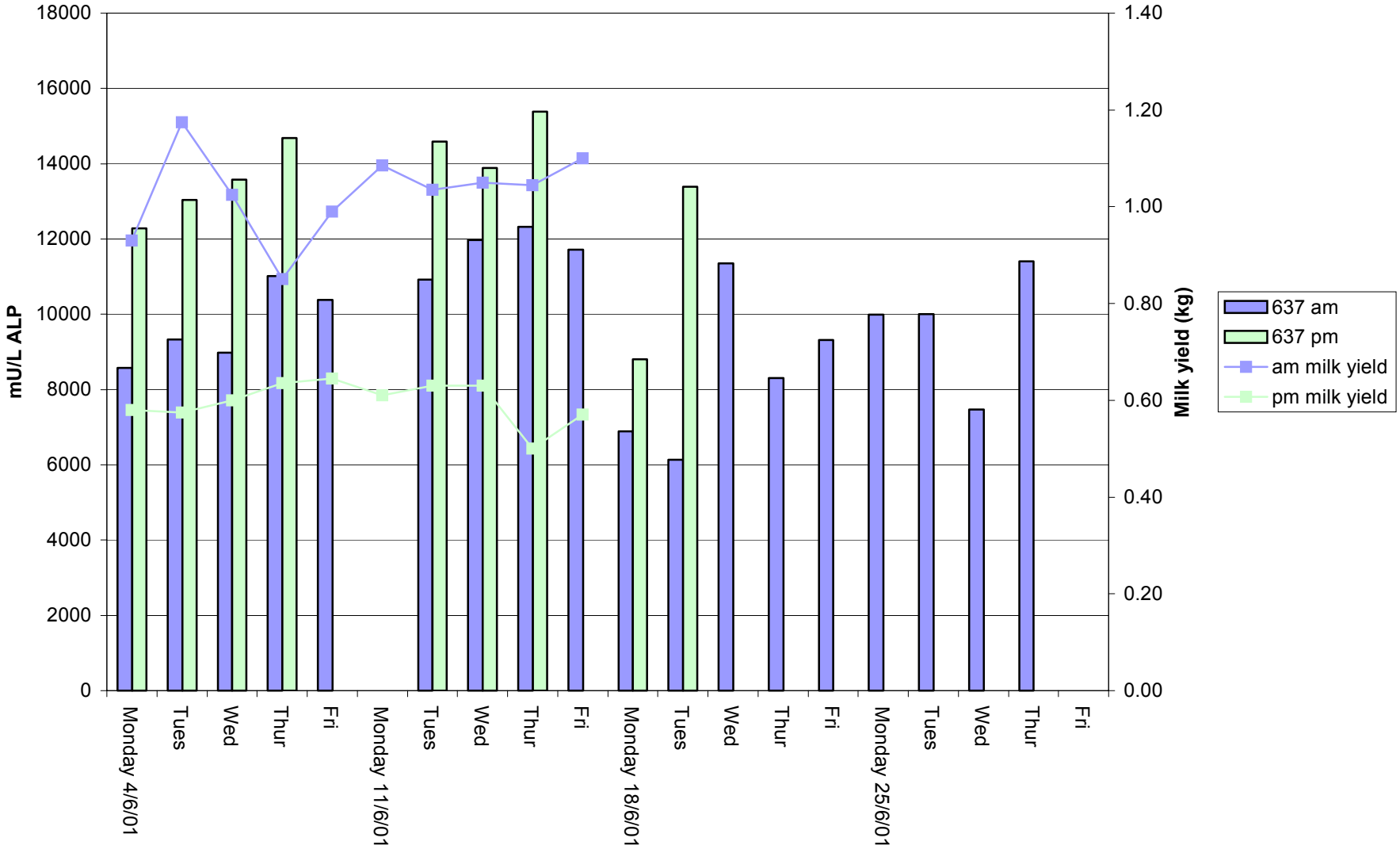


Figure 2p) Daily sampling of ALP in raw milk from goat 705 during June

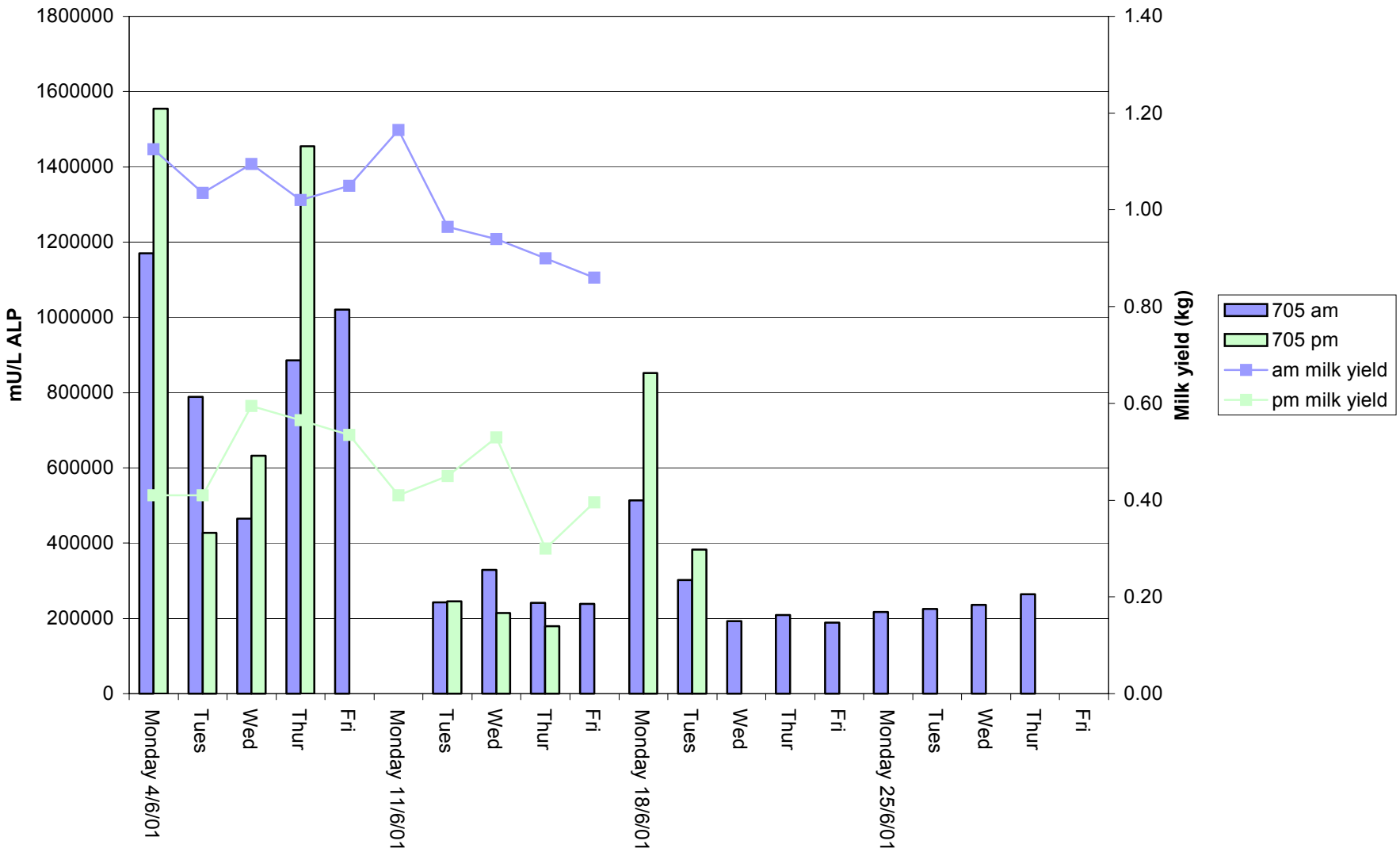


Figure 2r) Daily sampling of ALP in raw milk from goat 899 during June

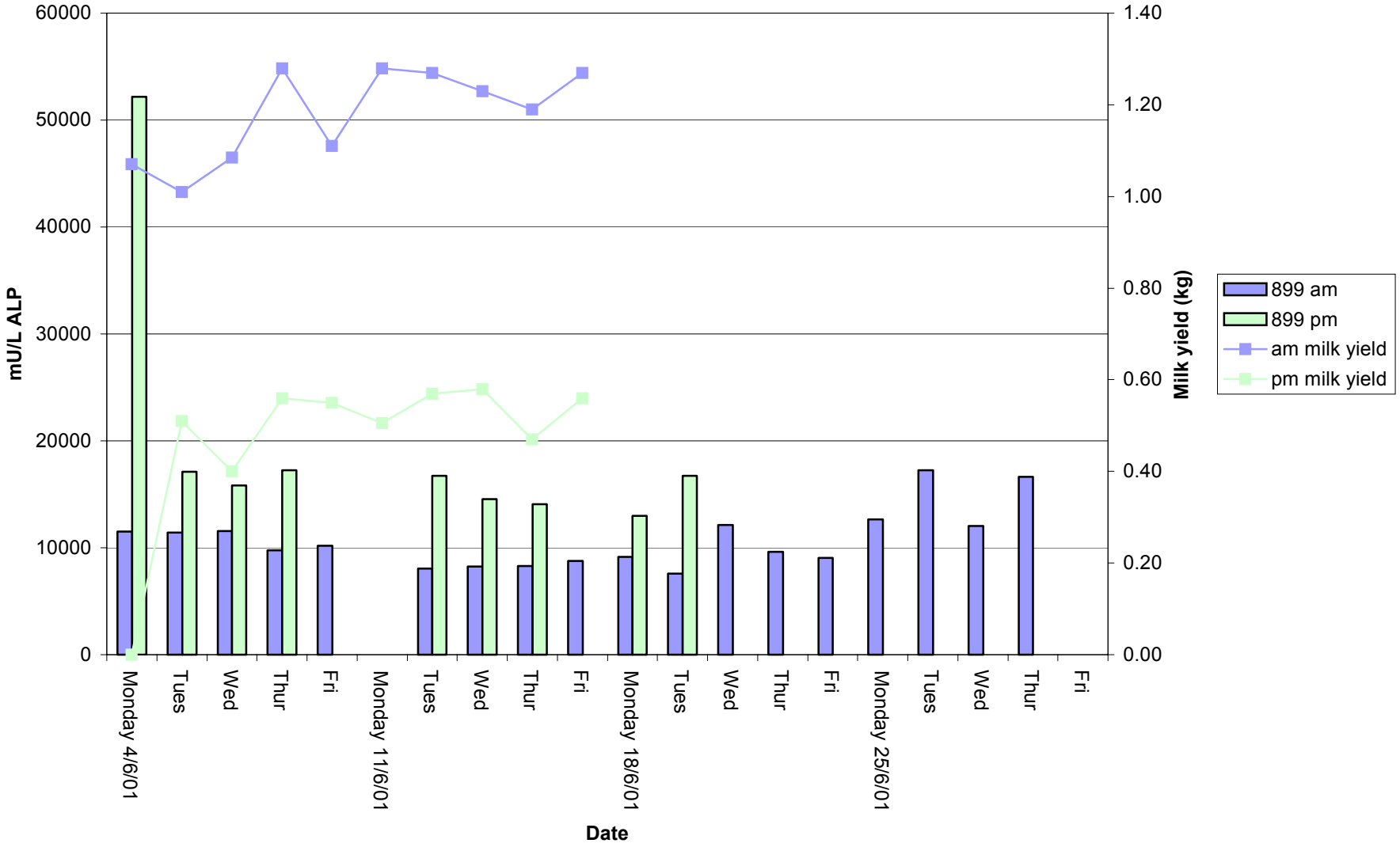


Figure 3 a) ALP in raw milk from goat 601

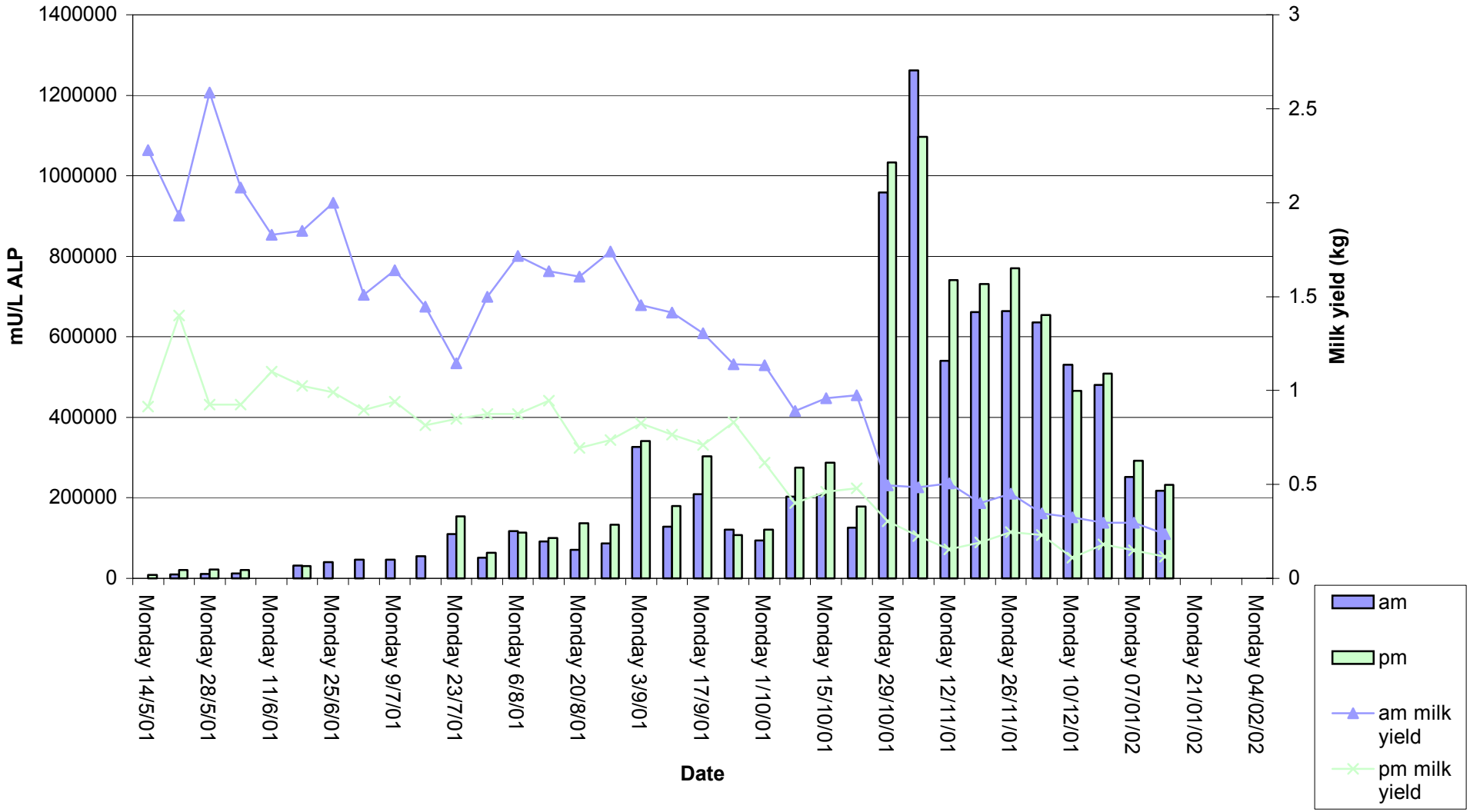


Figure 3 b) ALP in raw milk from goat 610

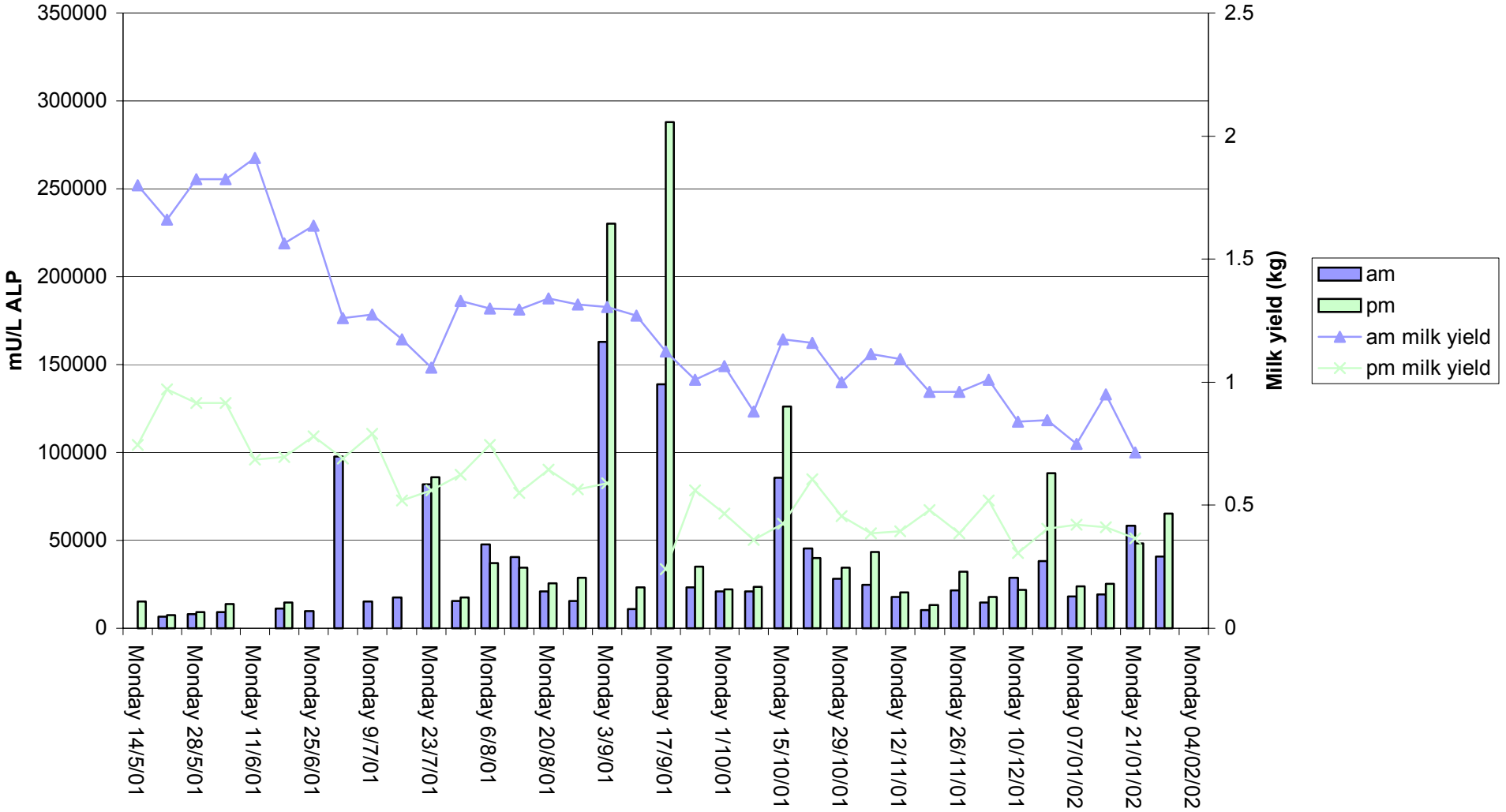


Figure 3 c) ALP in raw milk from goat 617

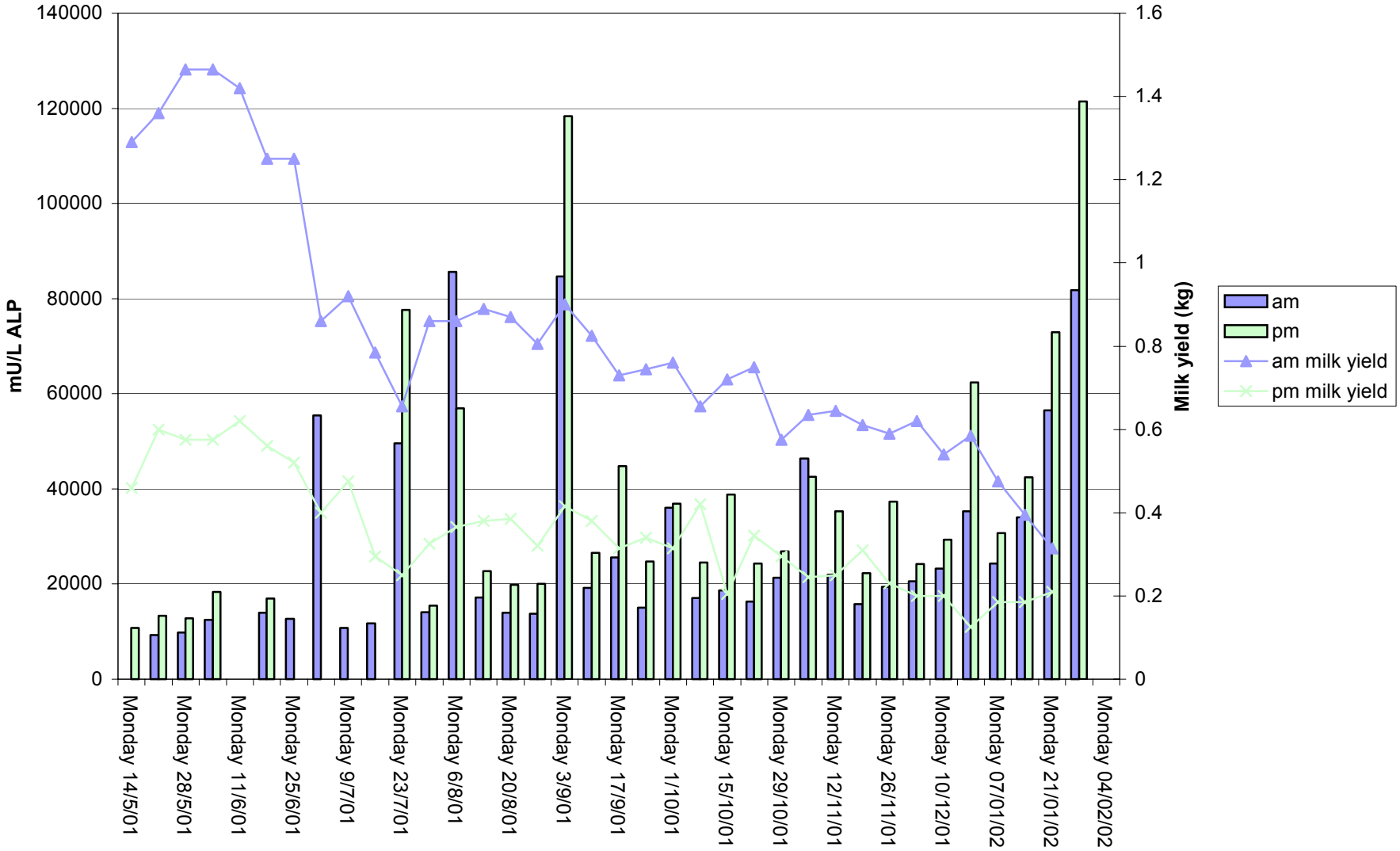


Figure 3 d) ALP in raw milk from goat 618

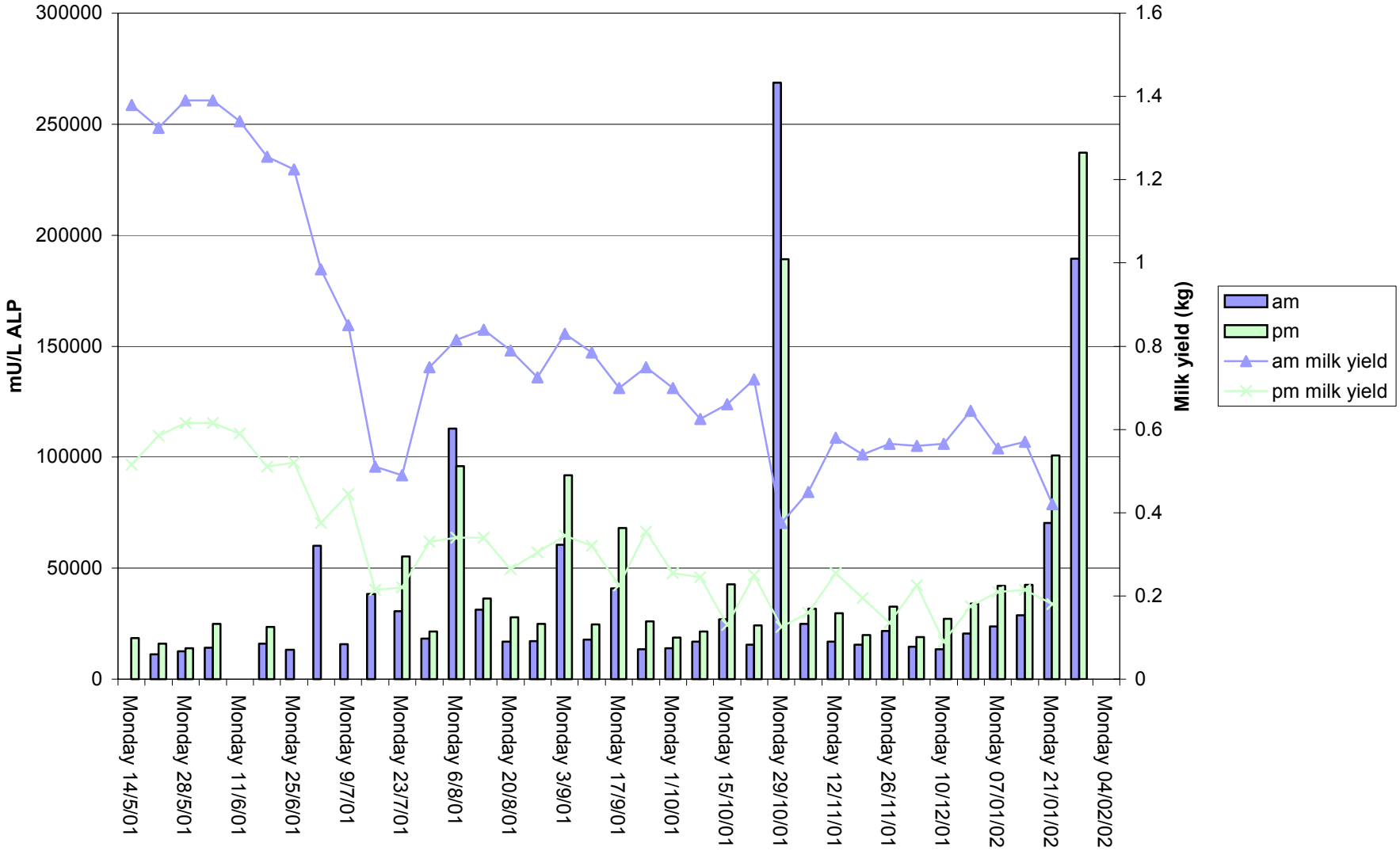


Figure 3 e) ALP in raw milk from goat 622

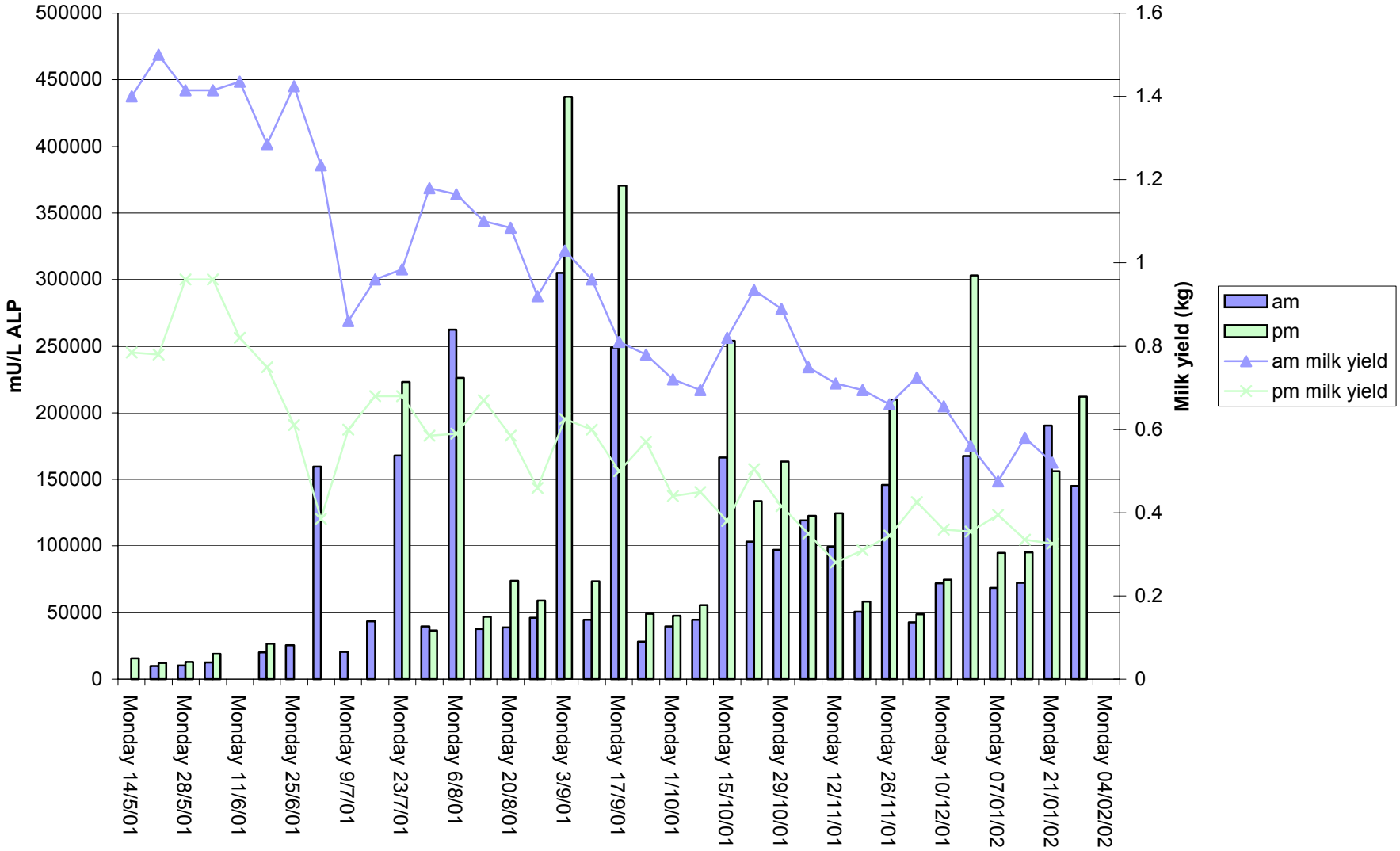


Figure 3 f) ALP in raw milk from goat 639

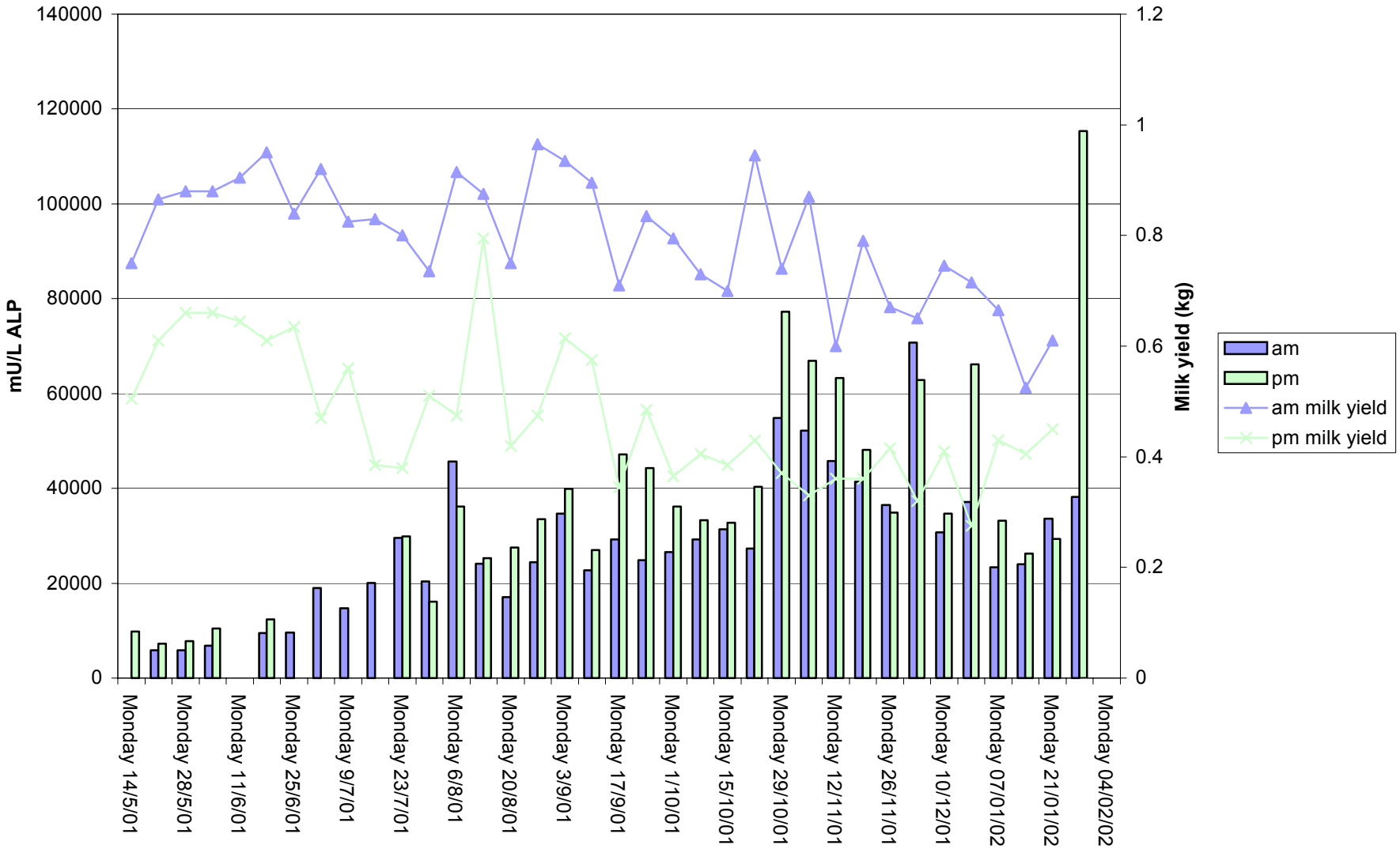


Figure 3 g) ALP in raw milk from goat 713

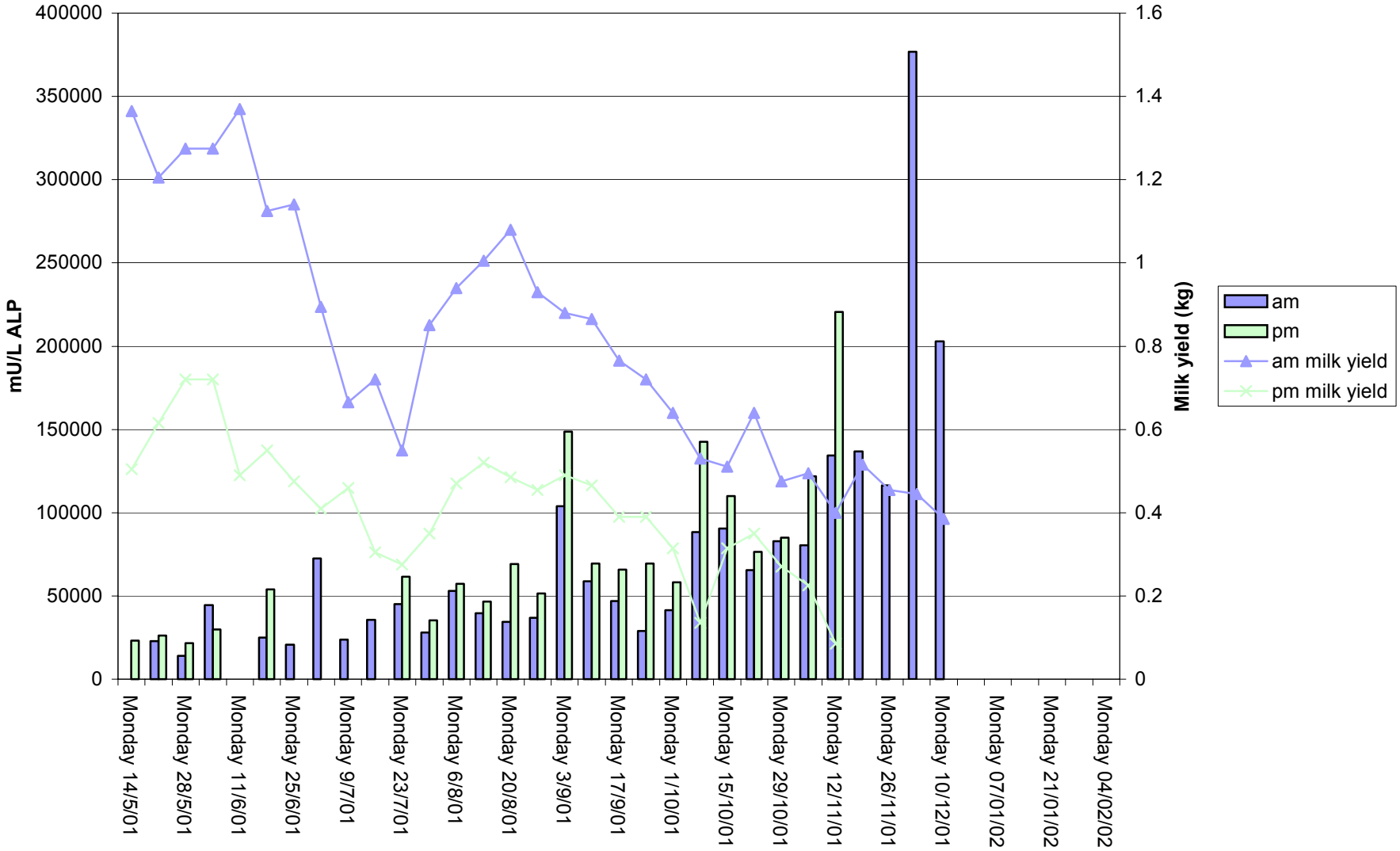


Figure 3 h) ALP in raw milk from goat 806

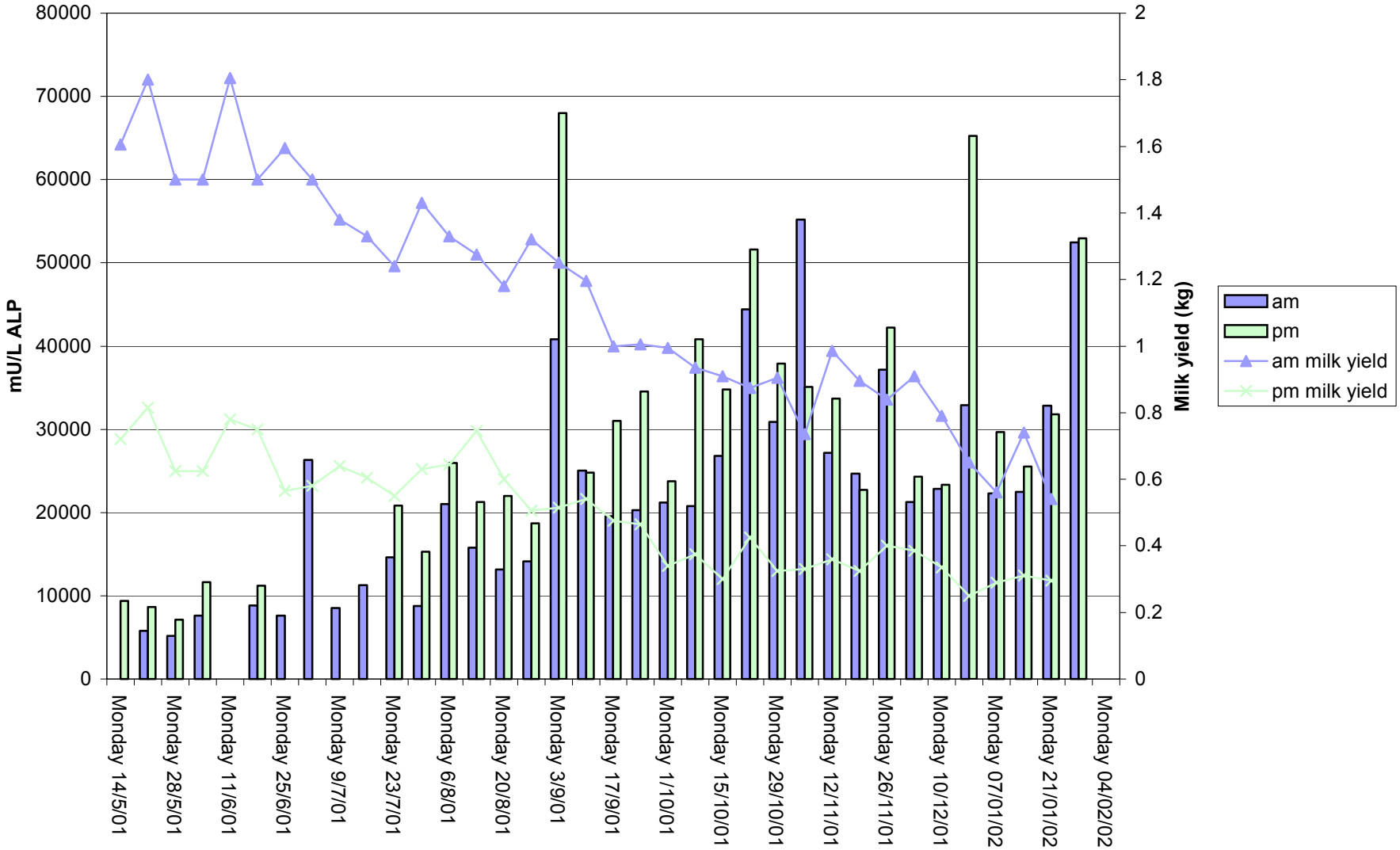


Figure 3 i) ALP in raw milk from goat 890

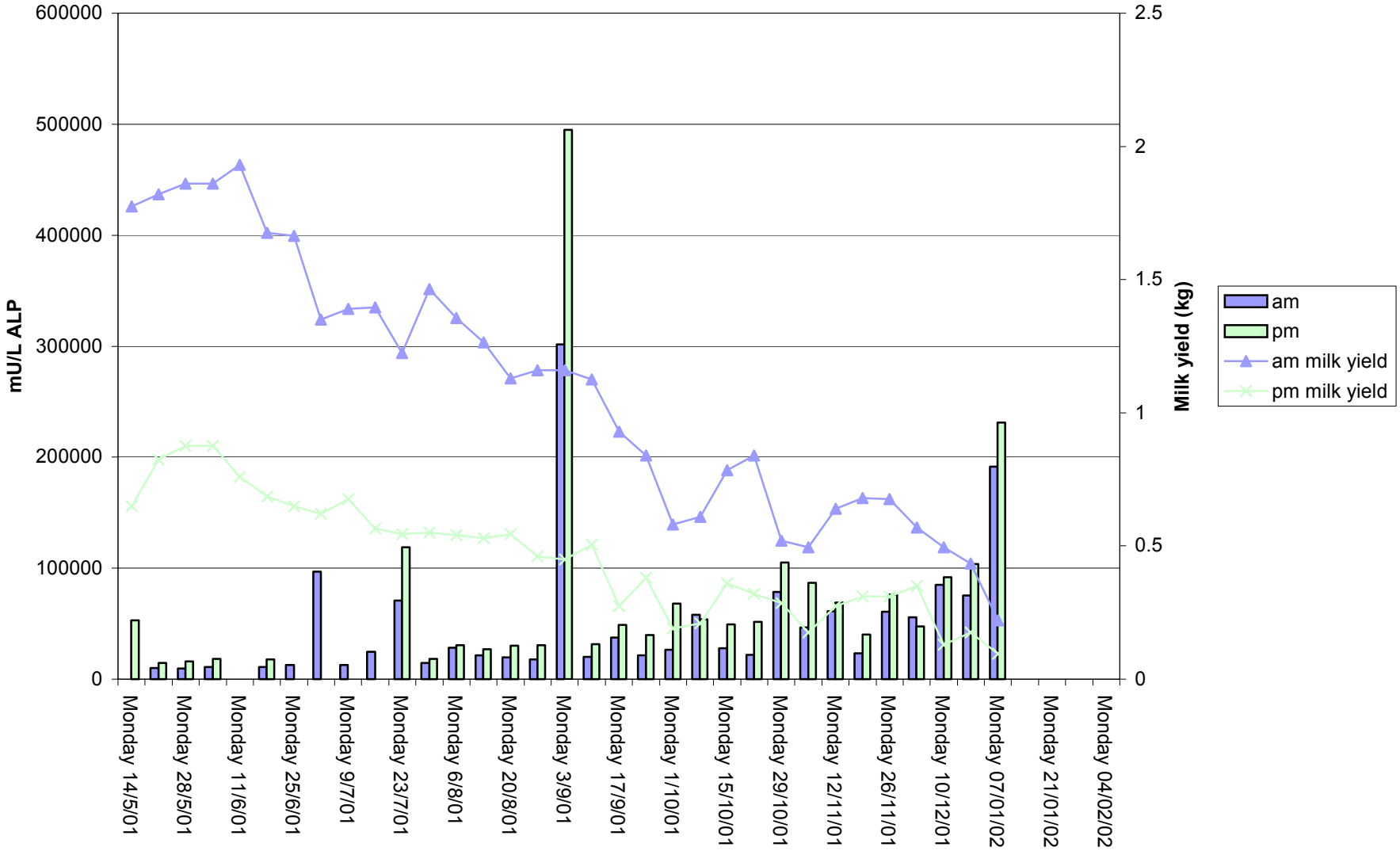


Figure 3 j) ALP in raw milk from goat 891

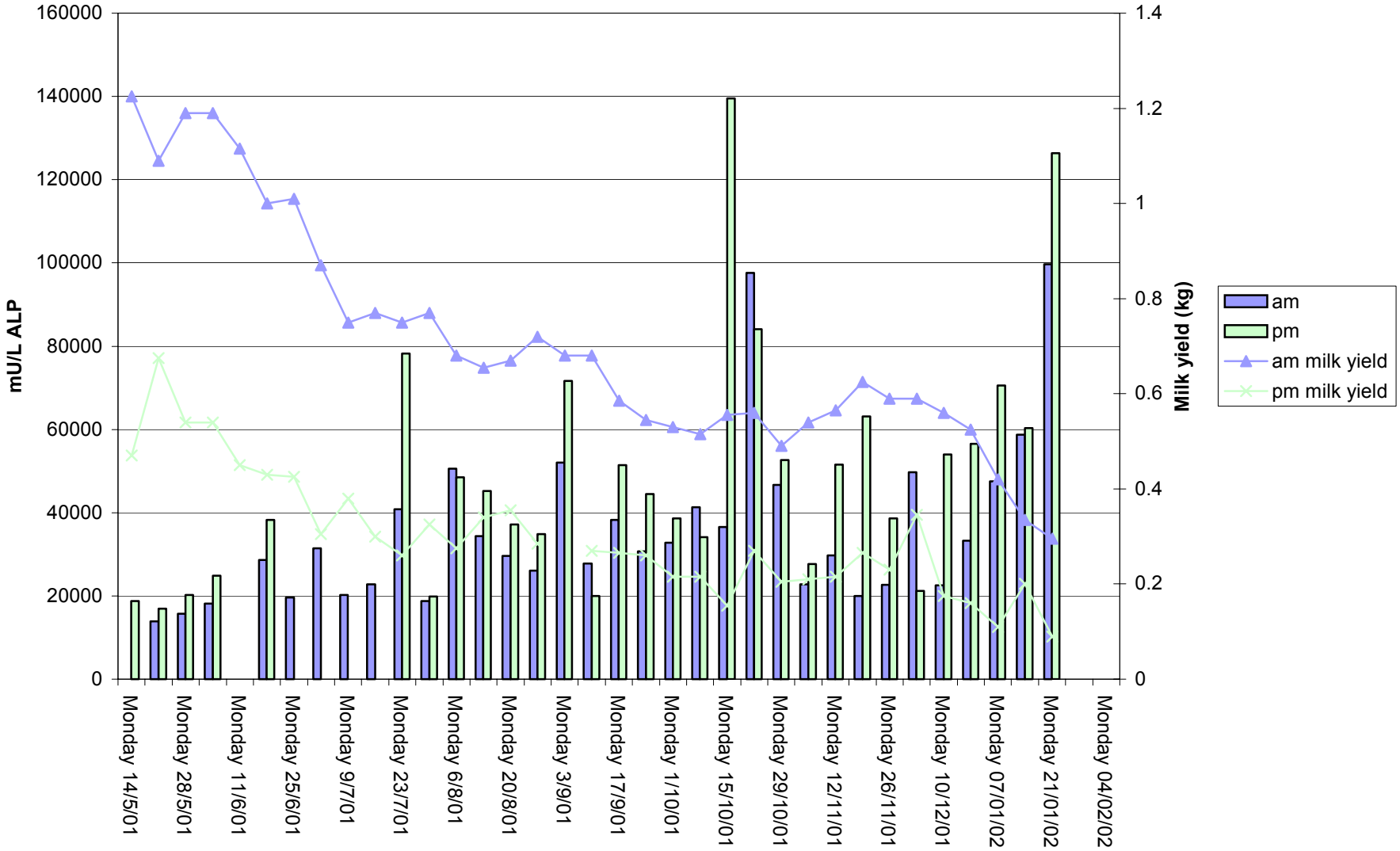


Figure 3 k) ALP in raw milk from goat 725

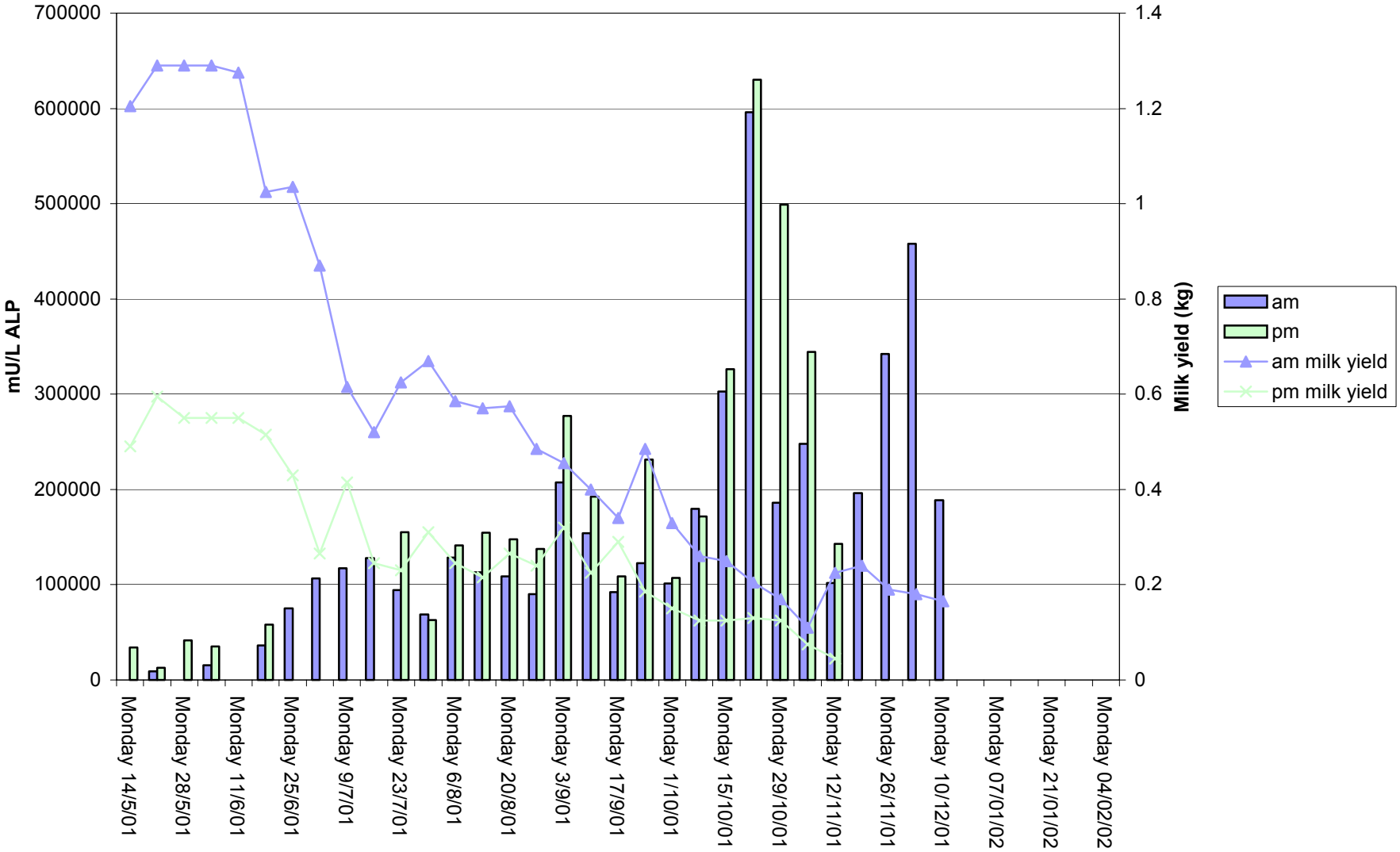


Figure 3 l) ALP in raw milk from goat 809

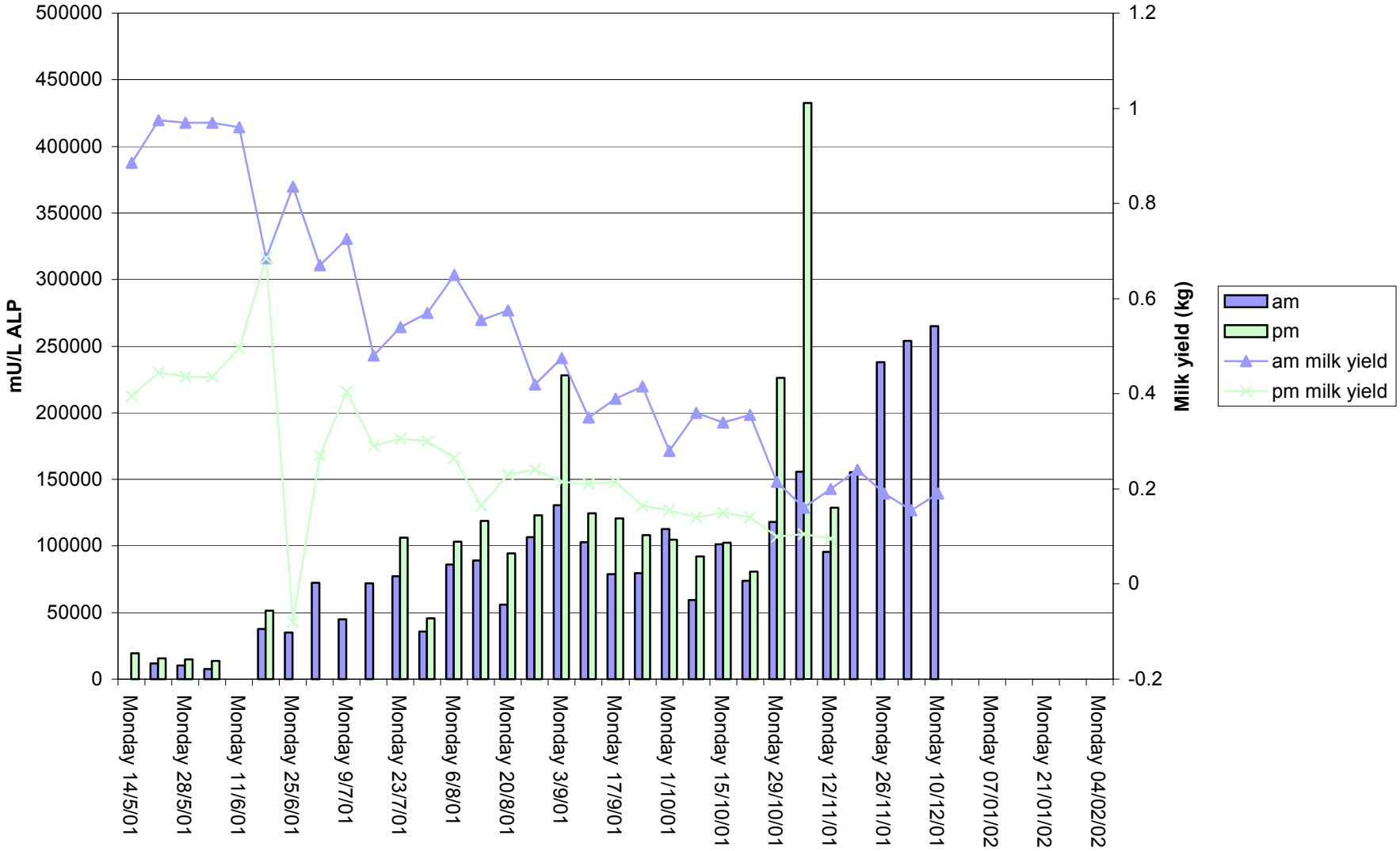


Figure 3 m) ALP in raw milk from goat 640

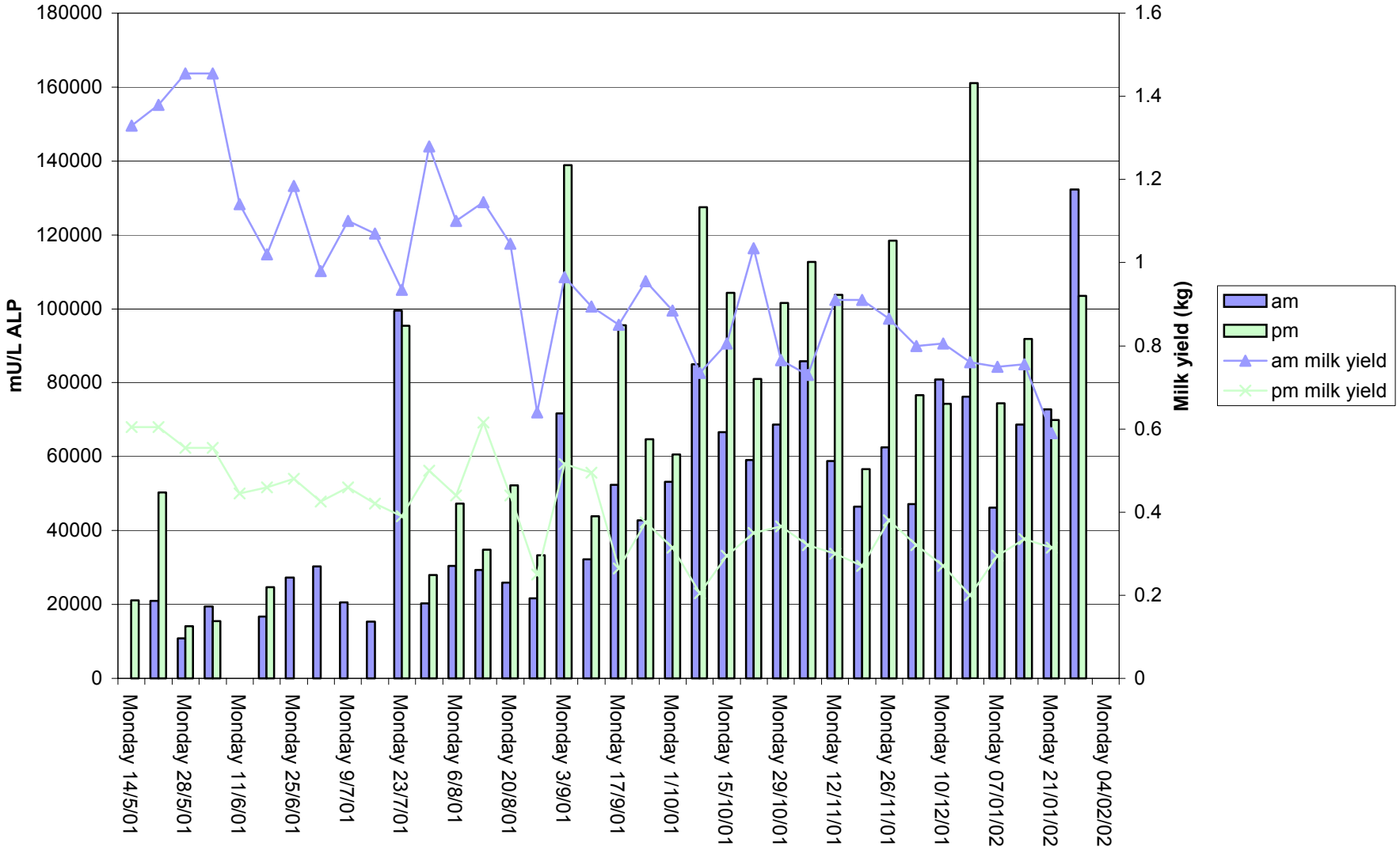


Figure 3 n) ALP in raw milk from goat 605

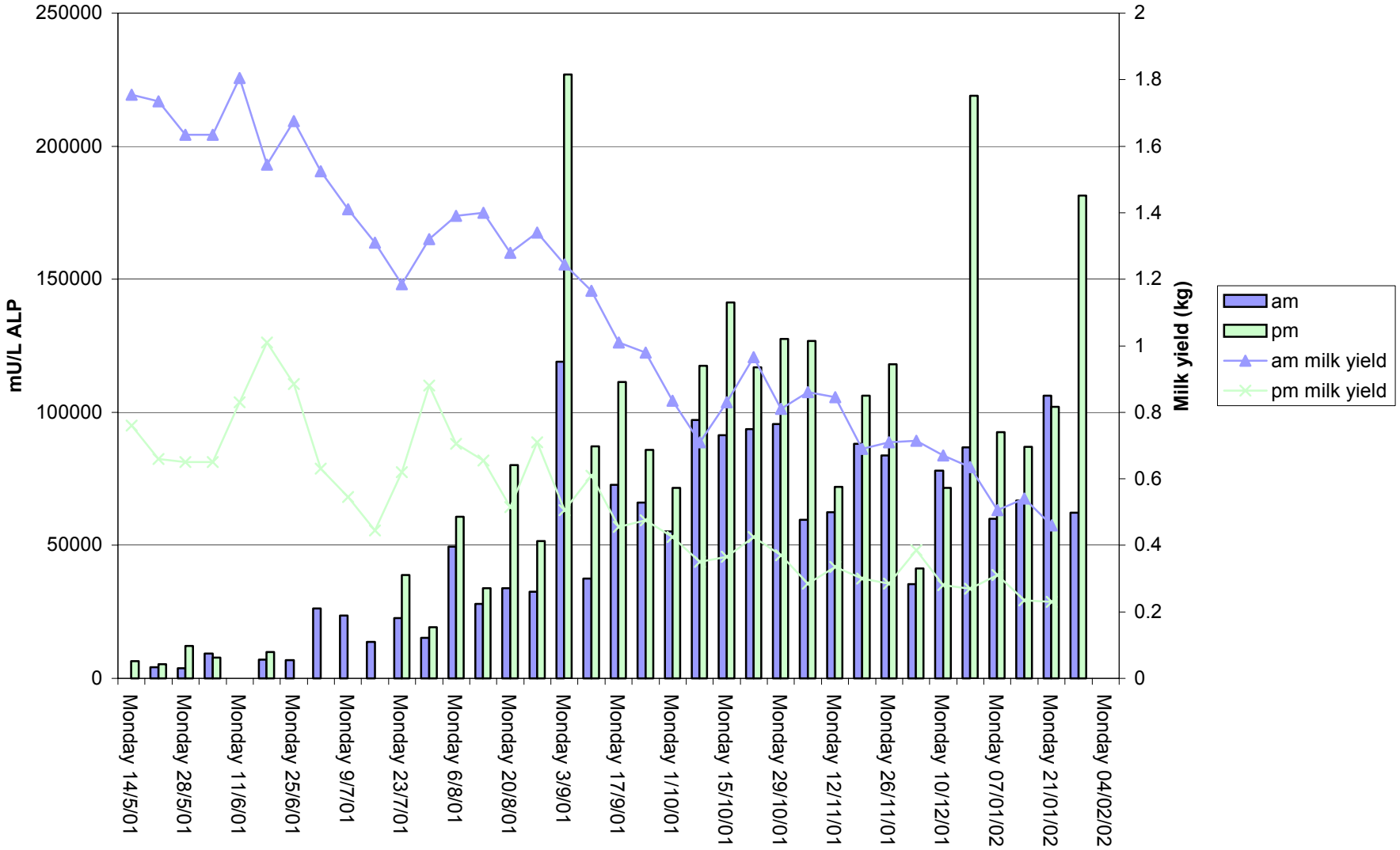


Figure 3 p) ALP in raw milk from goat 637

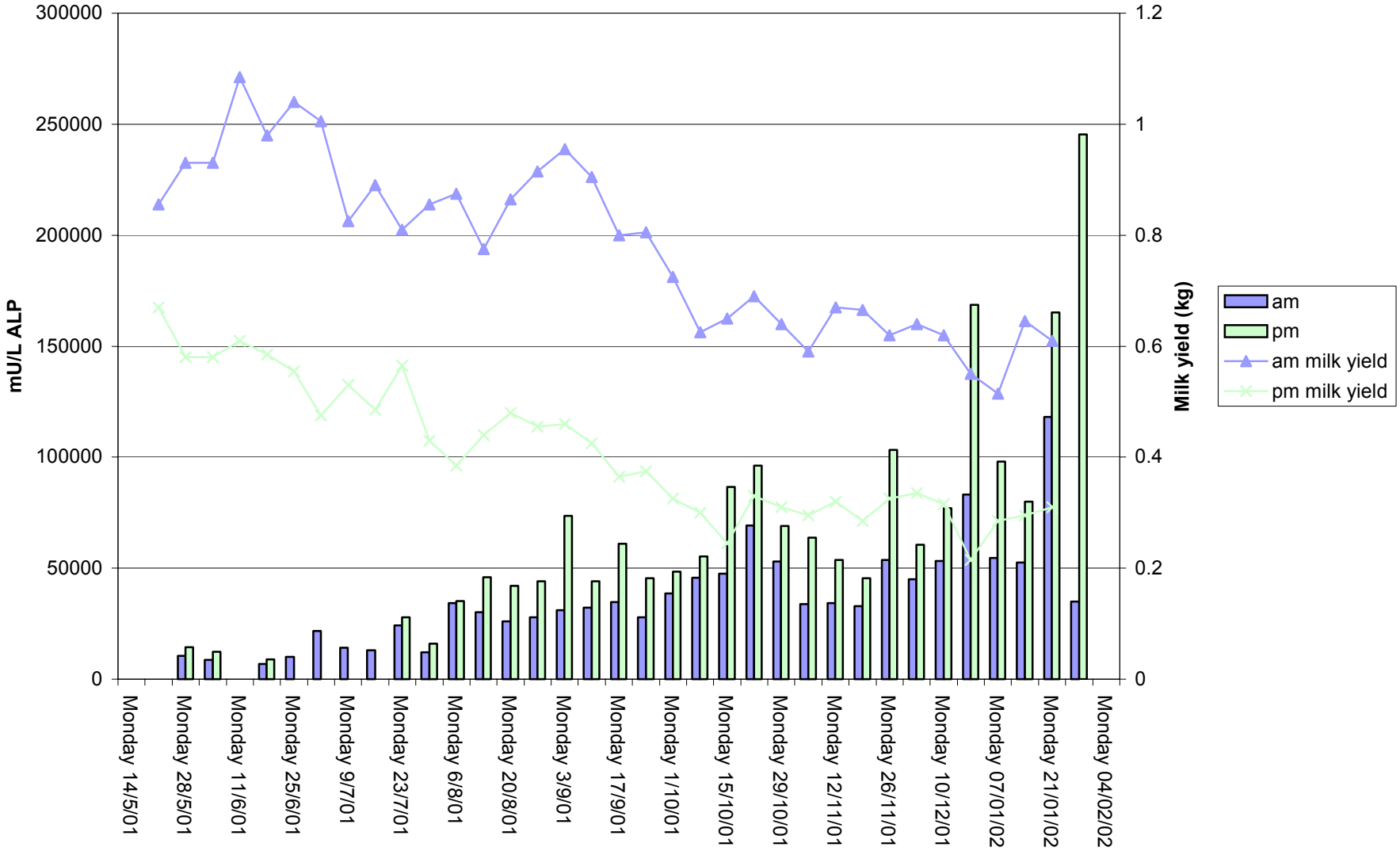


Figure 3 q) ALP in raw milk from goat 705

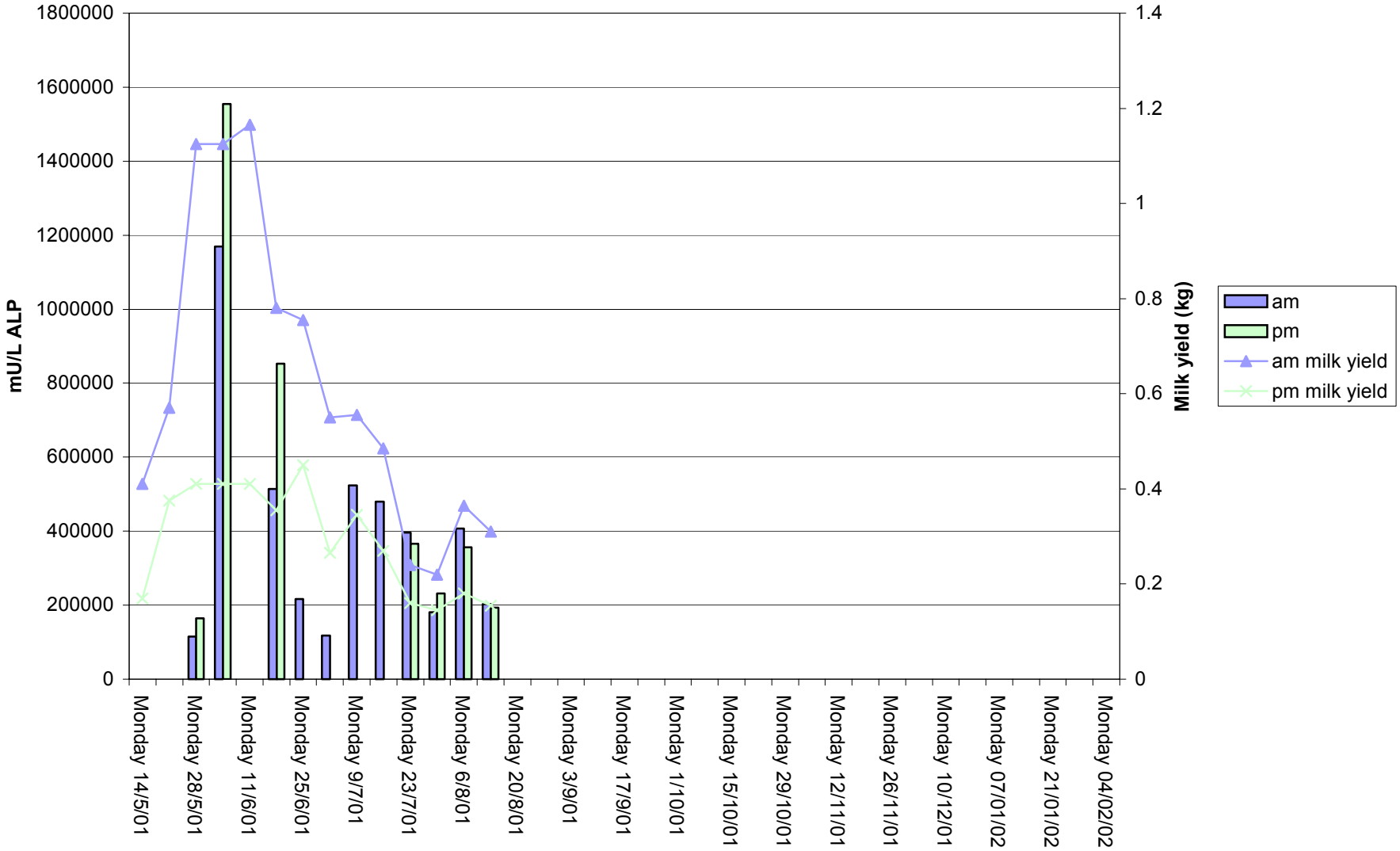


Figure 3 r) ALP in raw milk from goat 899

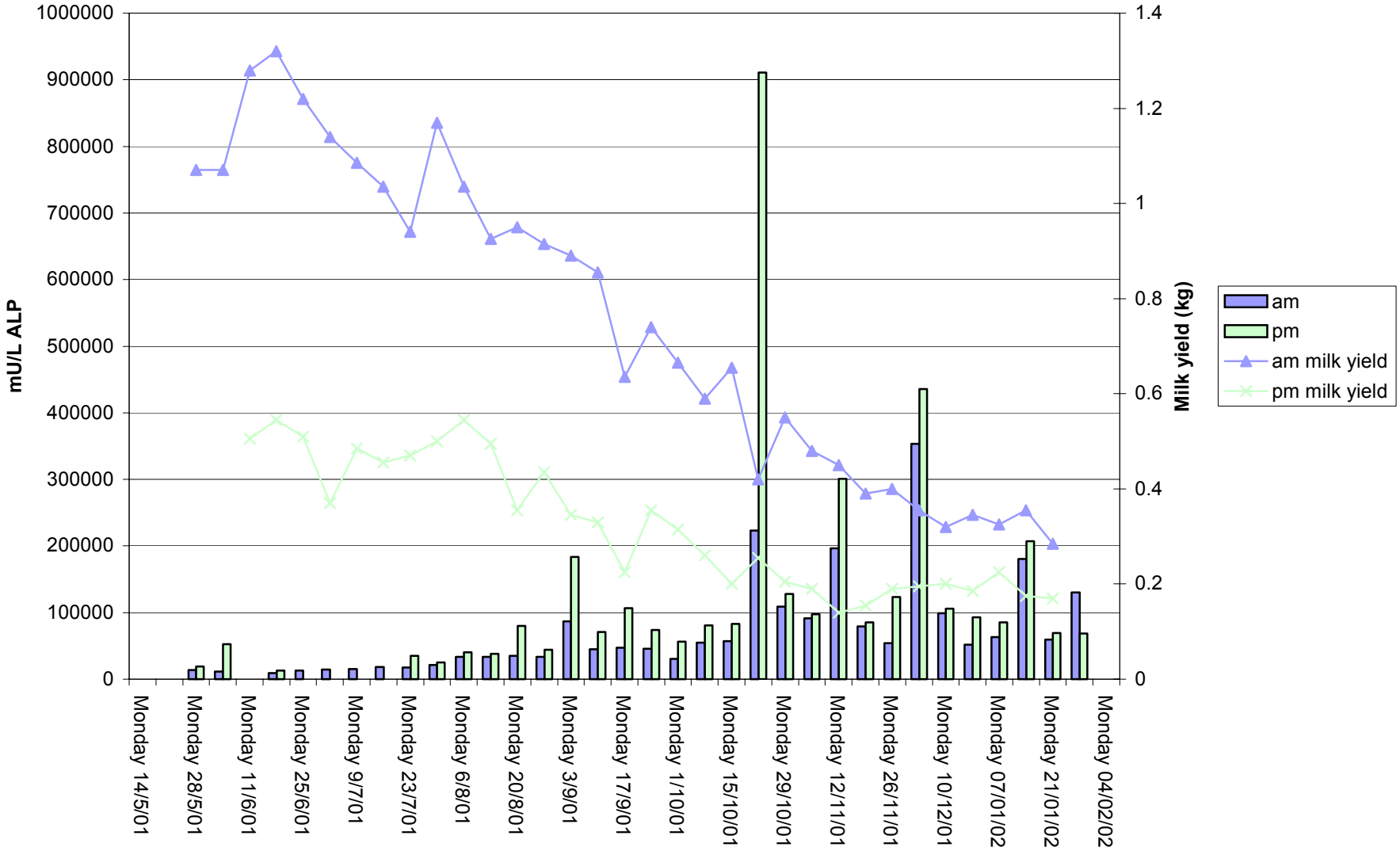


Fig 4 a)Total yield of ALP in morning and evening milk from 601

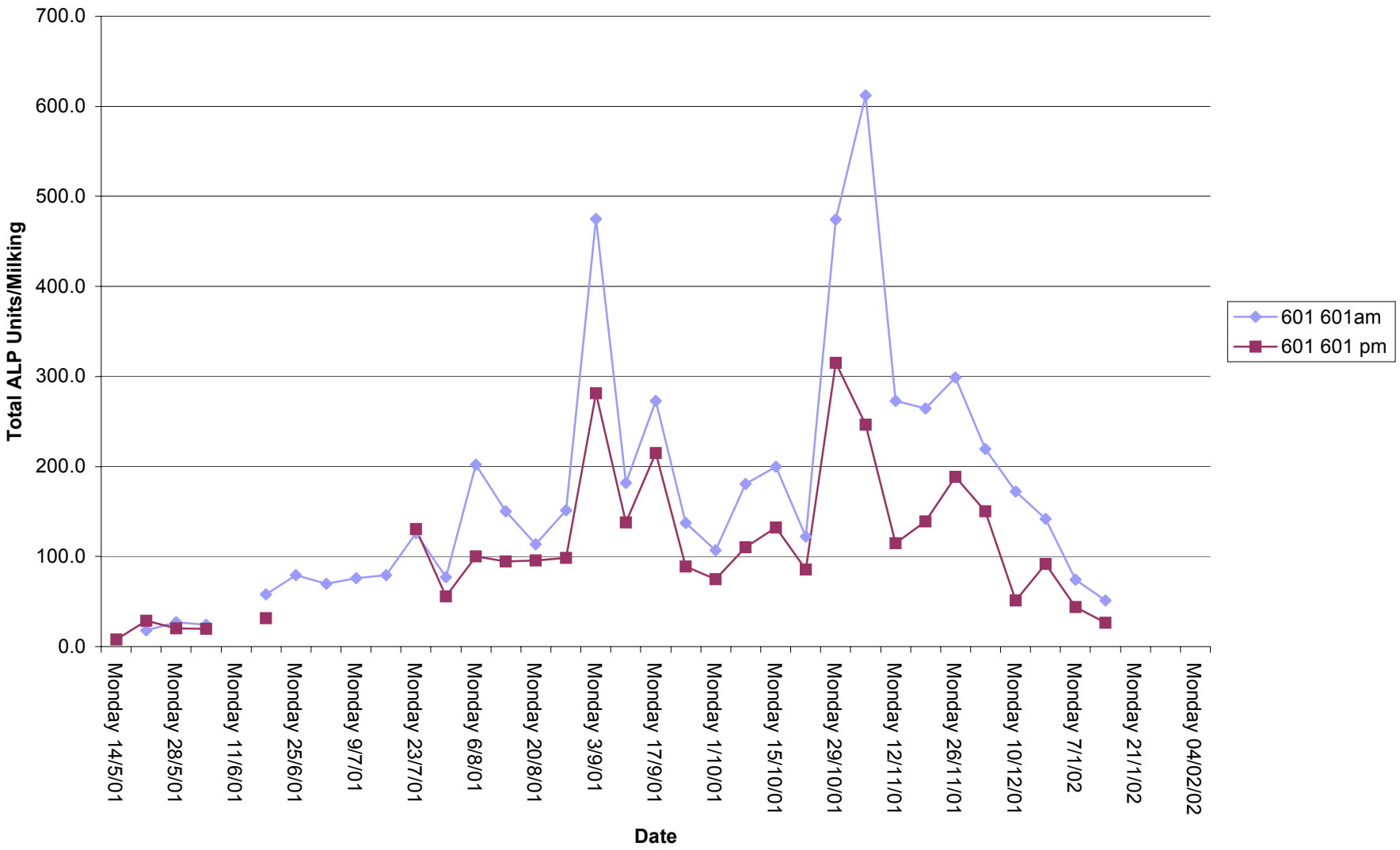


Fig 4 b)Total yield of ALP in morning and evening milk from 610

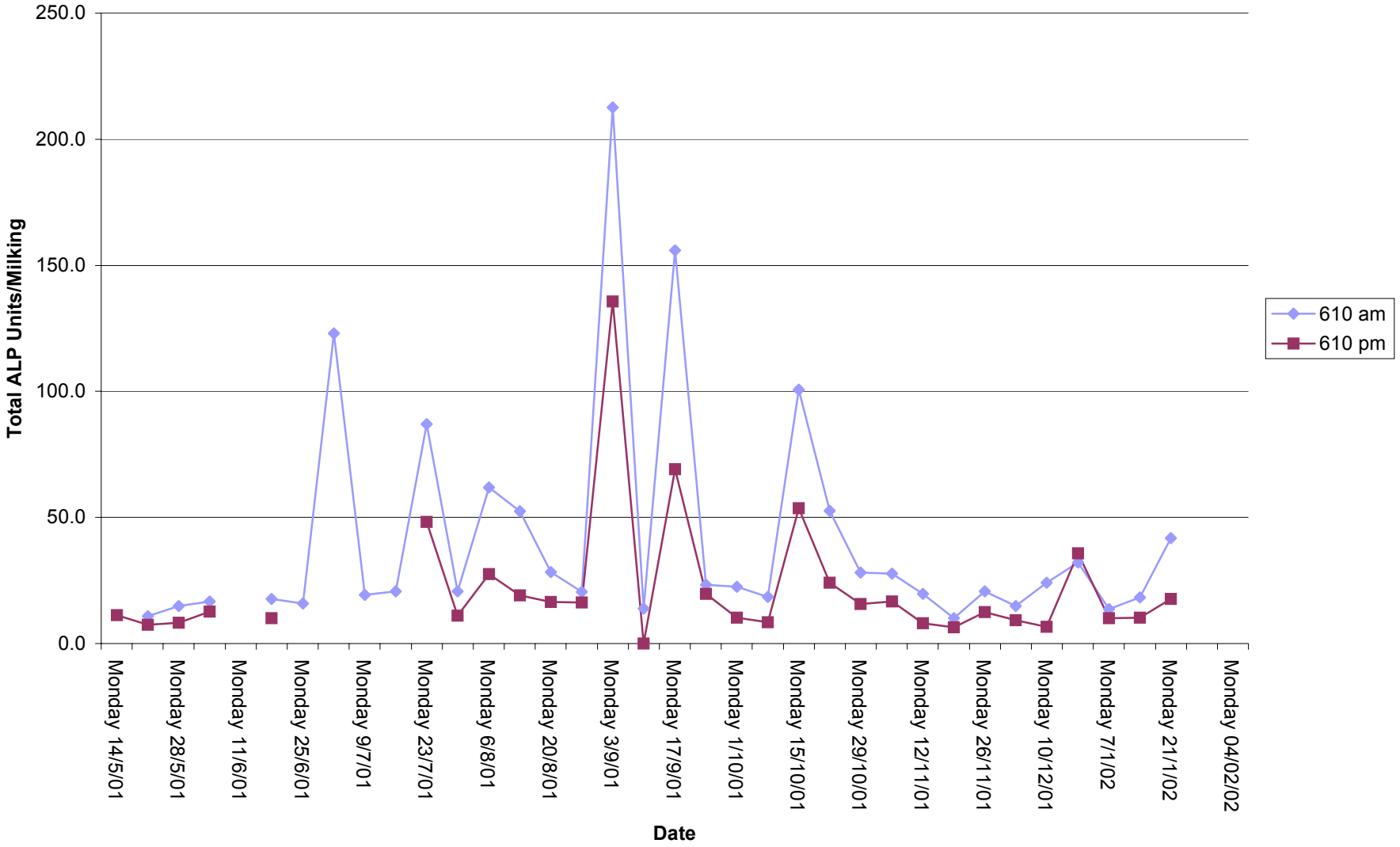


Fig 4 c)Total yield of ALP in morning and evening milk from 617

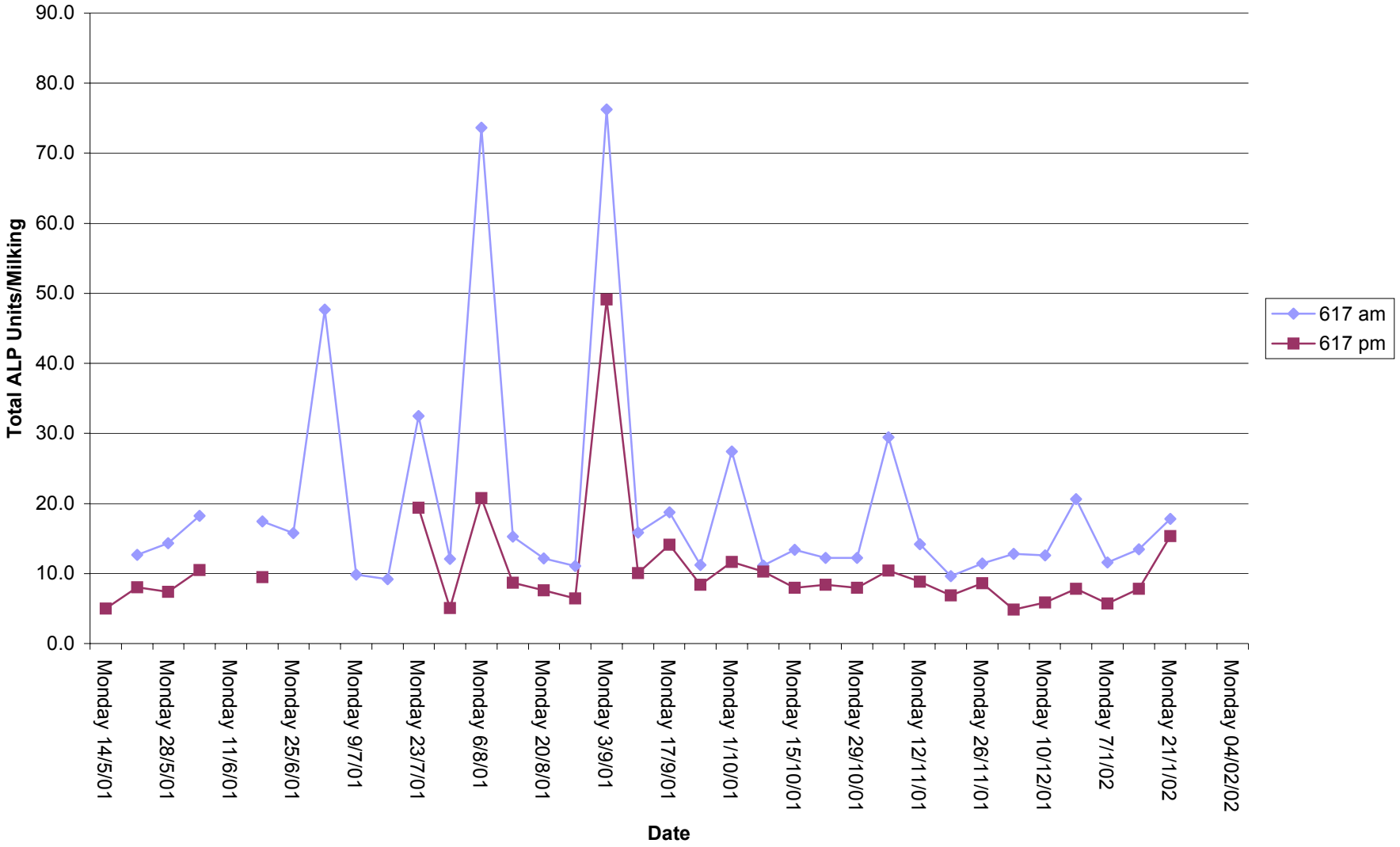


Fig 4 d)Total yield of ALP in morning and evening milk from 618

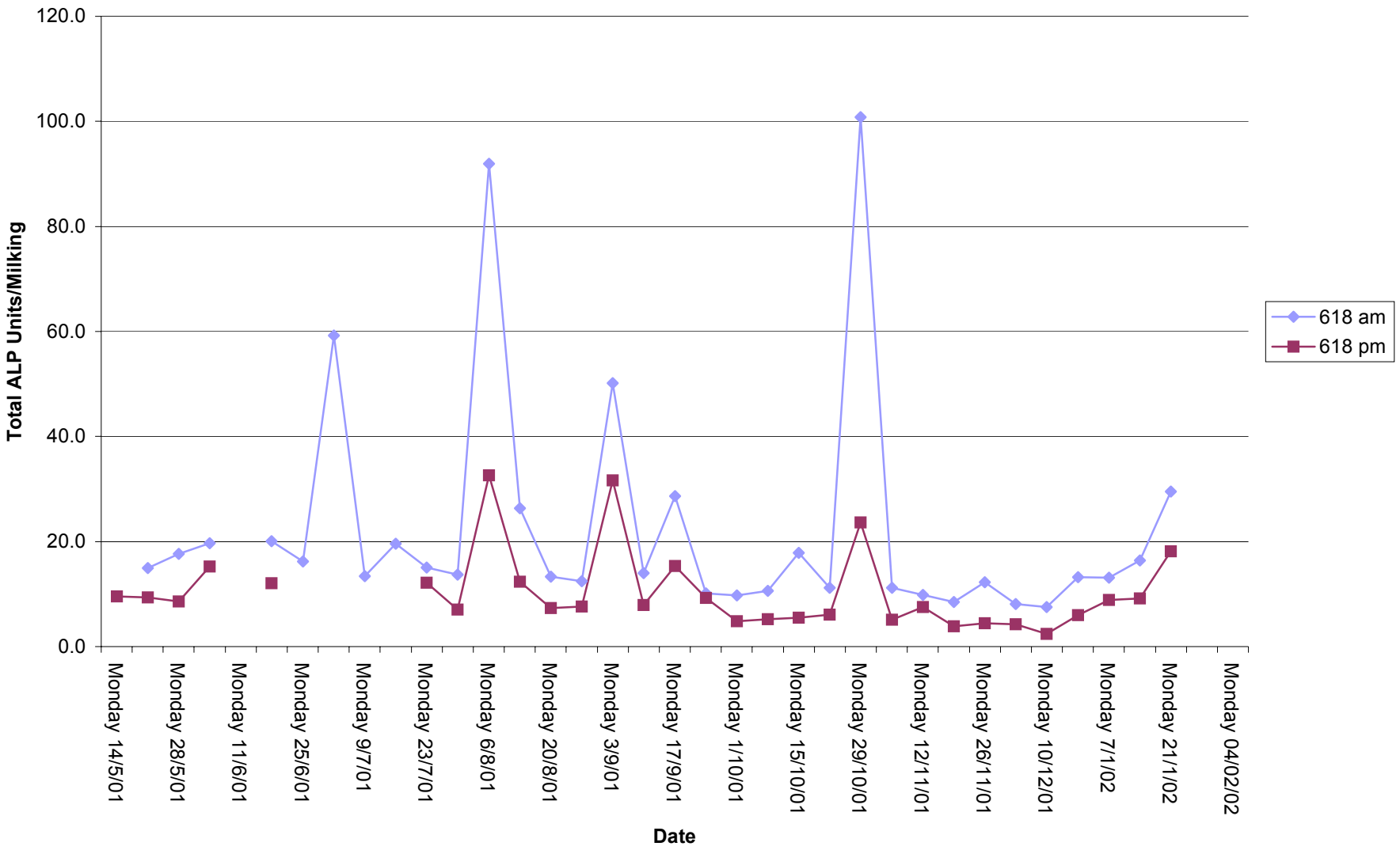


Fig 4 e)Total yield of ALP in morning and evening milk from 622

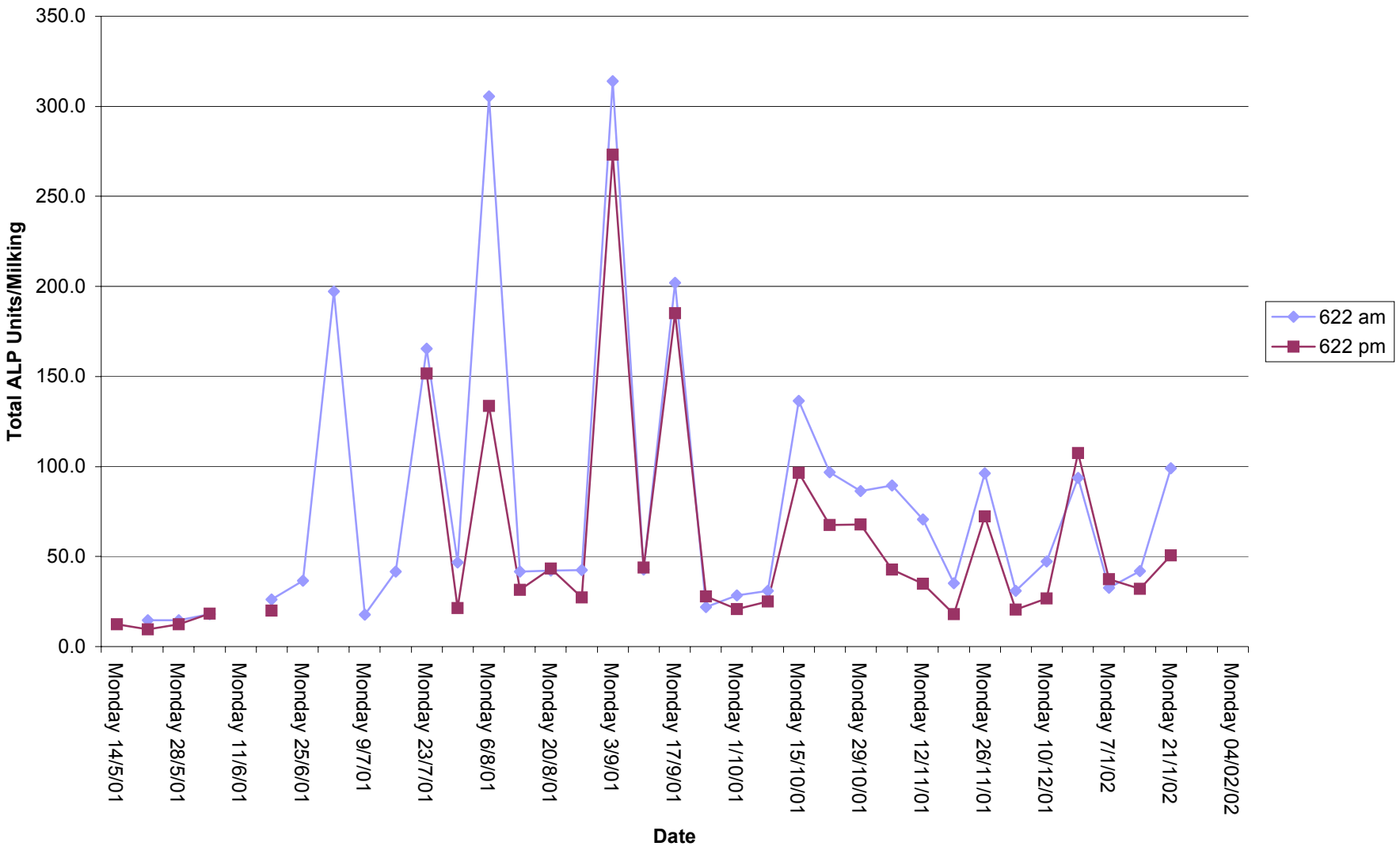


Fig 4 f)Total yield of ALP in morning and evening milk from 639

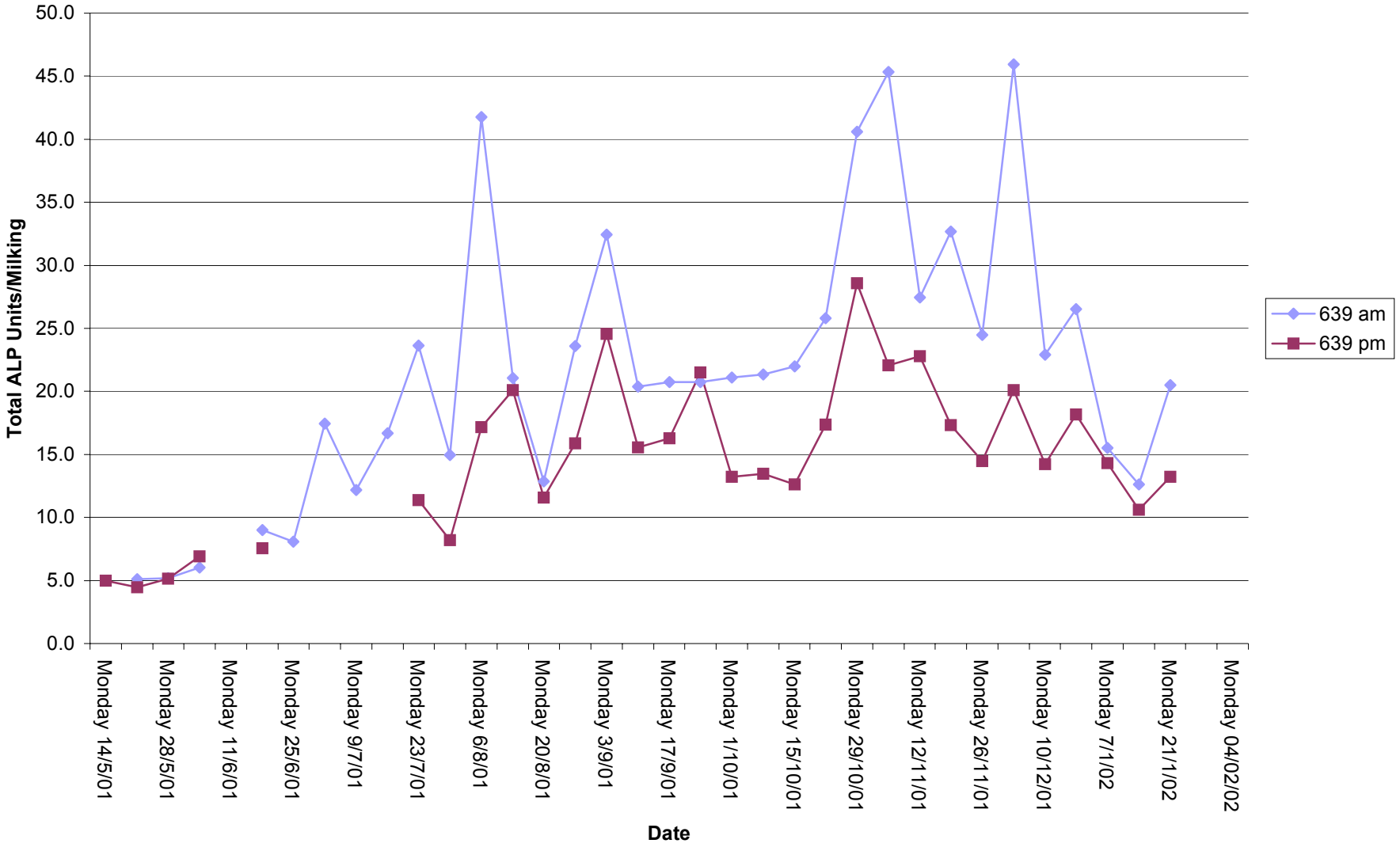


Fig 4 g)Total yield of ALP in morning and evening milk from 713

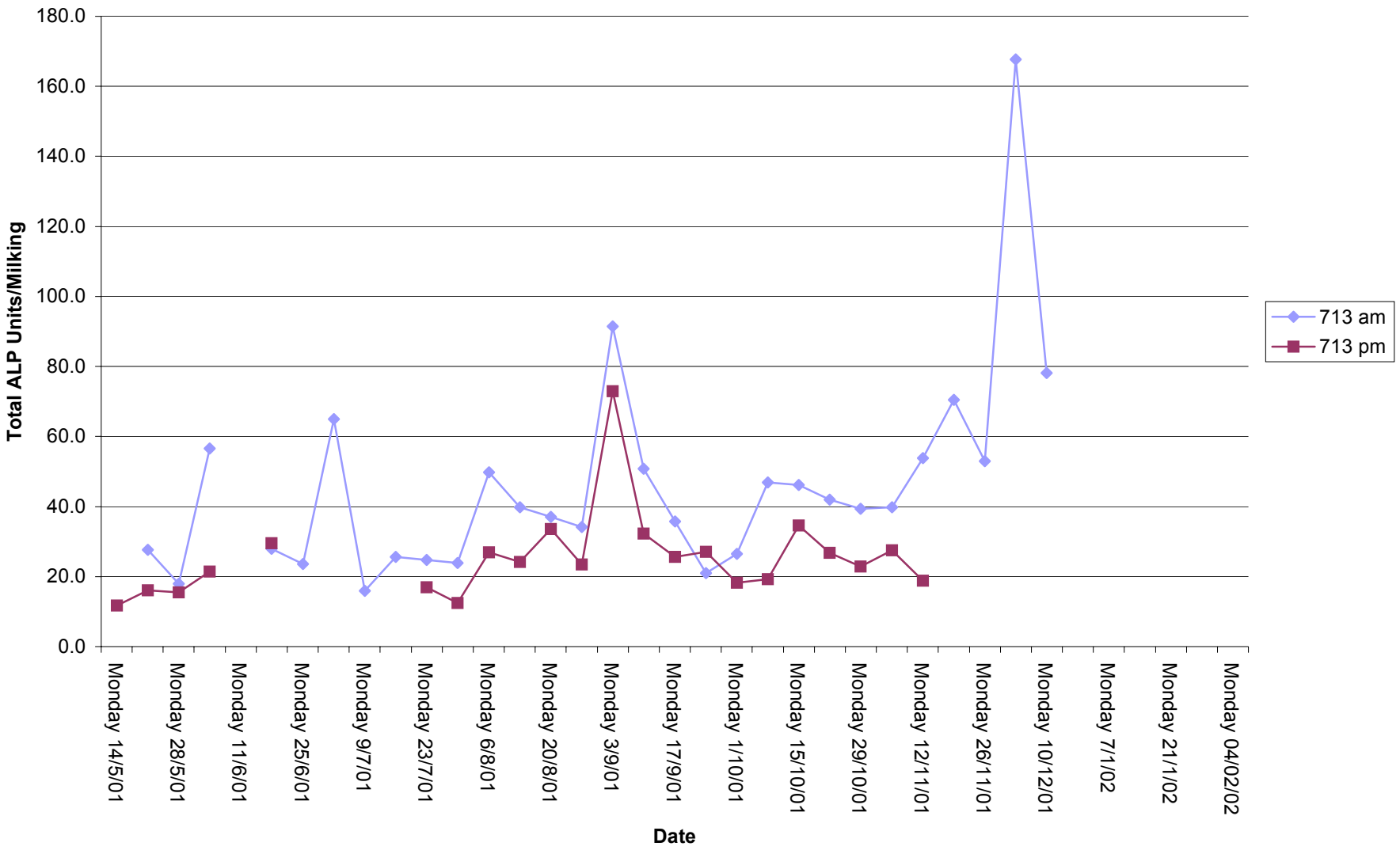


Fig 4 h)Total yield of ALP in morning and evening milk from 806

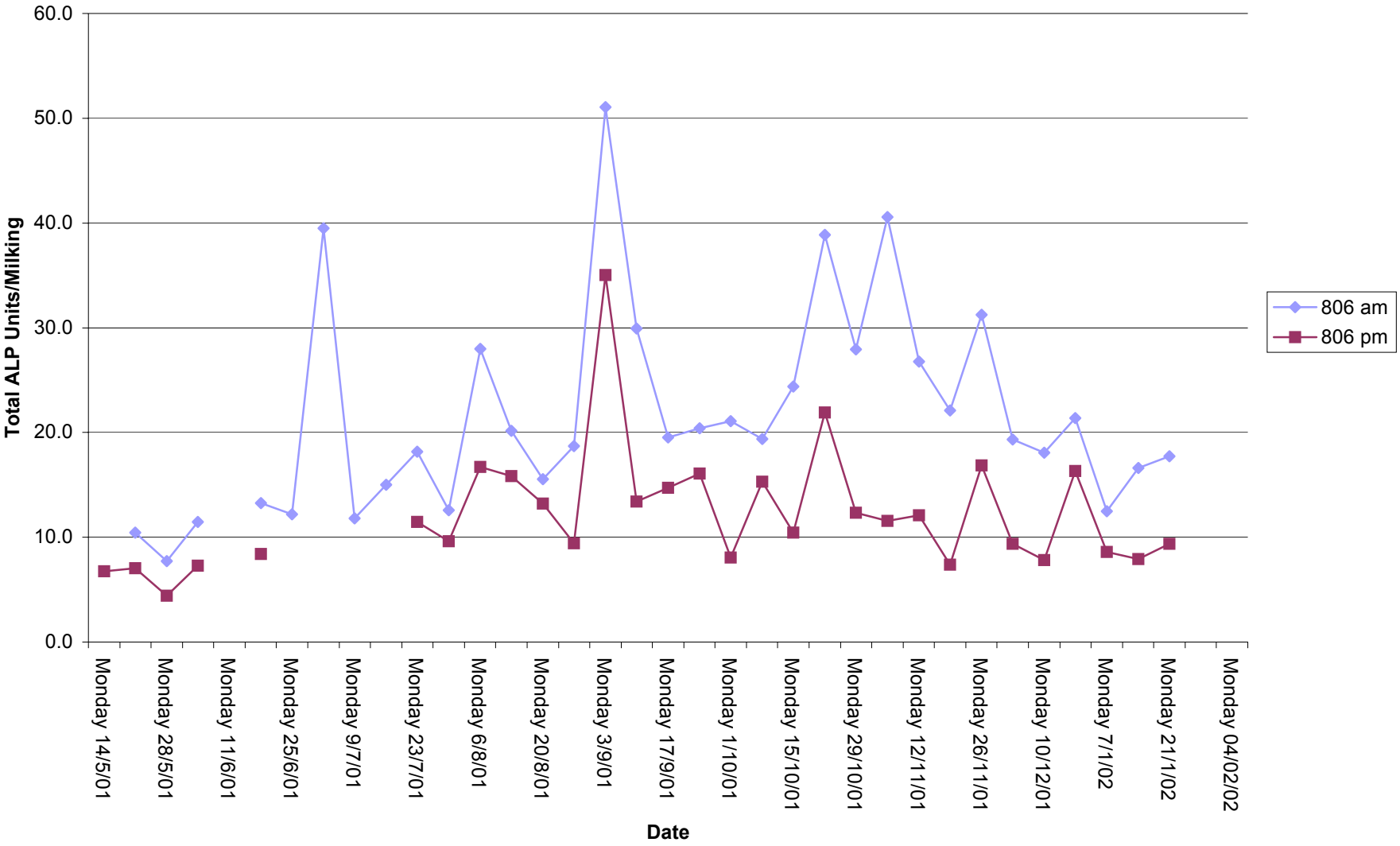


Fig 4 i)Total yield of ALP in morning and evening milk from 890

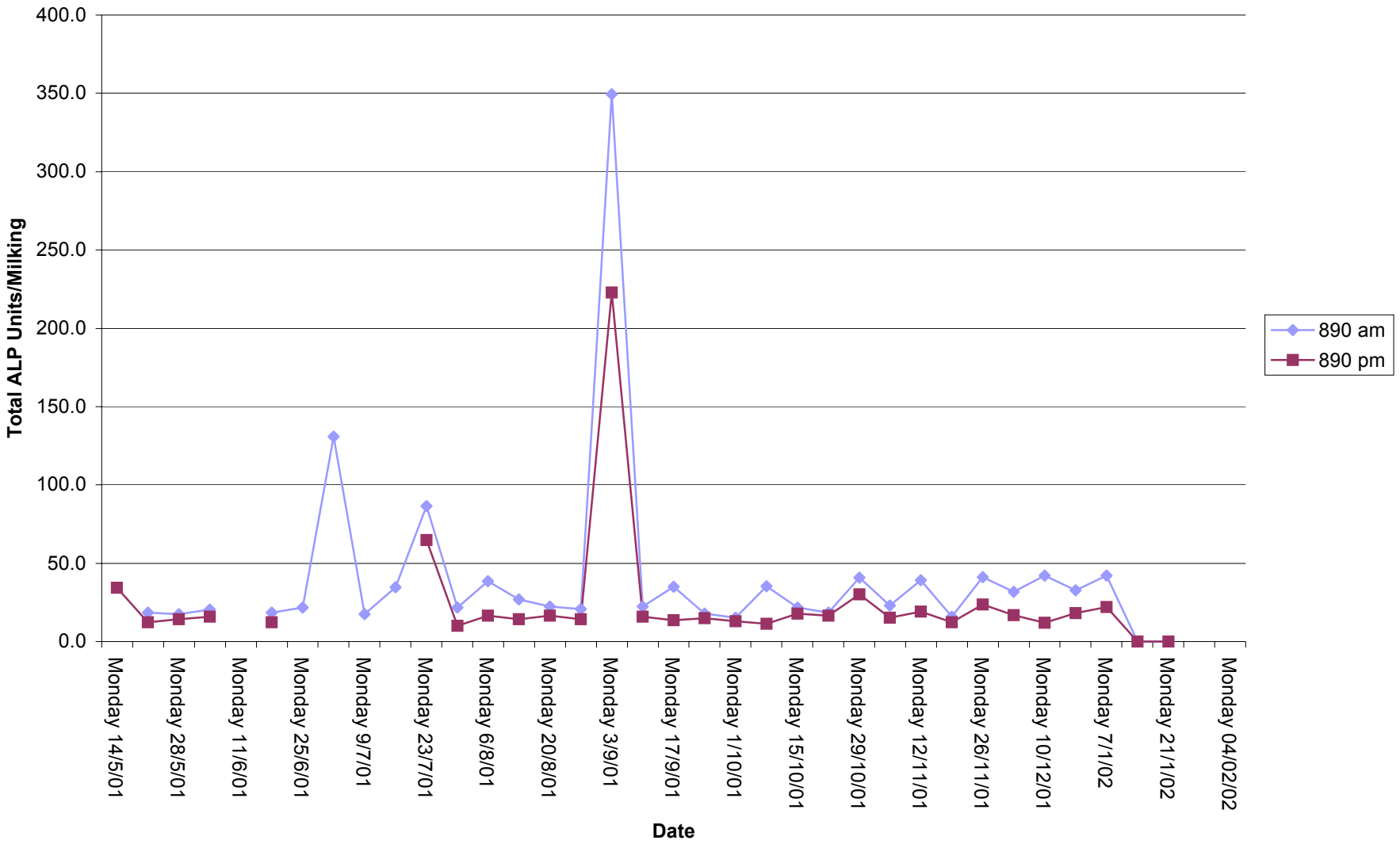


Fig 4 j)Total yield of ALP in morning and evening milk from 891

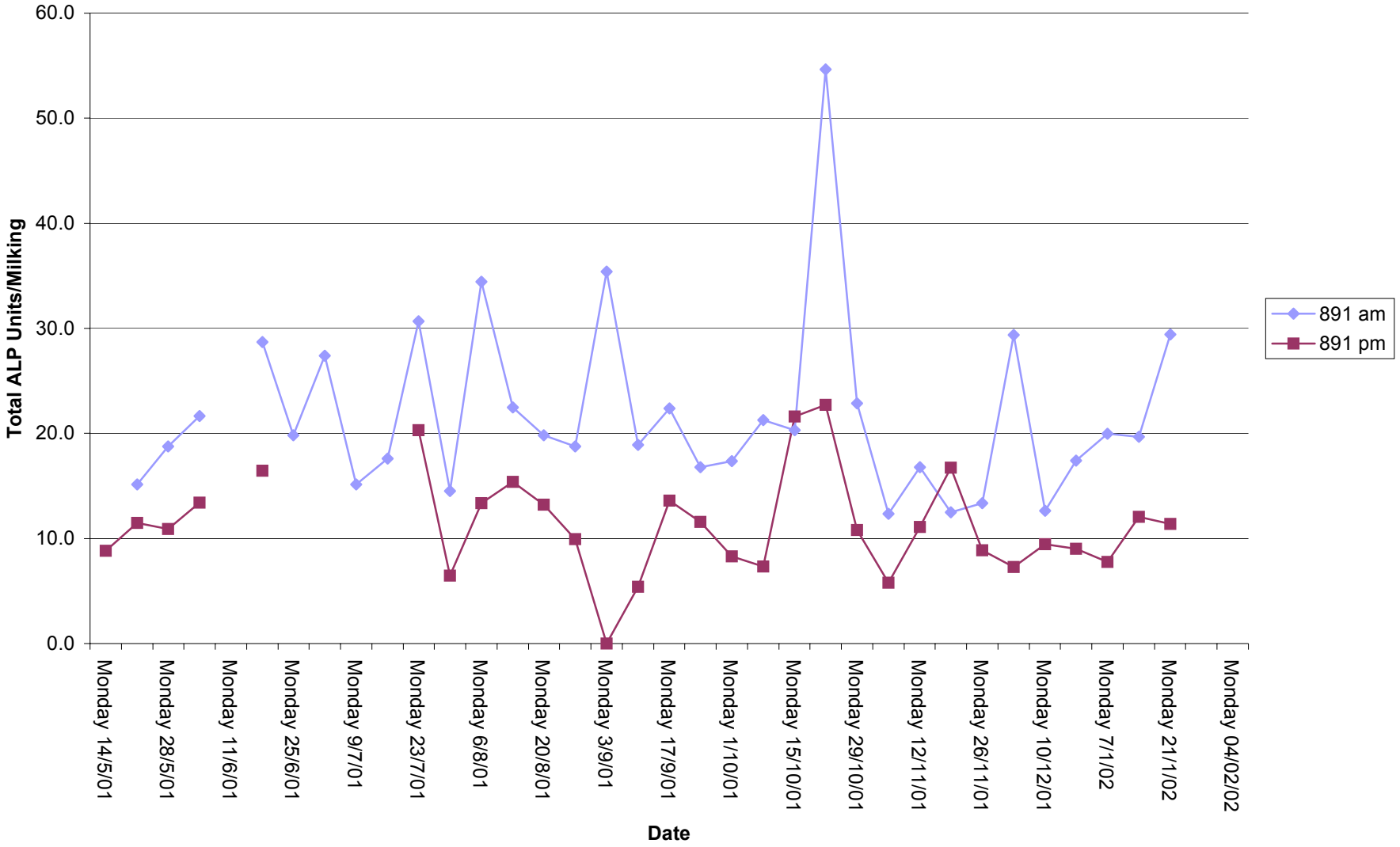


Fig 4 k)Total yield of ALP in morning and evening milk from 725

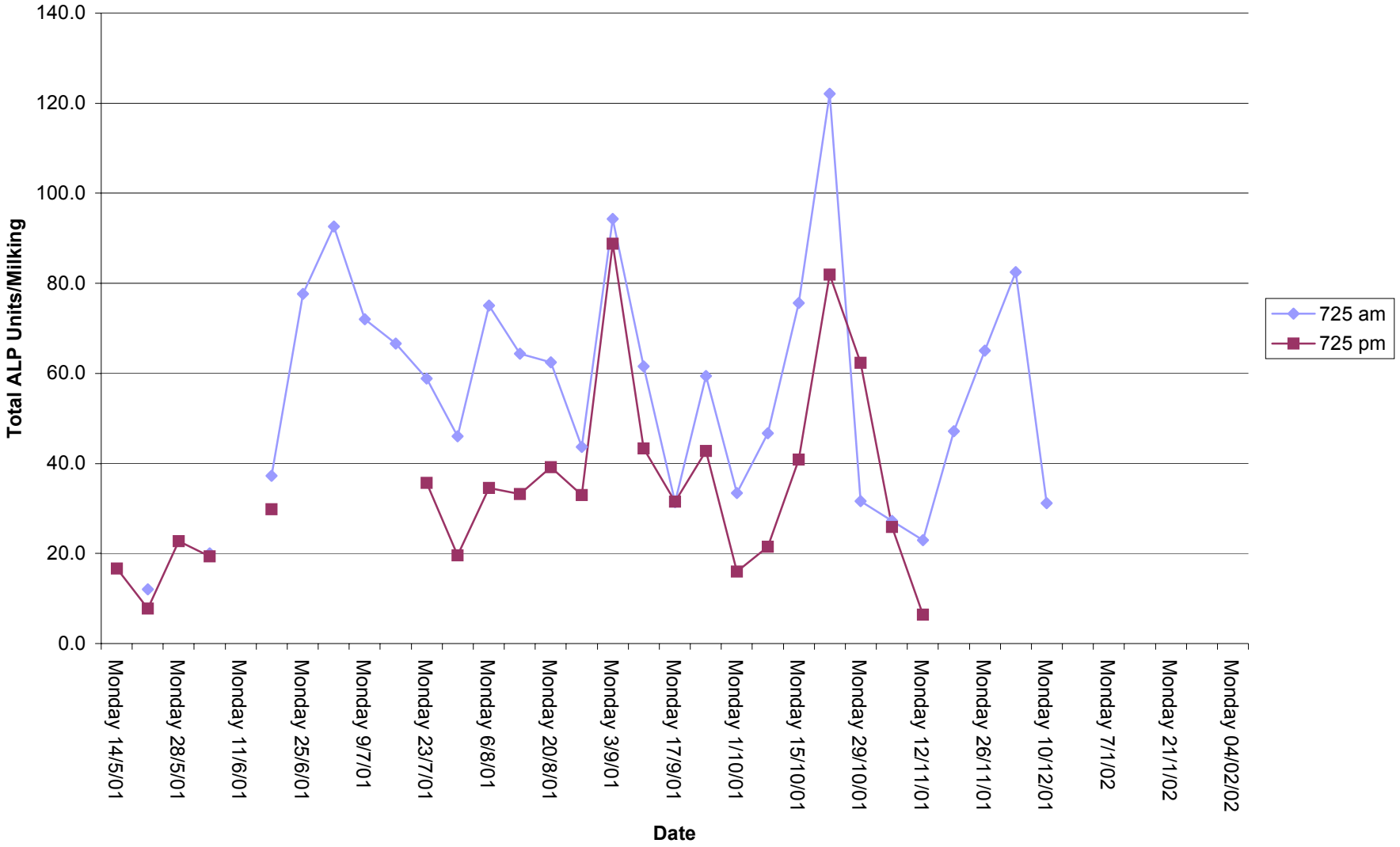


Fig 4 l)Total yield of ALP in morning and evening milk from 809

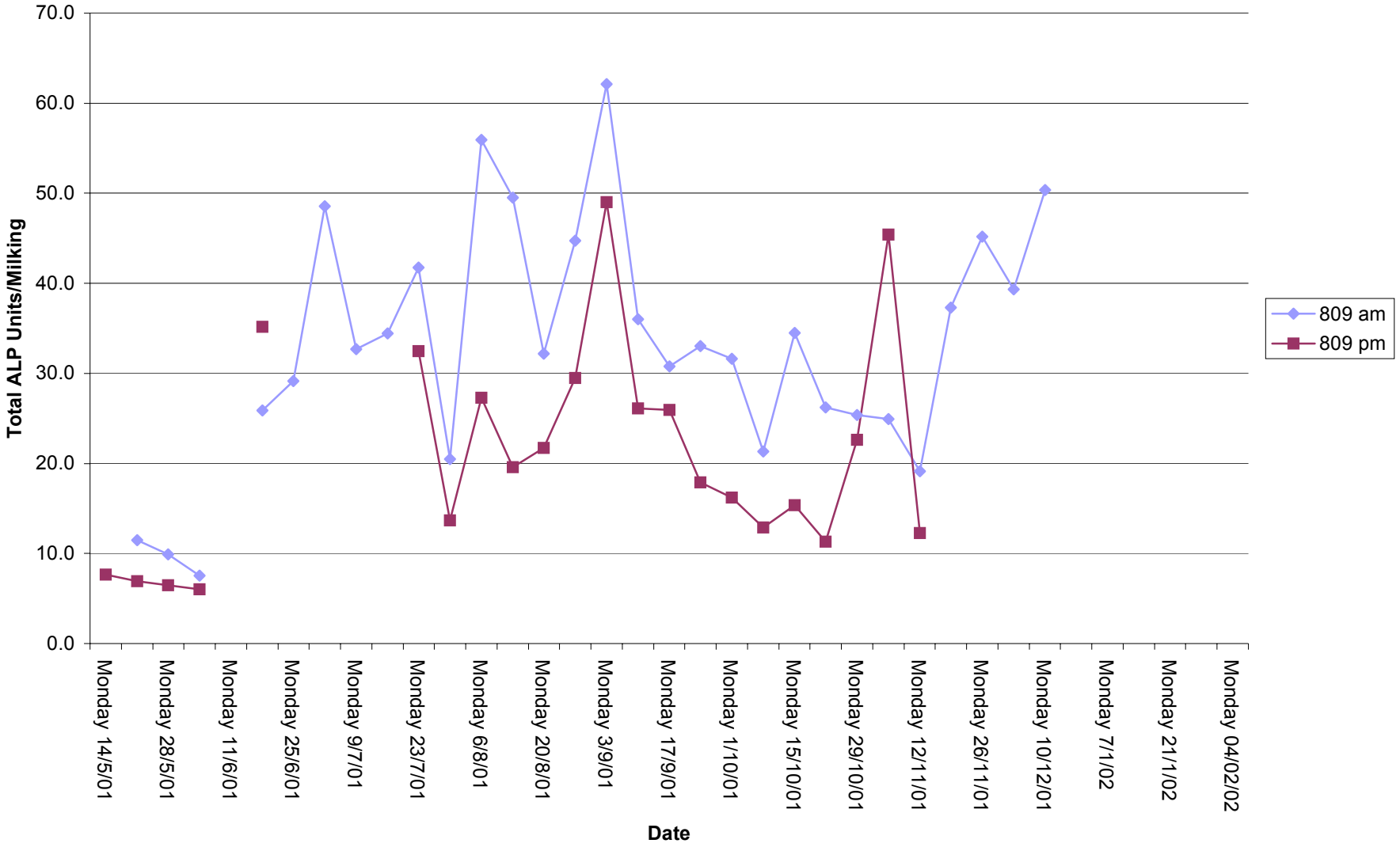


Fig 4 m)Total yield of ALP in morning and evening milk from 640

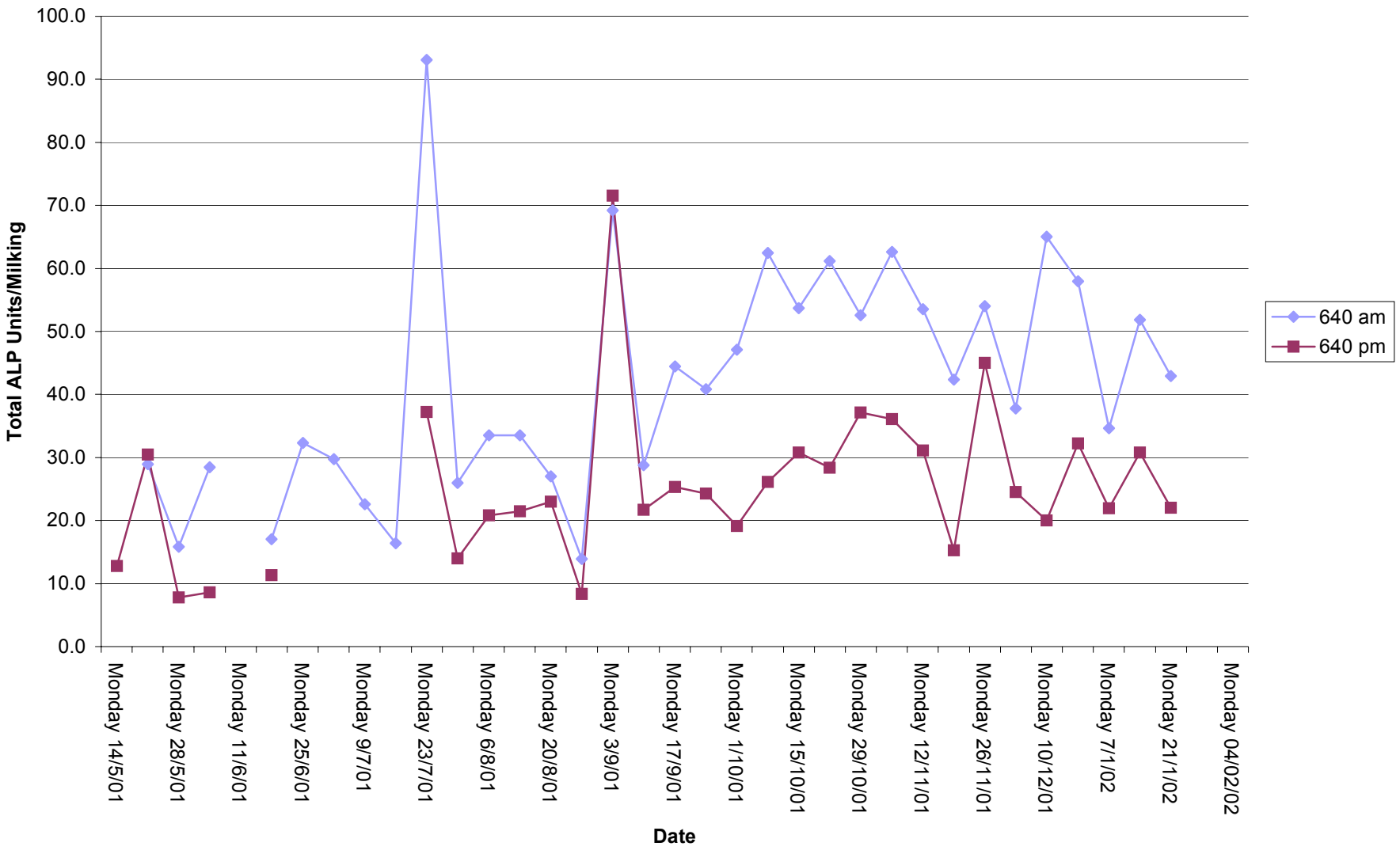


Fig 4 n)Total yield of ALP in morning and evening milk from 605

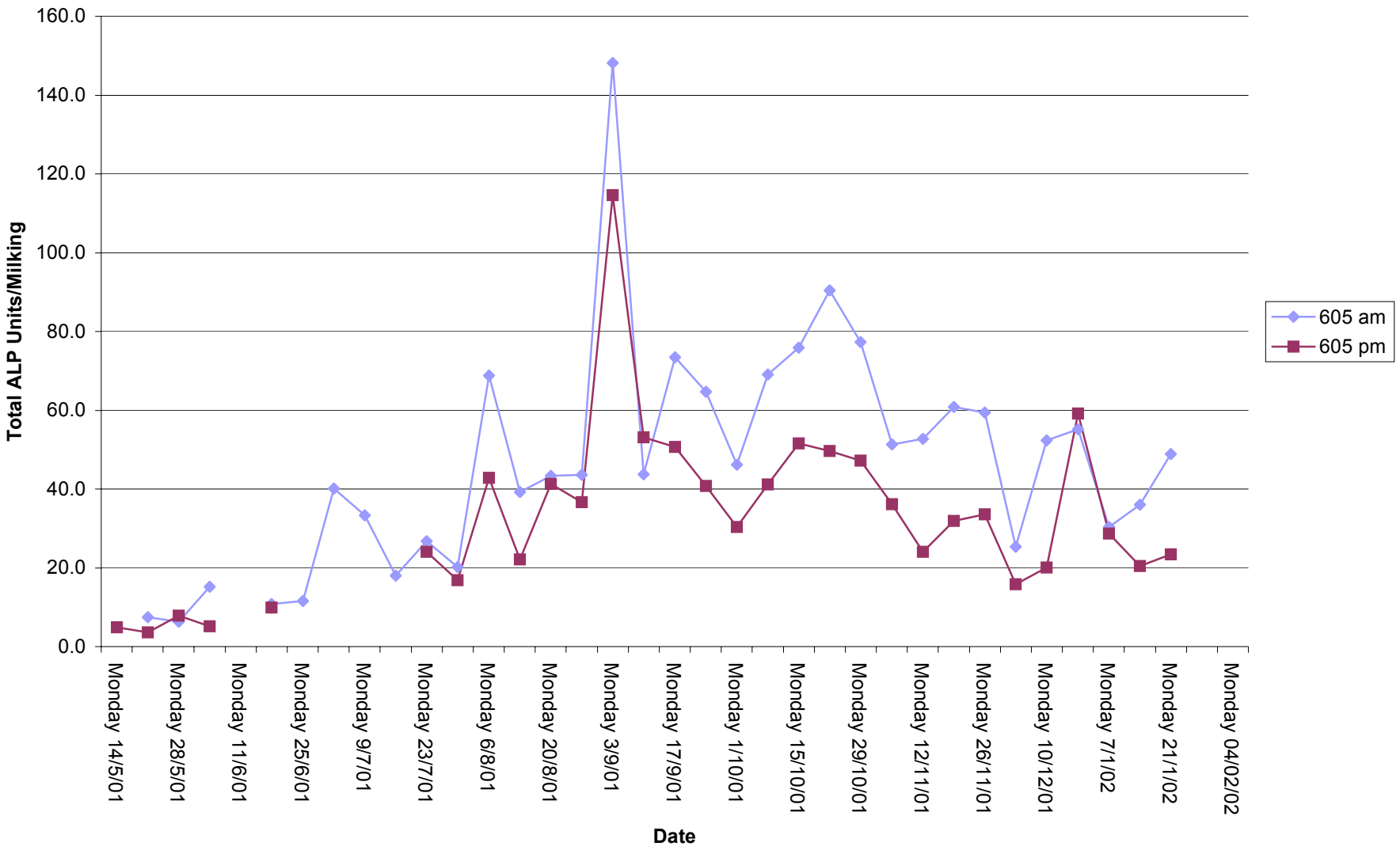


Fig 4 n)Total yield of ALP in morning and evening milk from 605

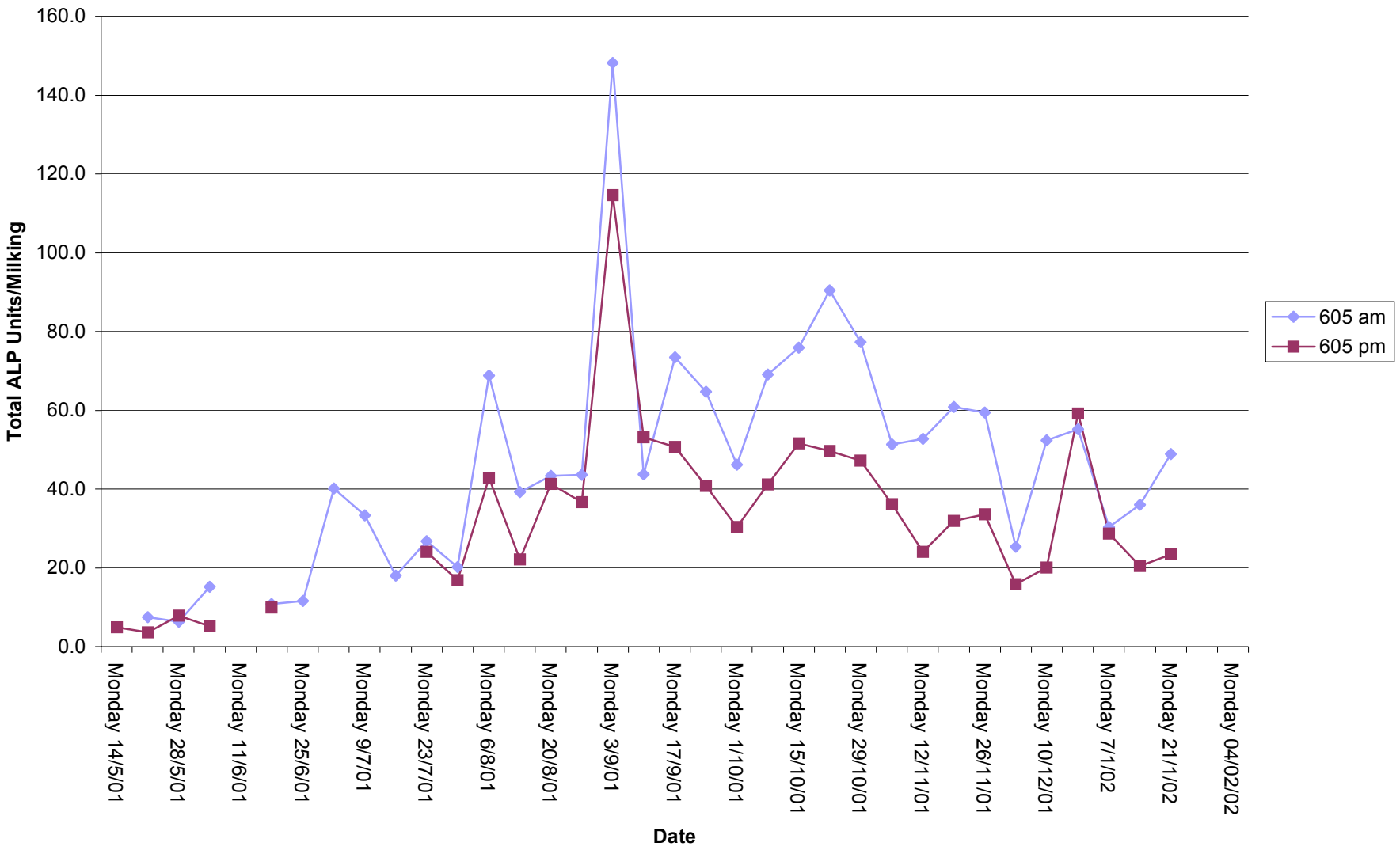


Fig 4 p)Total yield of ALP in morning and evening milk from 637

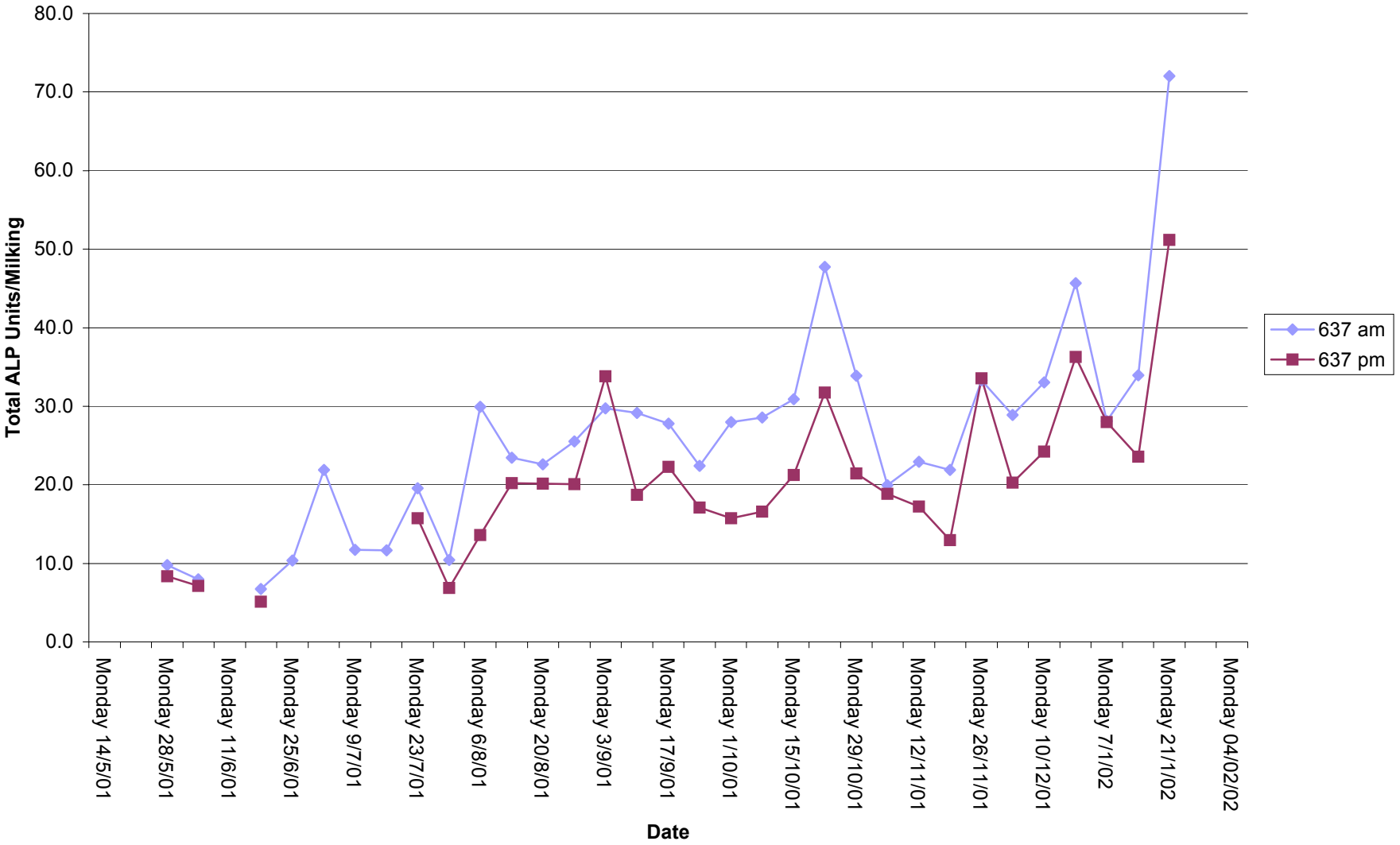


Fig 4 q)Total yield of ALP in morning and evening milk from 705

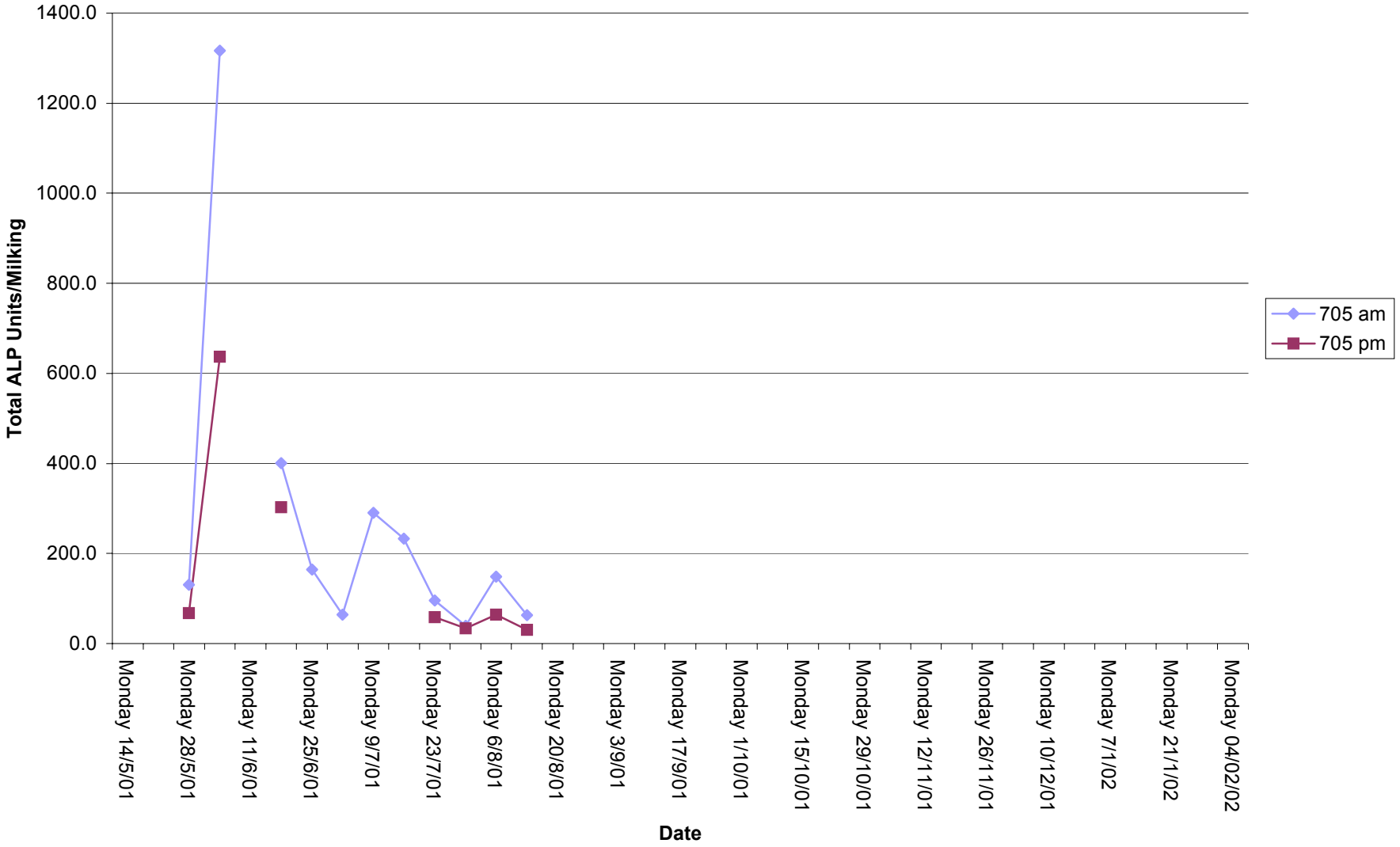


Fig 4 r)Total yield of ALP in morning and evening milk from 899

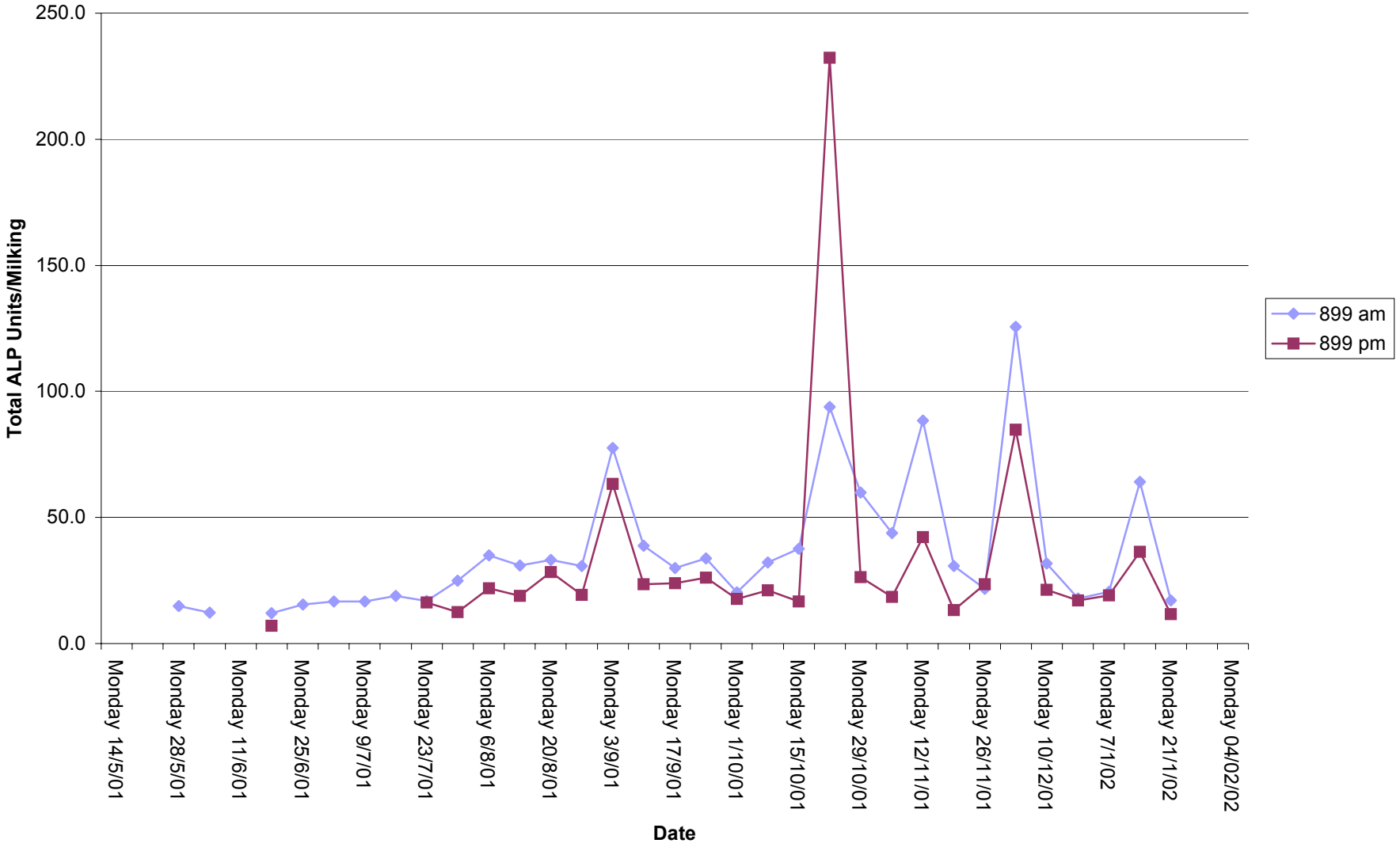


Fig 5a) Milk Composition Goat 601

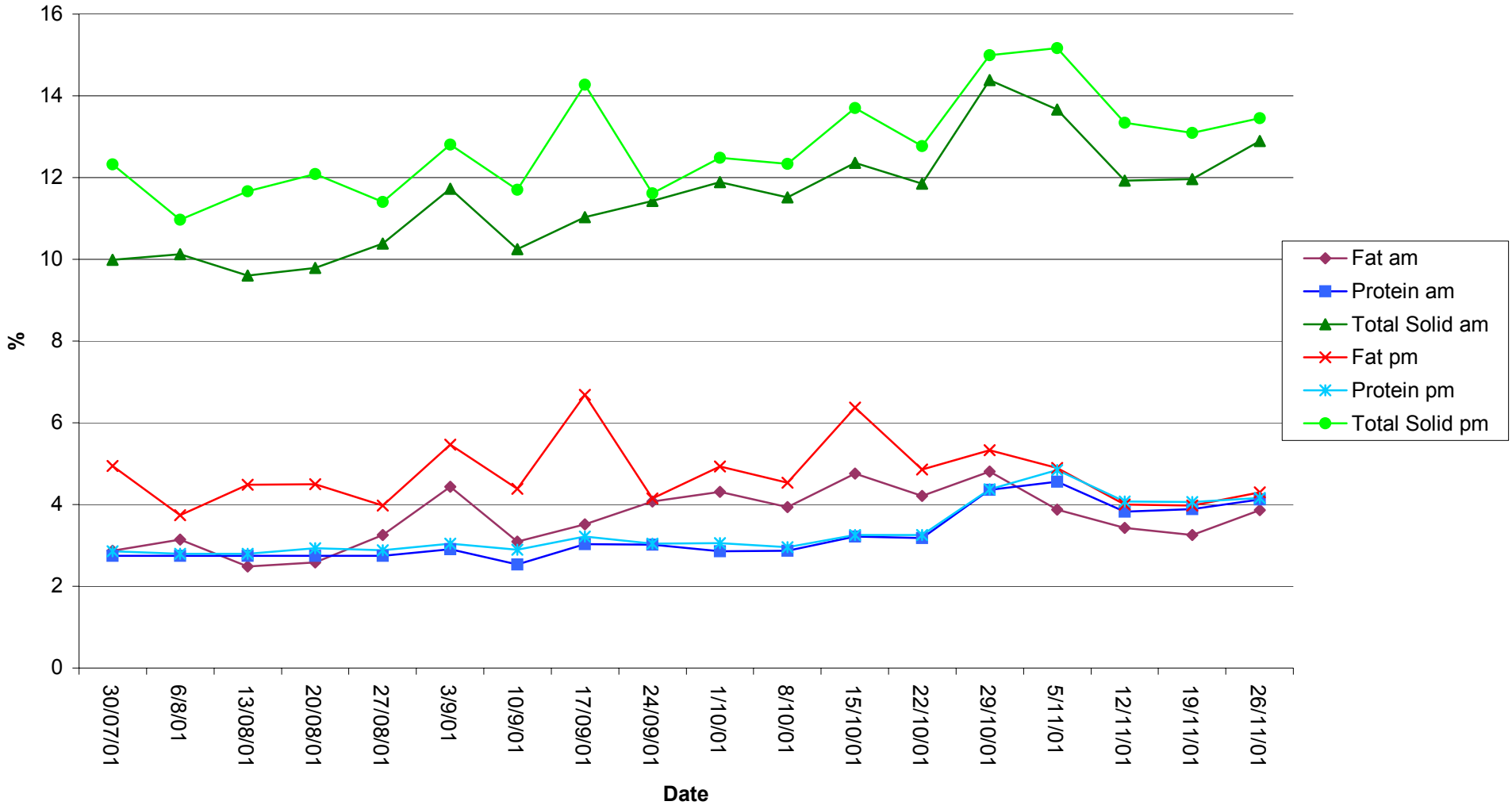


Fig 5b) Milk Composition Goat 610

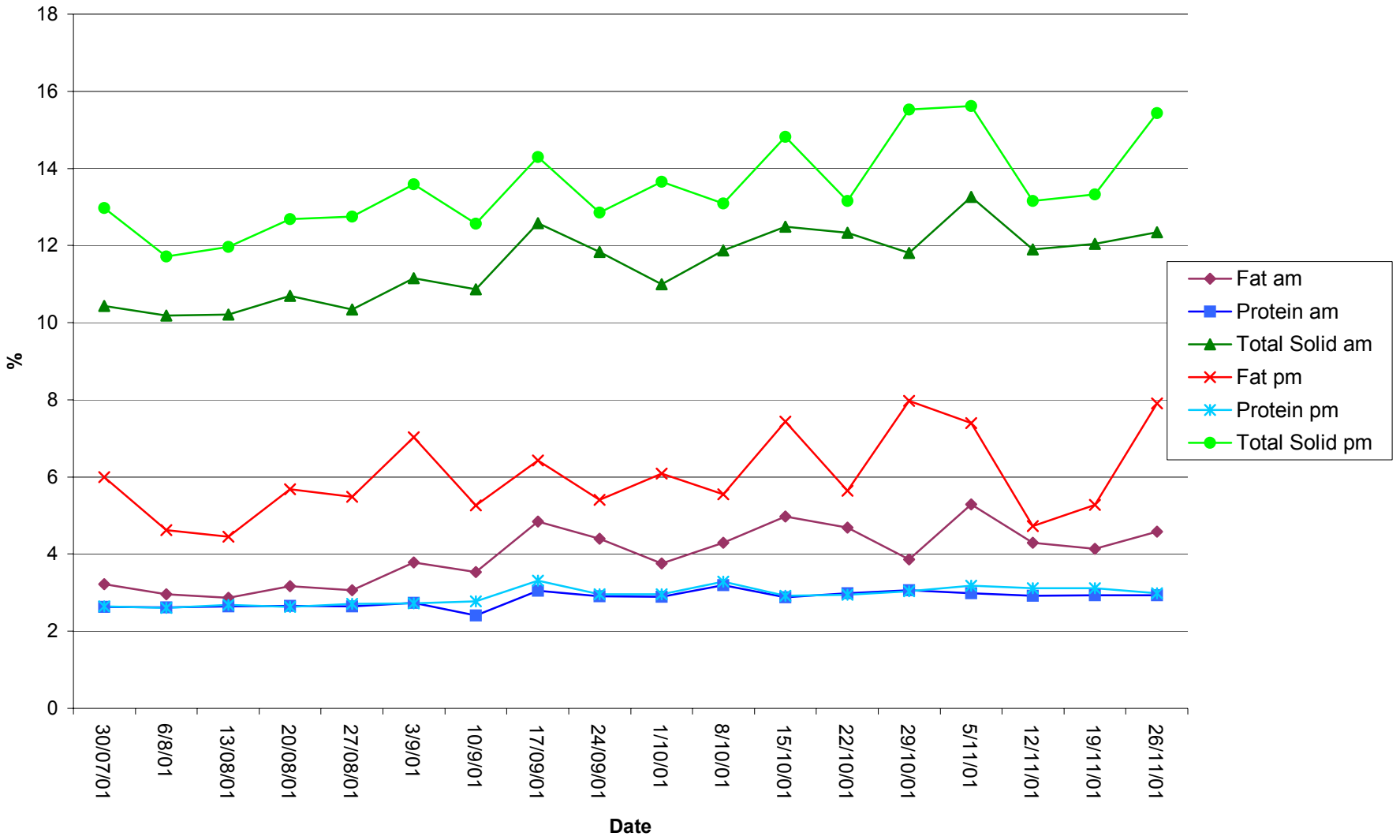


Fig 5c) Milk Composition Goat 617

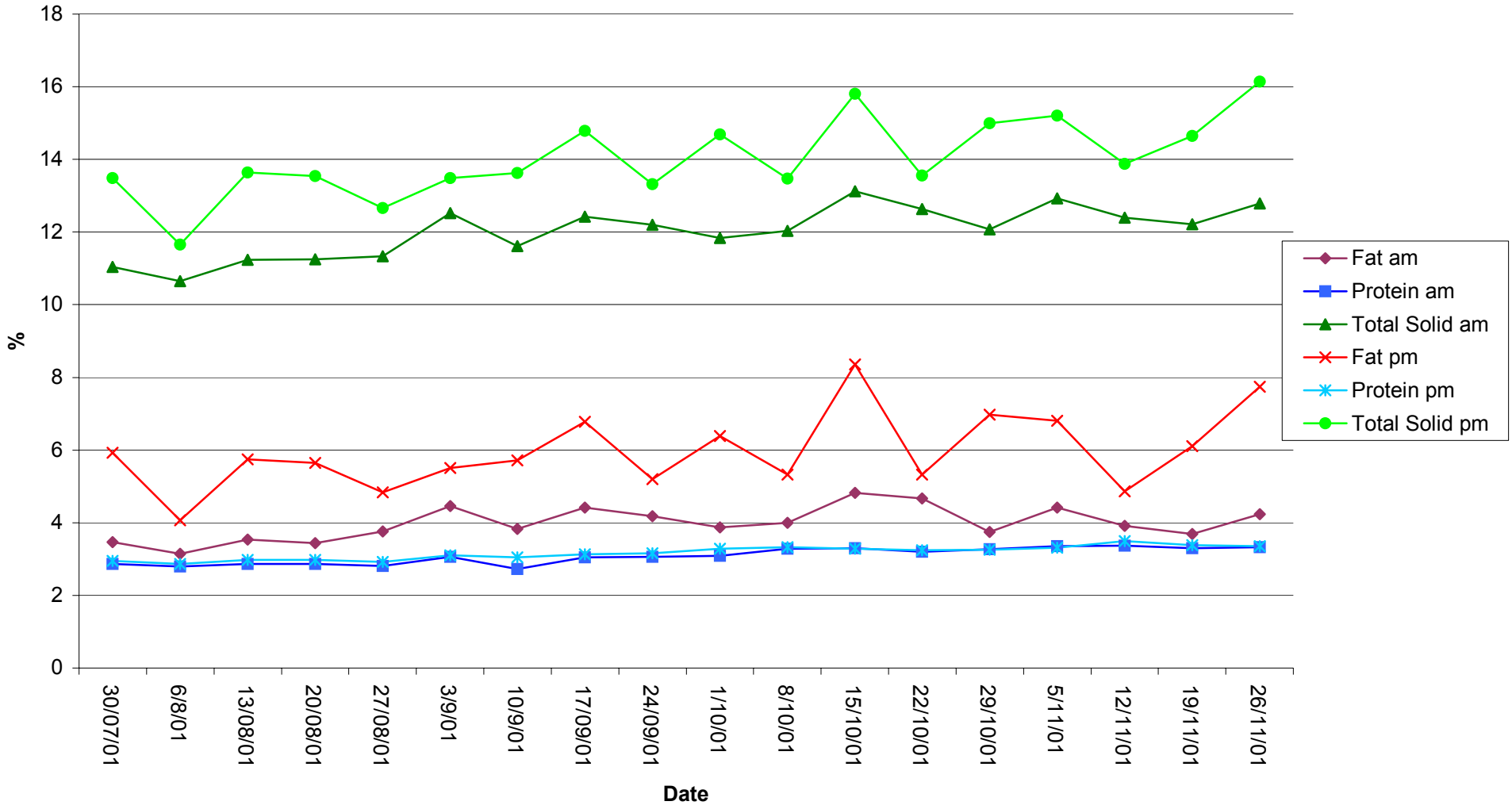


Fig 5d) Milk Composition Goat 618

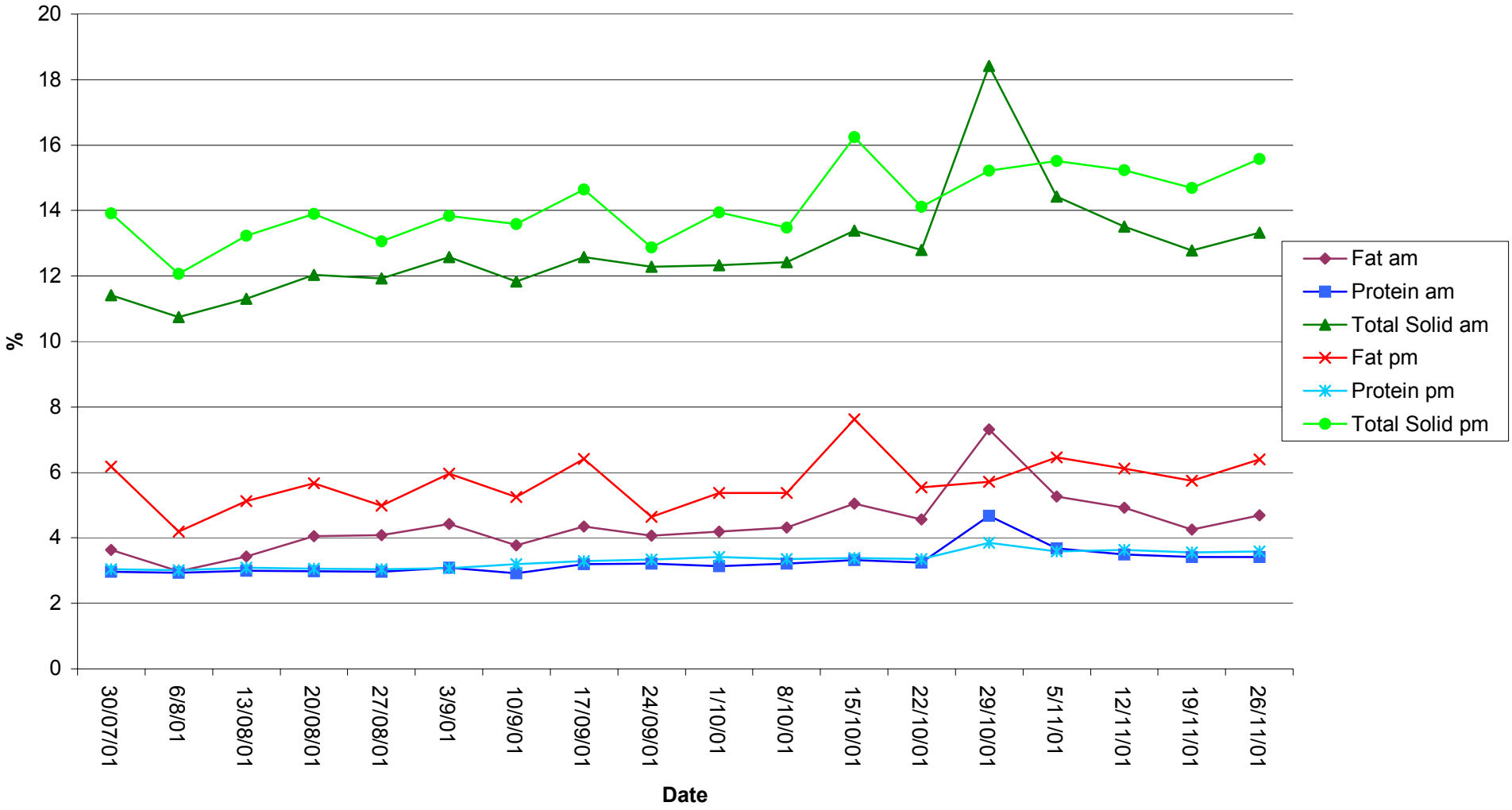


Fig 5e) Milk Composition Goat 622

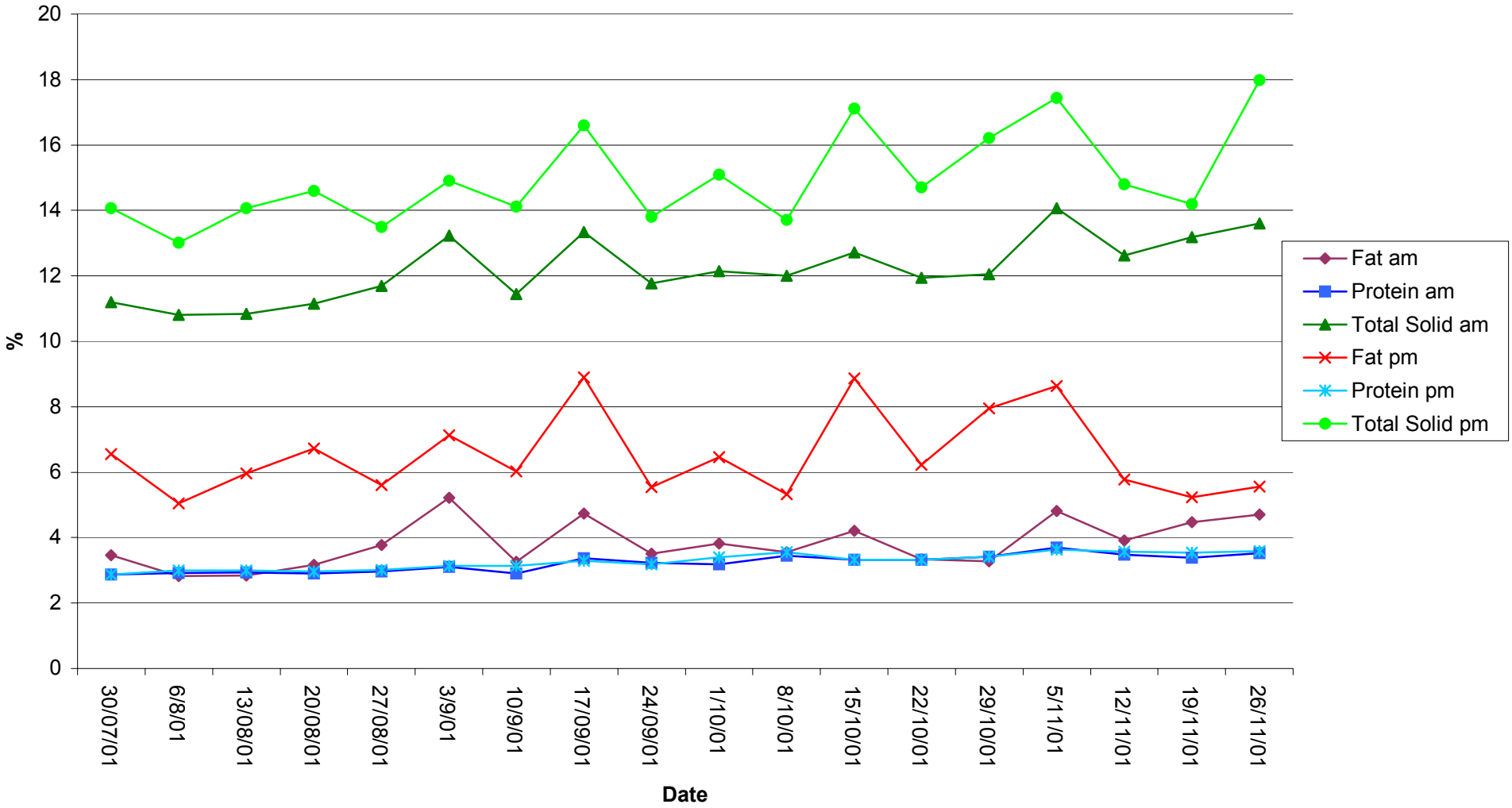


Fig 5f) Milk Composition Goat 639

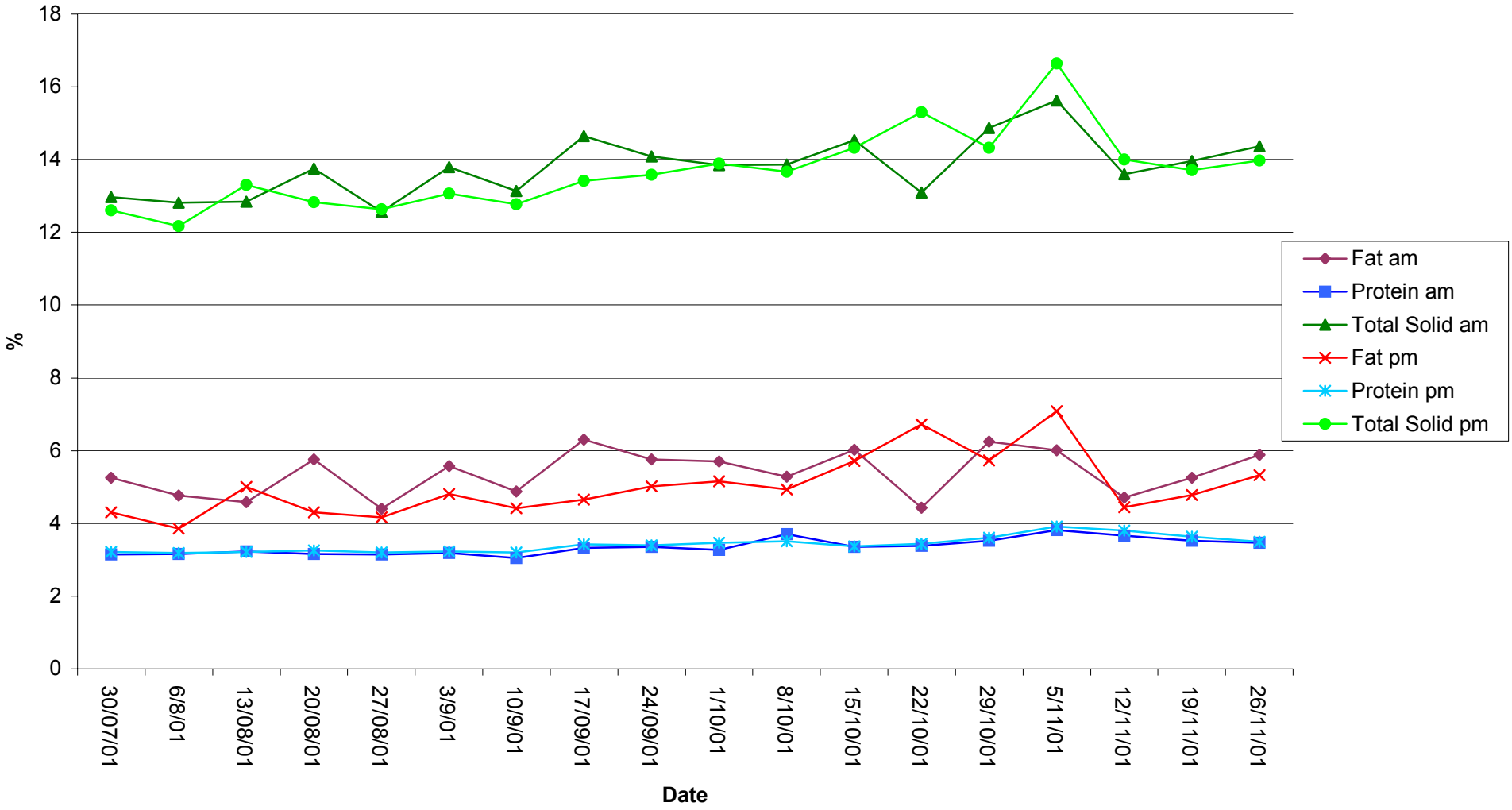


Fig 5g) Milk Composition Goat 713

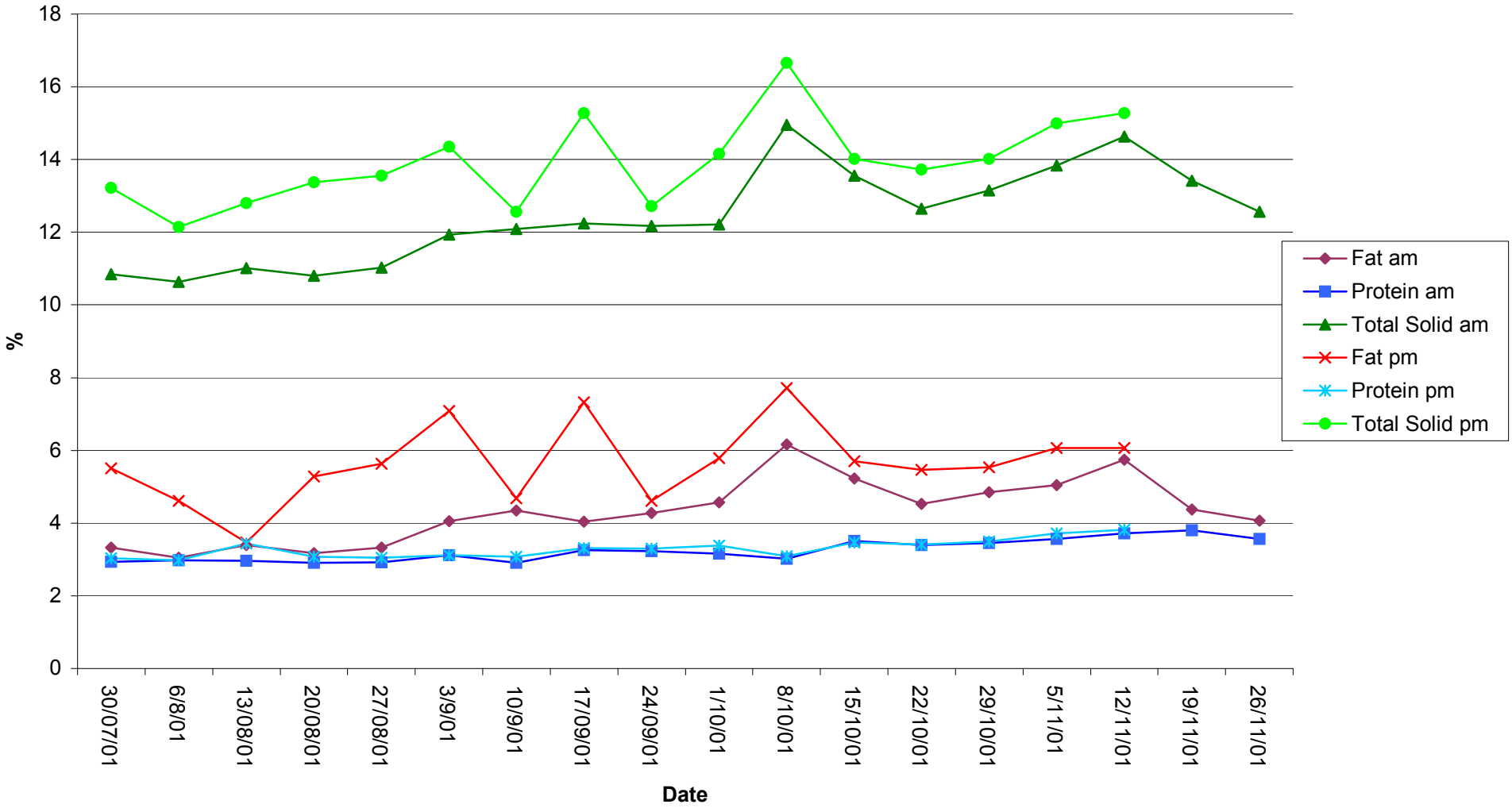


Fig 5h) Milk Composition Goat 806

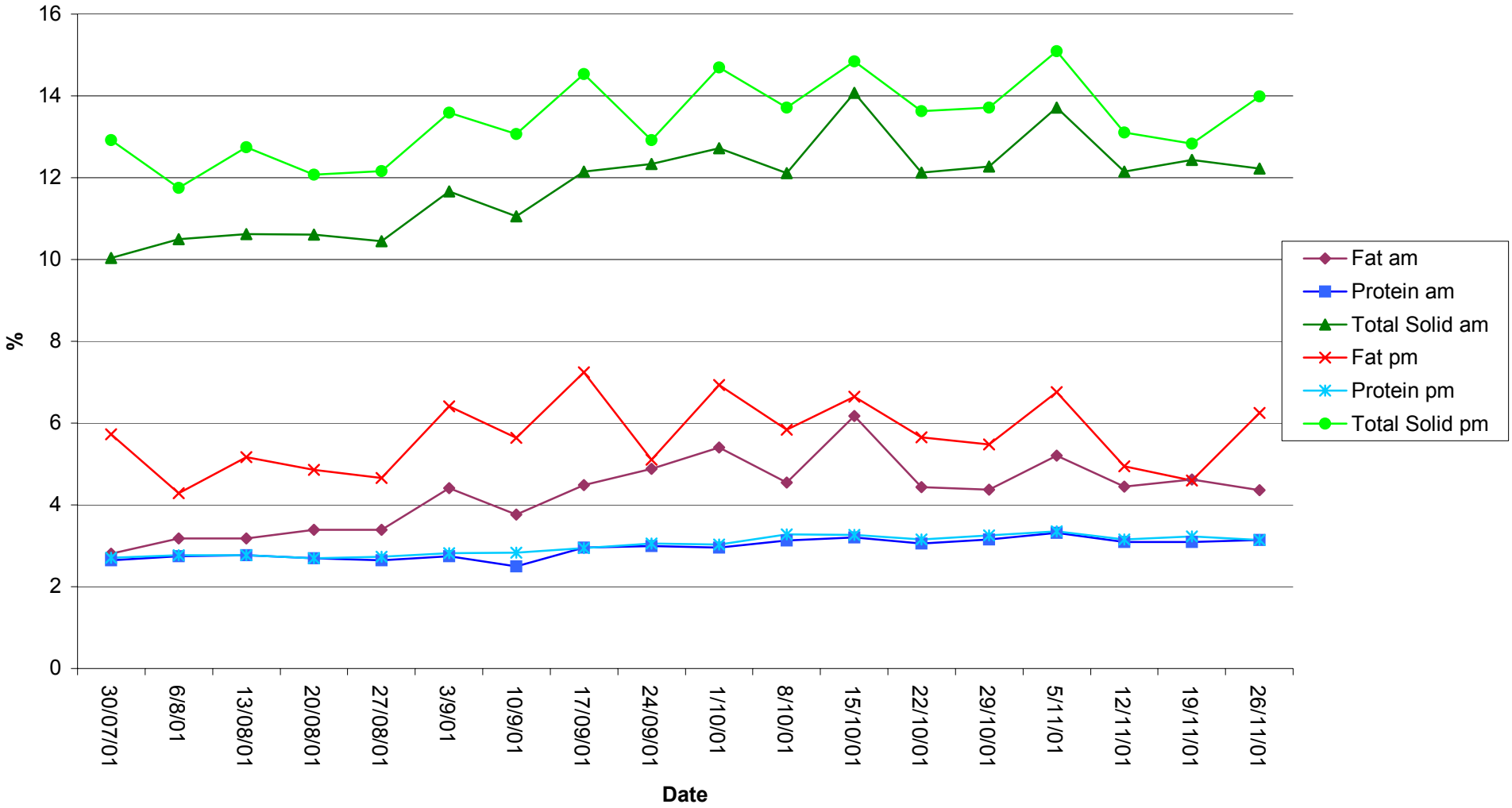


Fig 5i) Milk Composition Goat 890

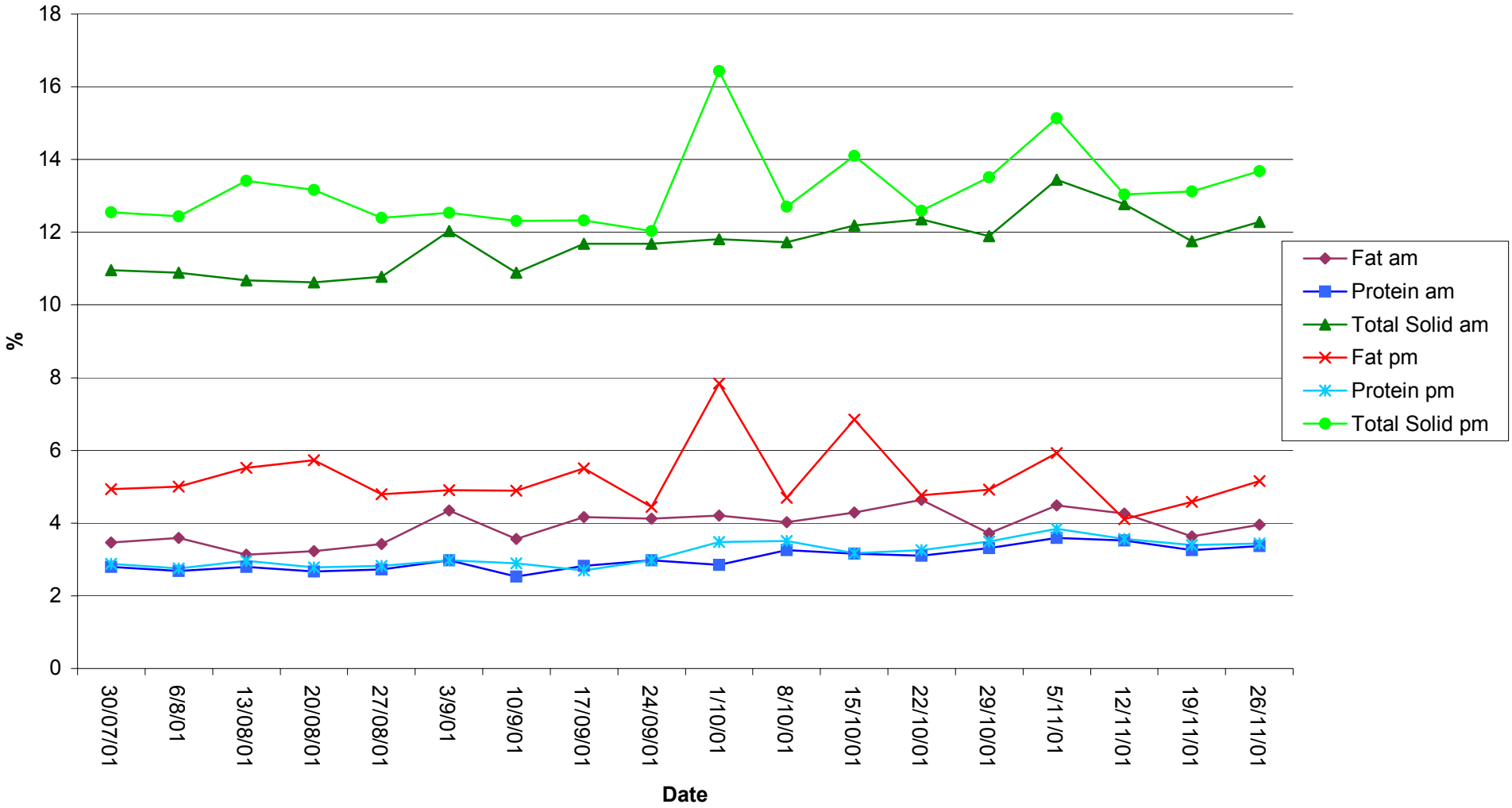


Fig 5j) Milk Composition Goat 891

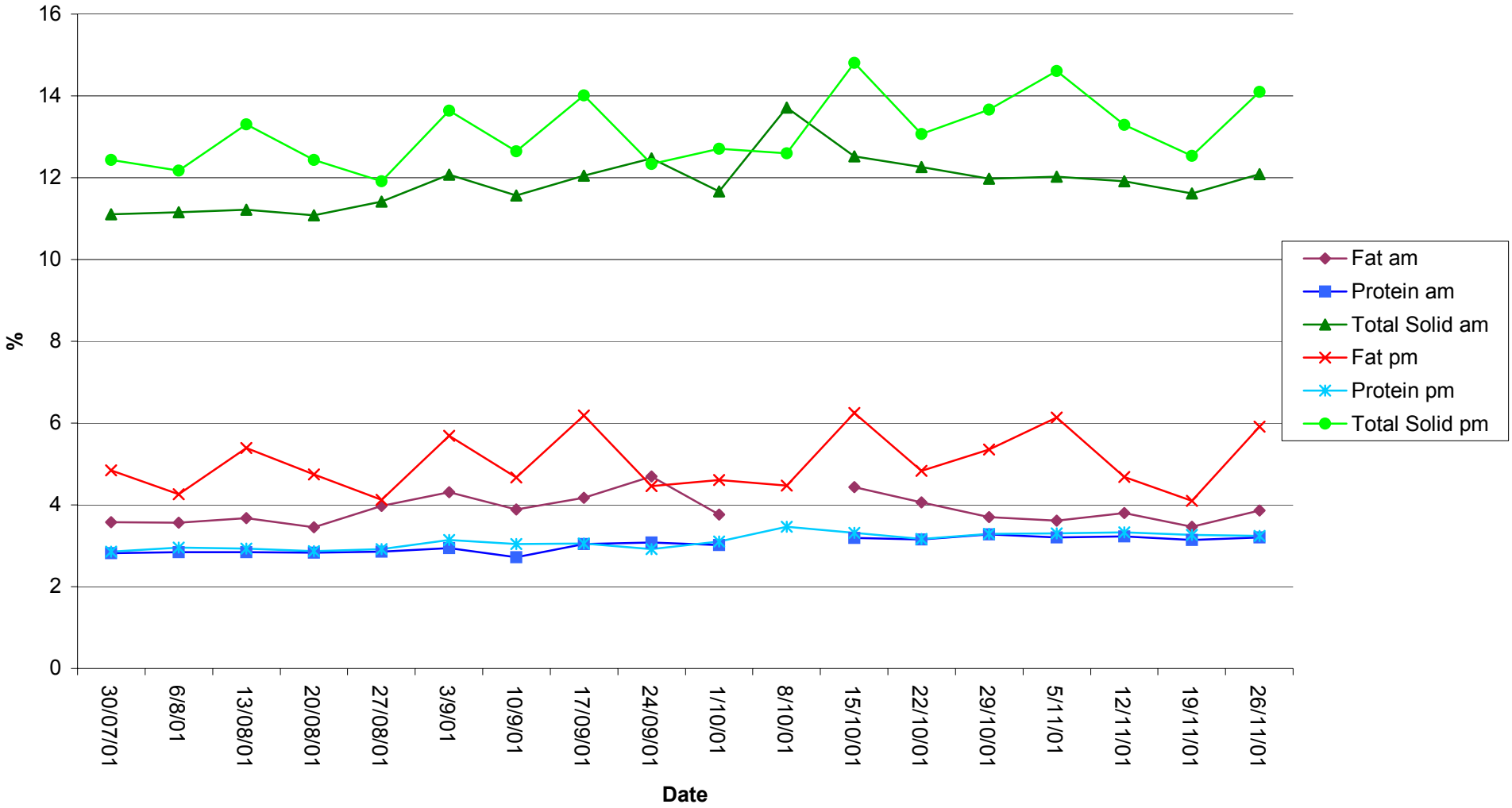


Fig 5k) Milk Composition Goat 725

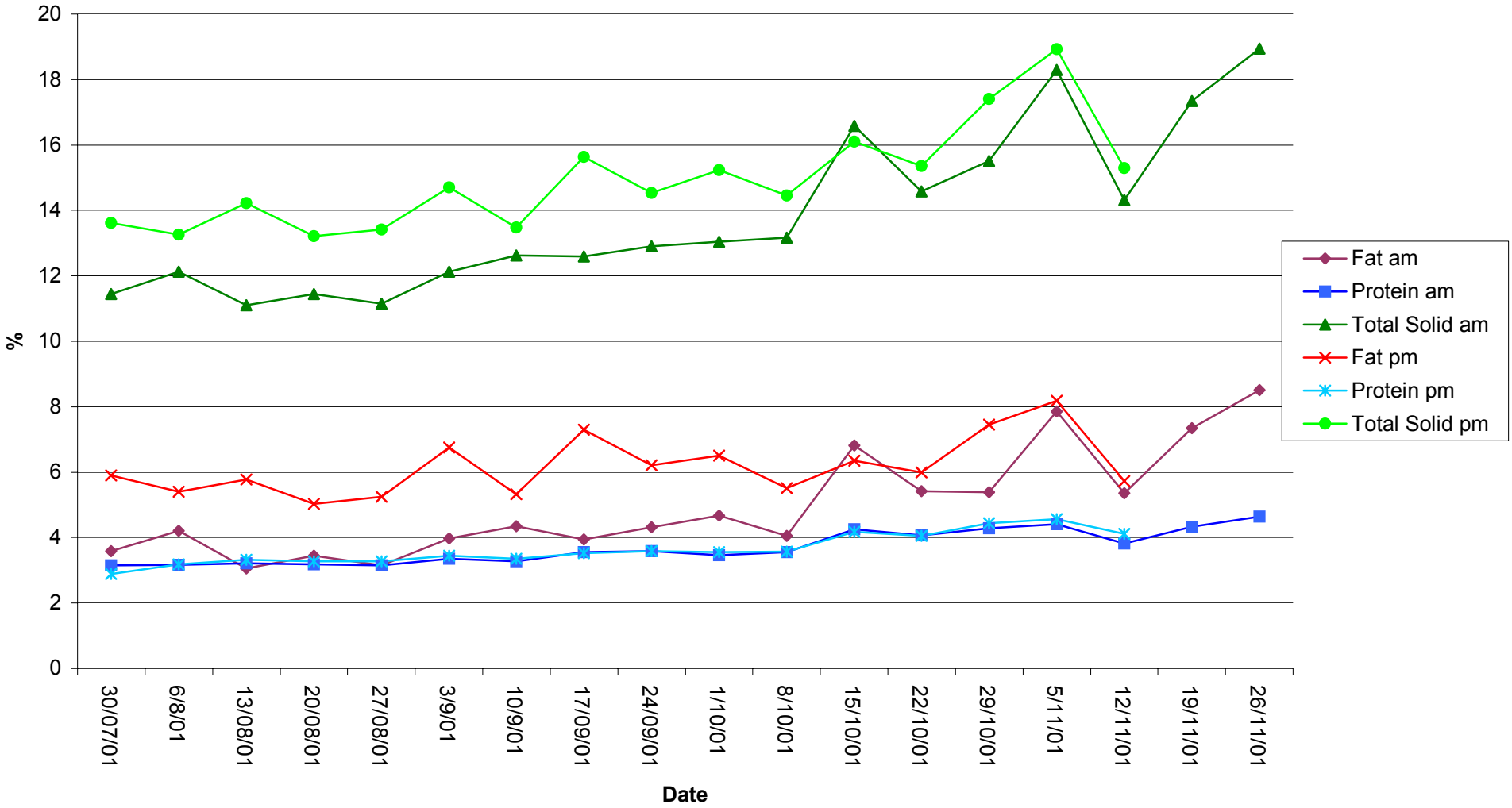


Fig 5l) Milk Composition Goat 809

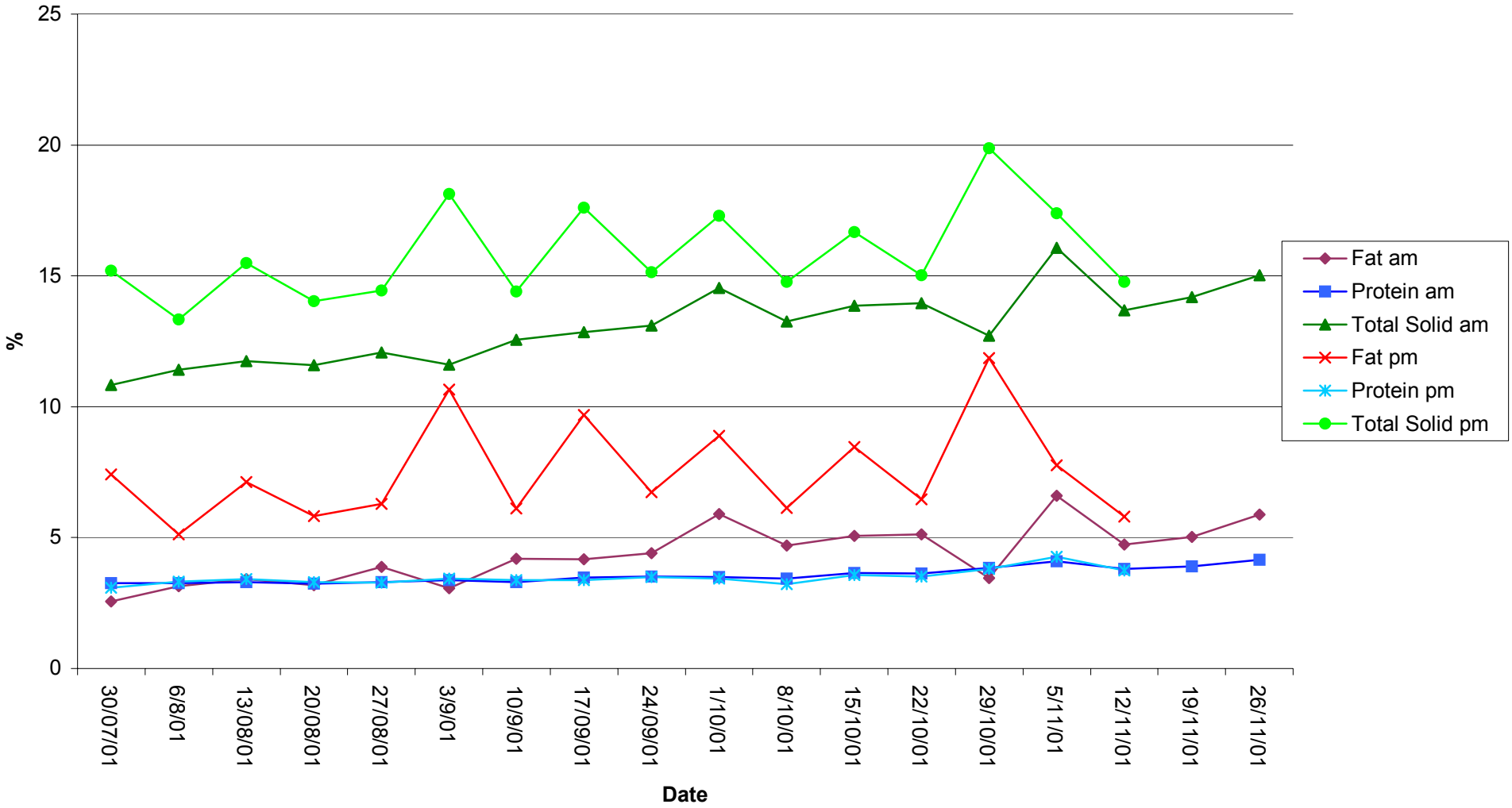


Fig 5m) Milk Composition Goat 640

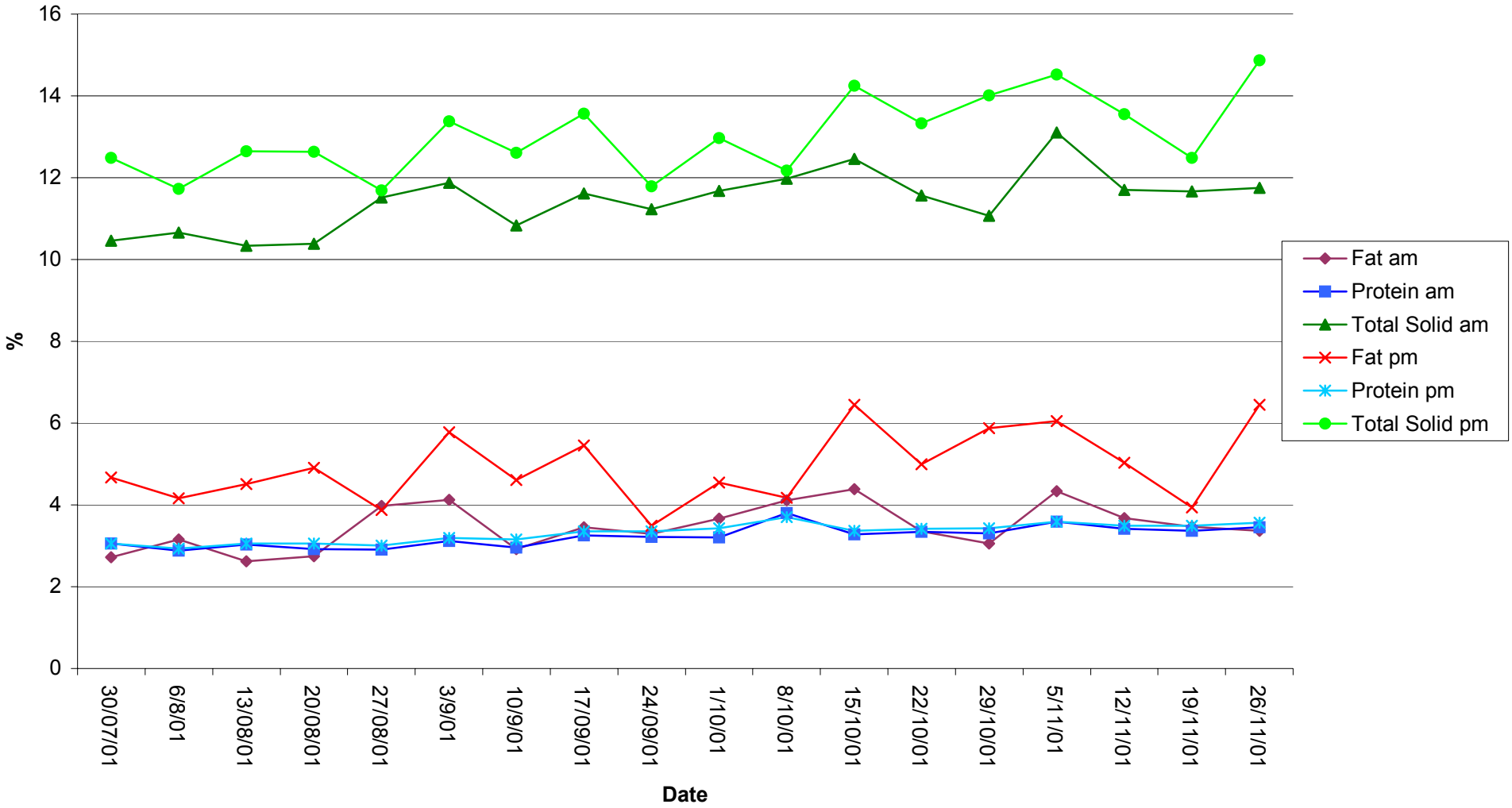


Fig 5n) Milk Composition Goat 605

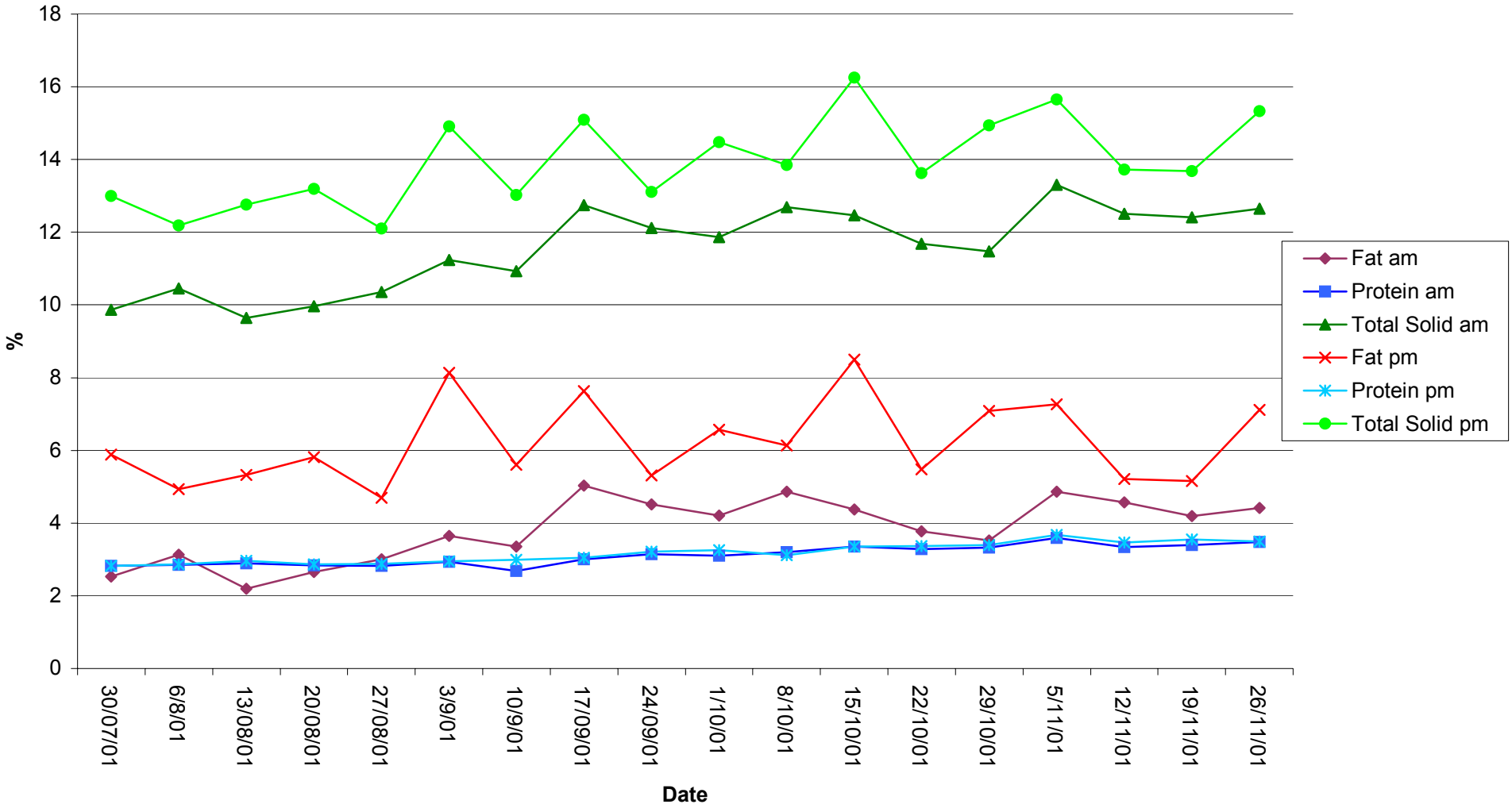


Fig 5o) Milk Composition Goat 637

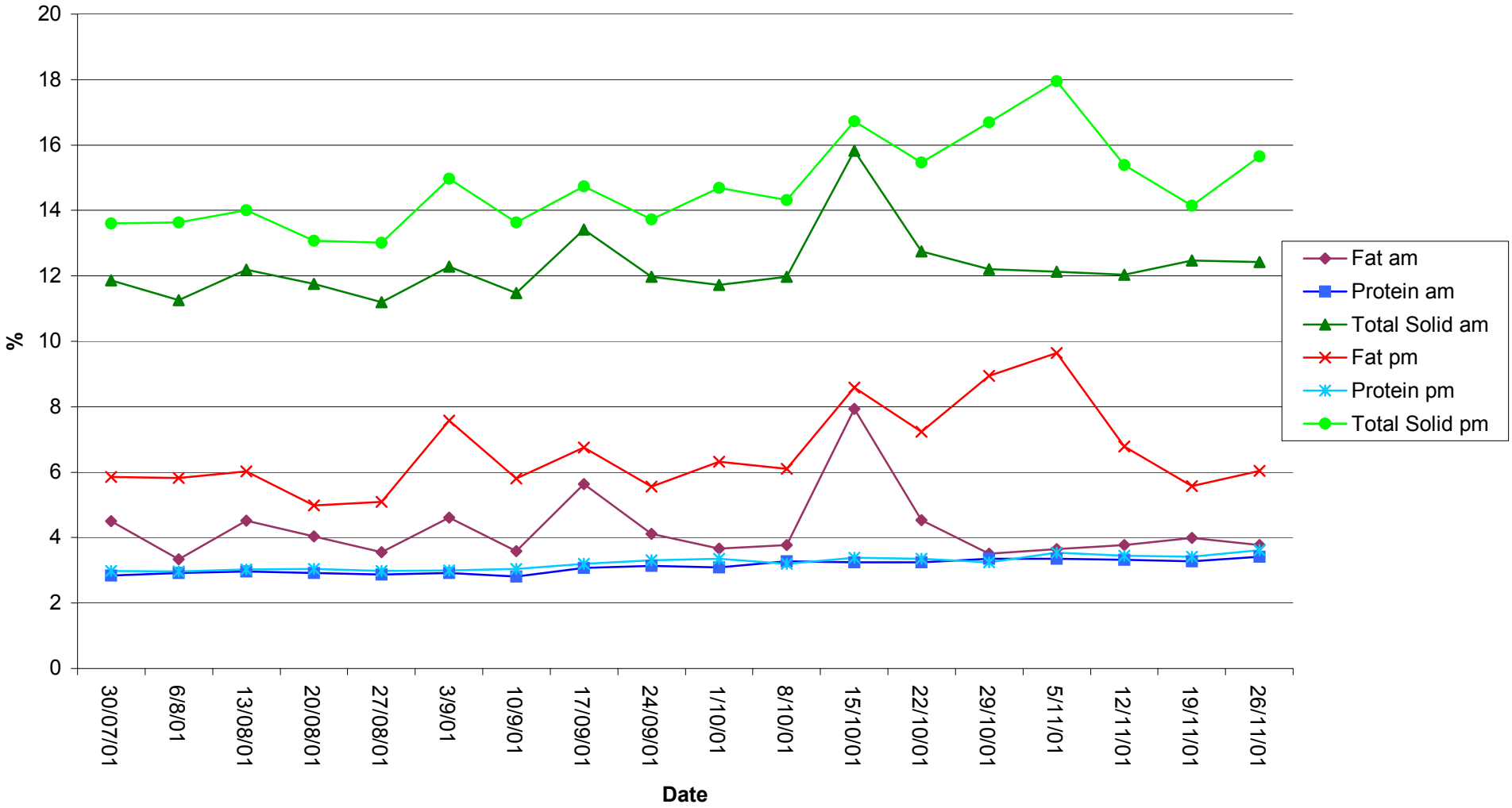


Fig 5p) Milk Composition Goat 705

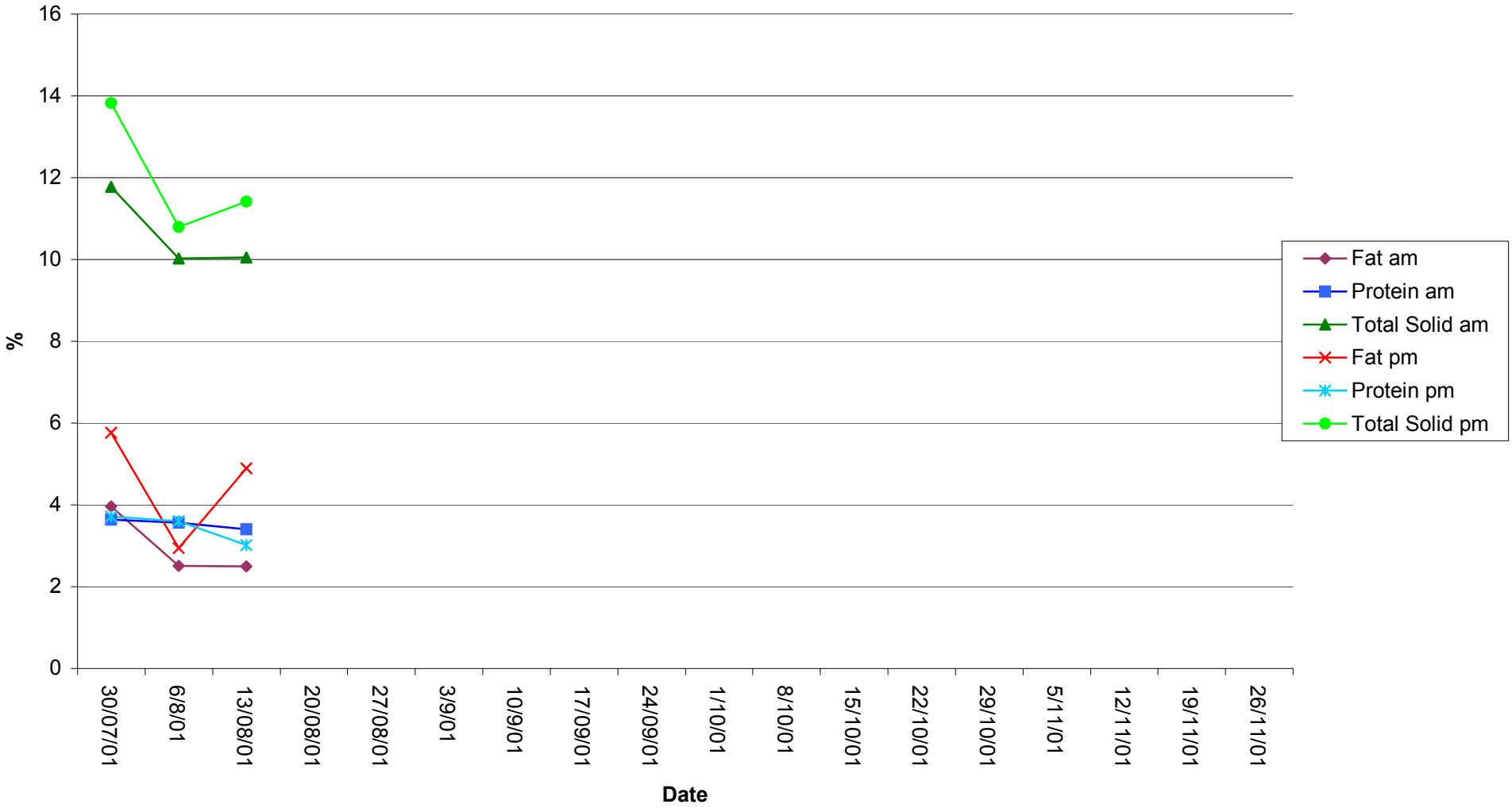


Fig 5q) Milk Composition Goat 899

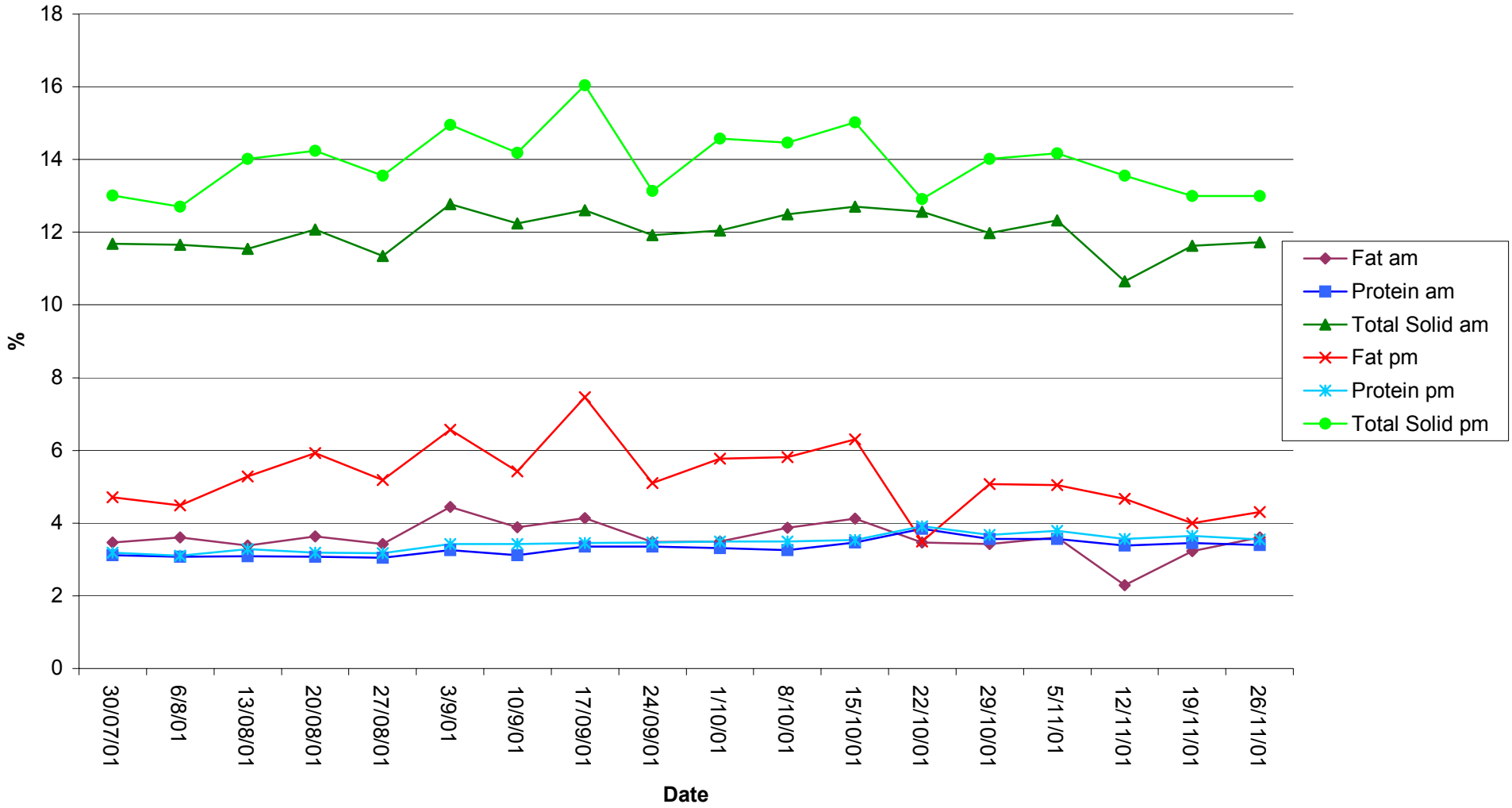


Fig 6a) Somatic Cell Count Goat 601

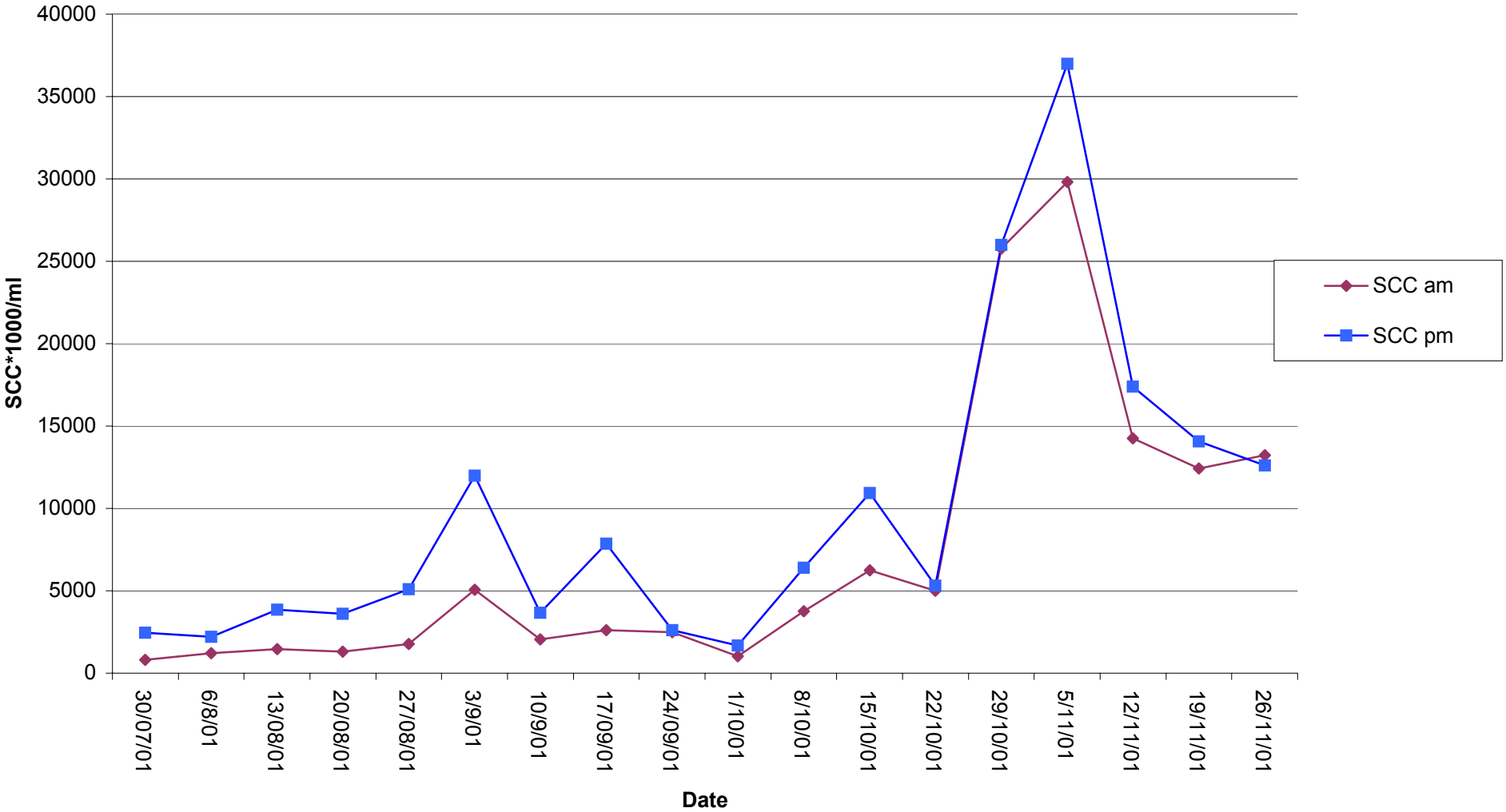


Fig 6b) Somatic Cell Count Goat 610

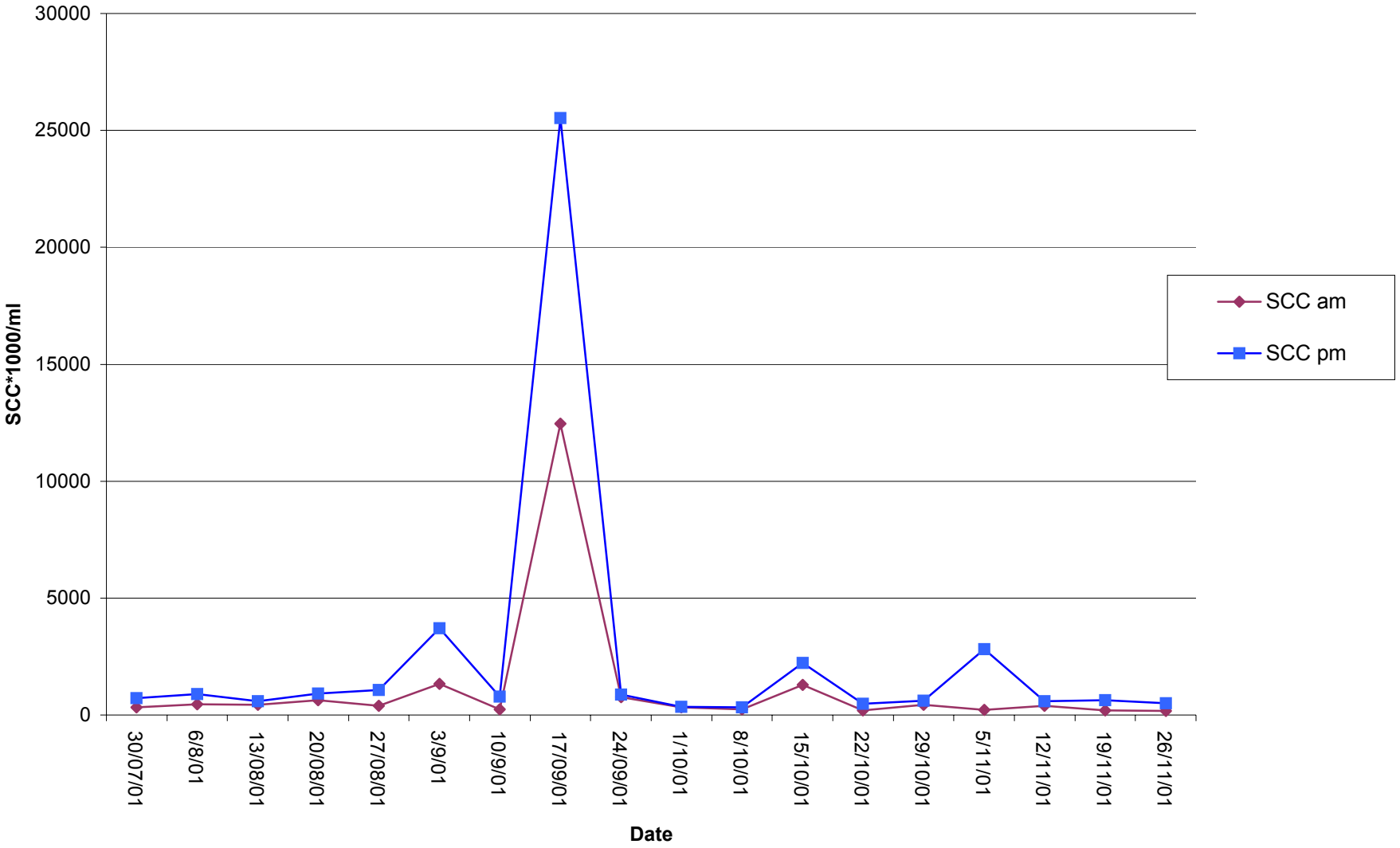


Fig 6c) Somatic Cell Count Goat 617

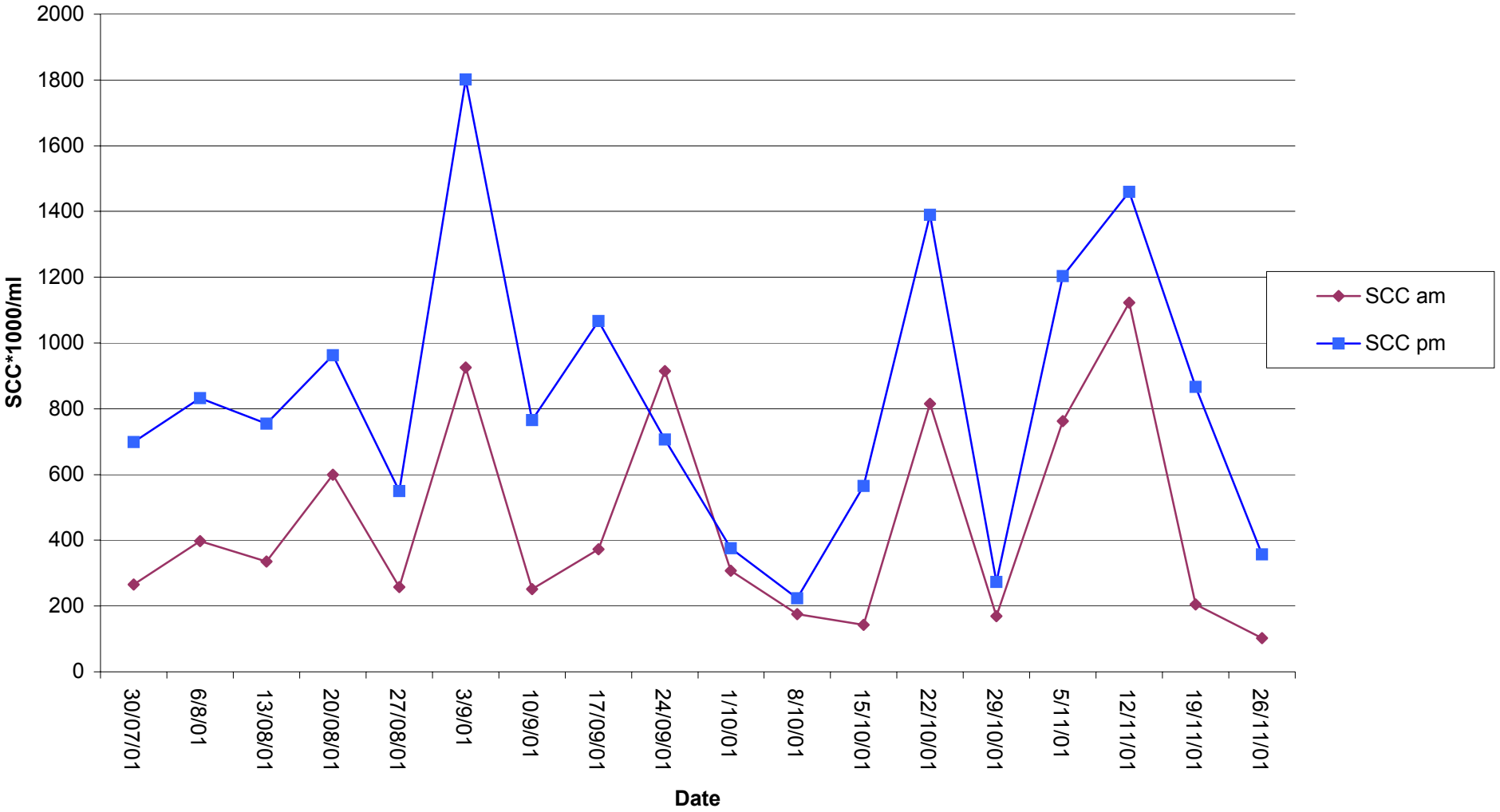


Fig 6d) Somatic Cell Count Goat 618

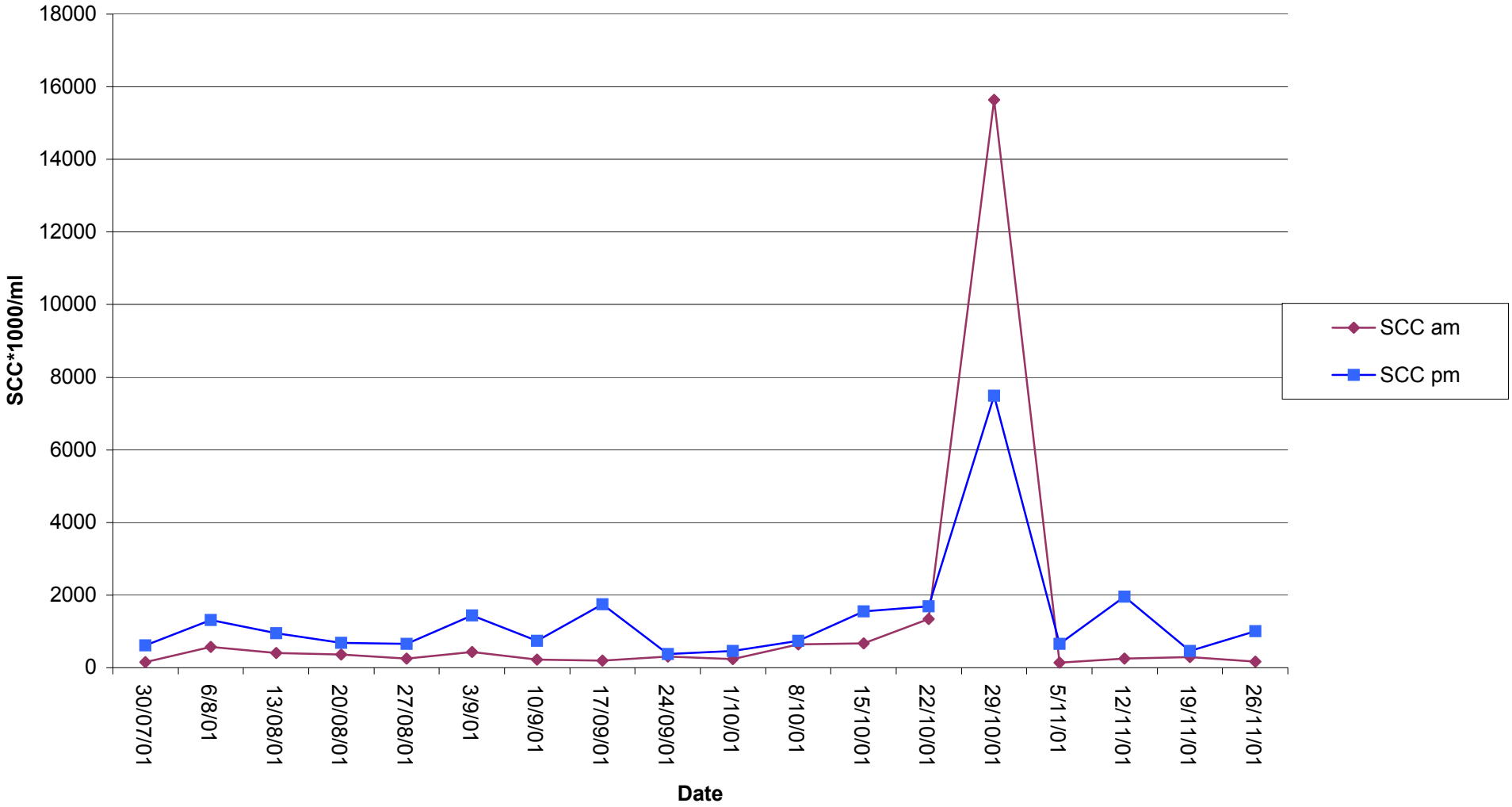


Fig 6e) Somatic Cell Count Goat 622

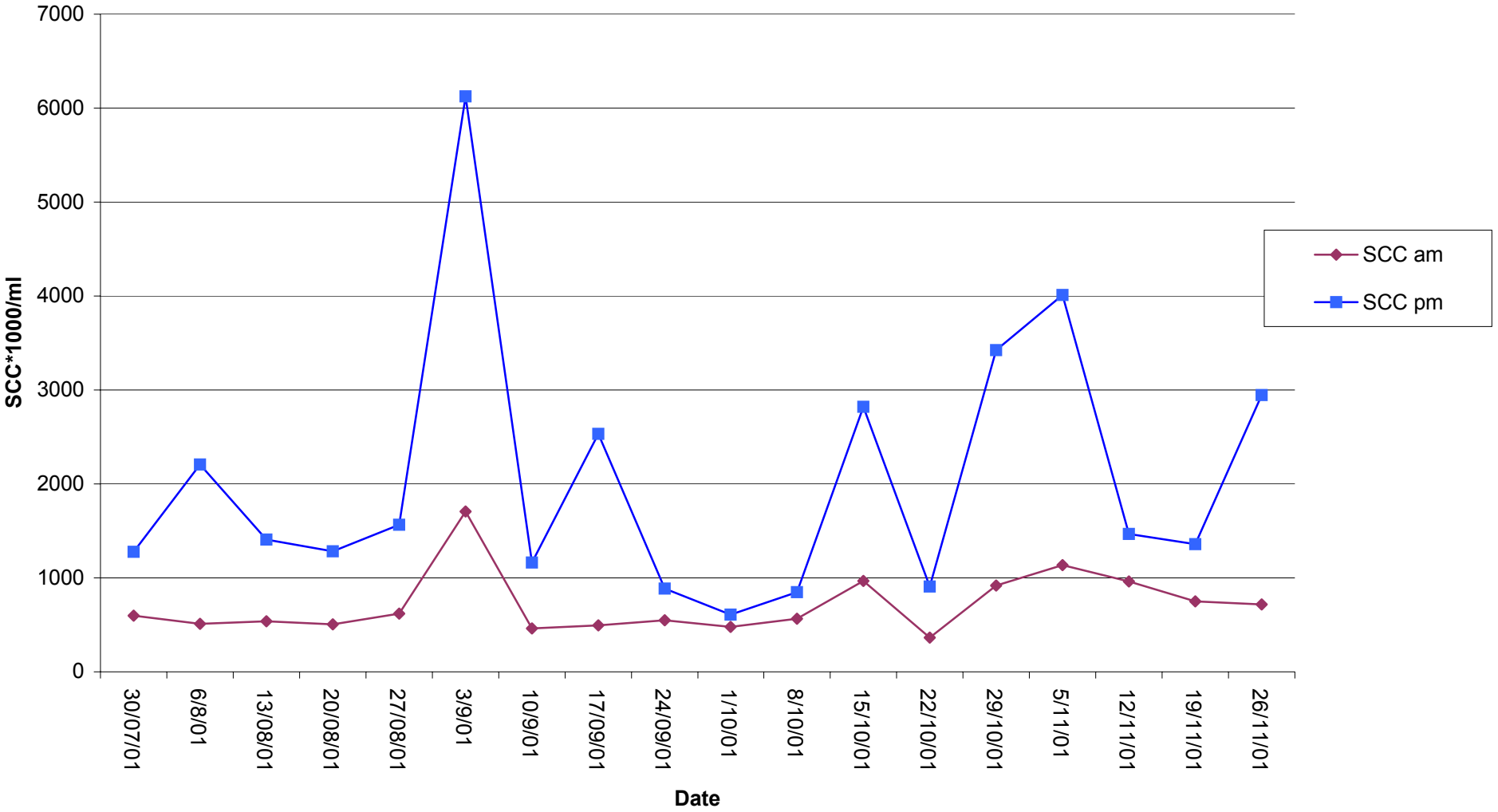


Fig 6f) Somatic Cell Count Goat 639

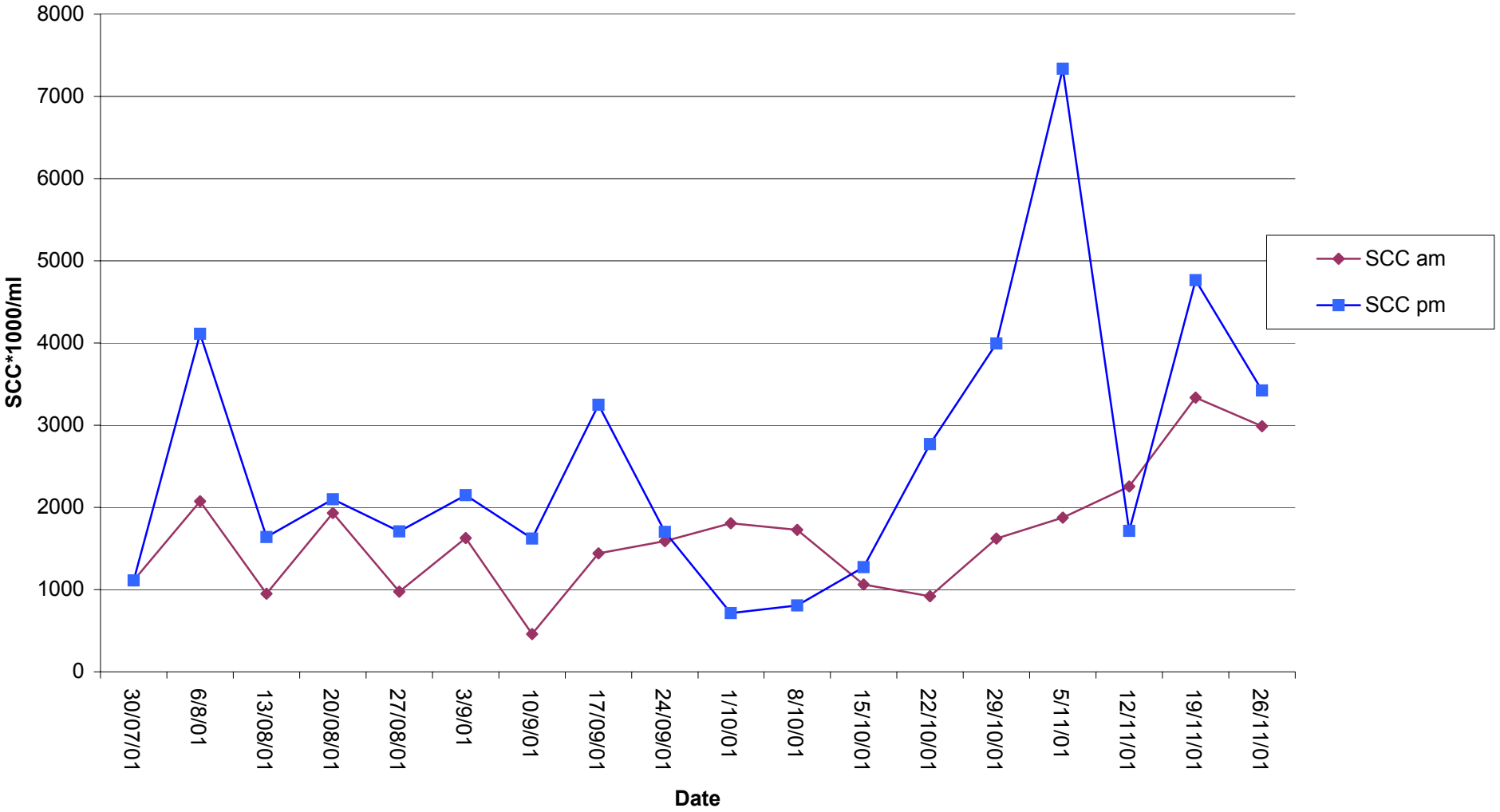


Fig 6g) Somatic Cell Count Goat 713

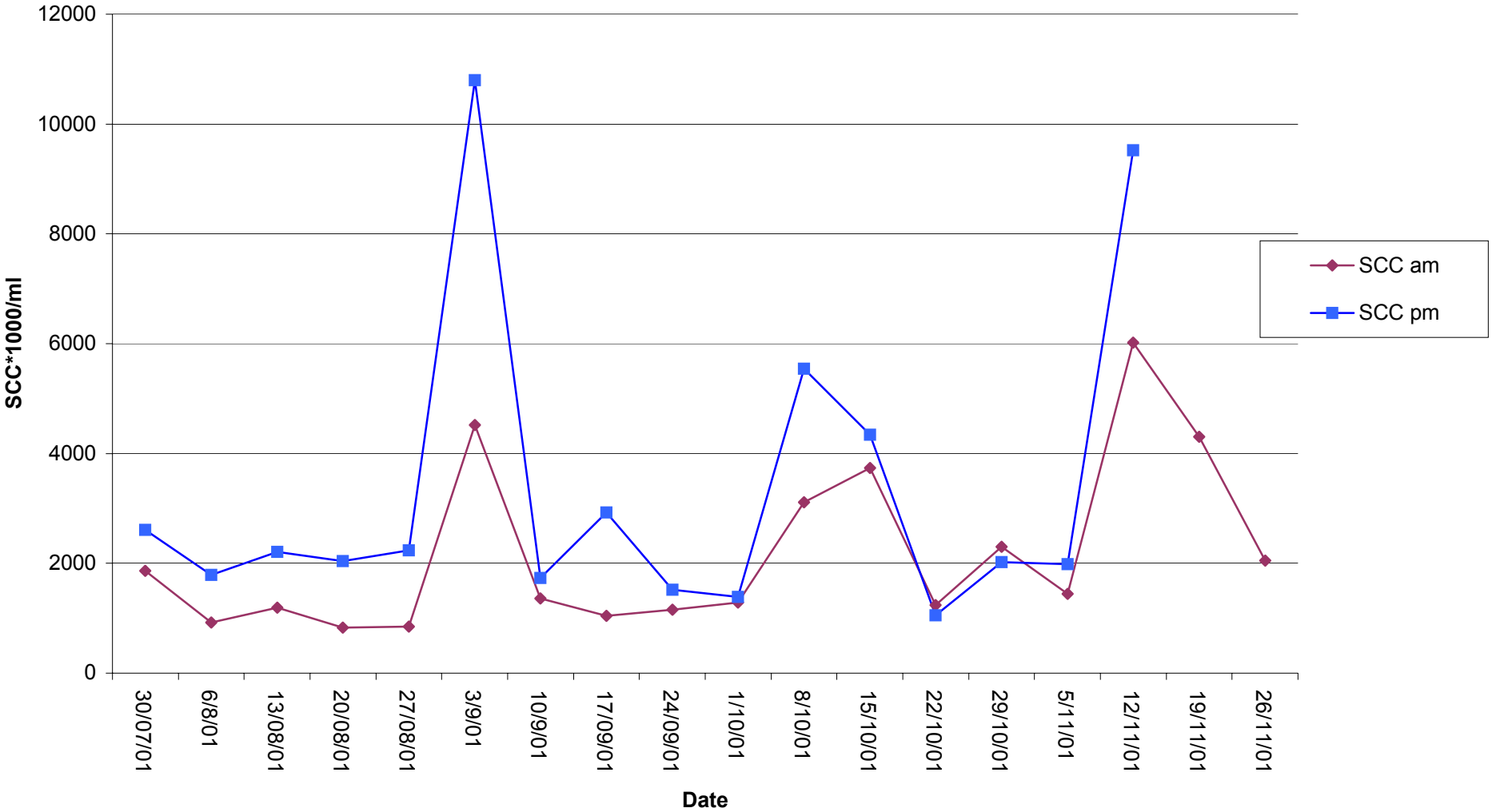


Fig 6h) Somatic Cell Count Goat 806

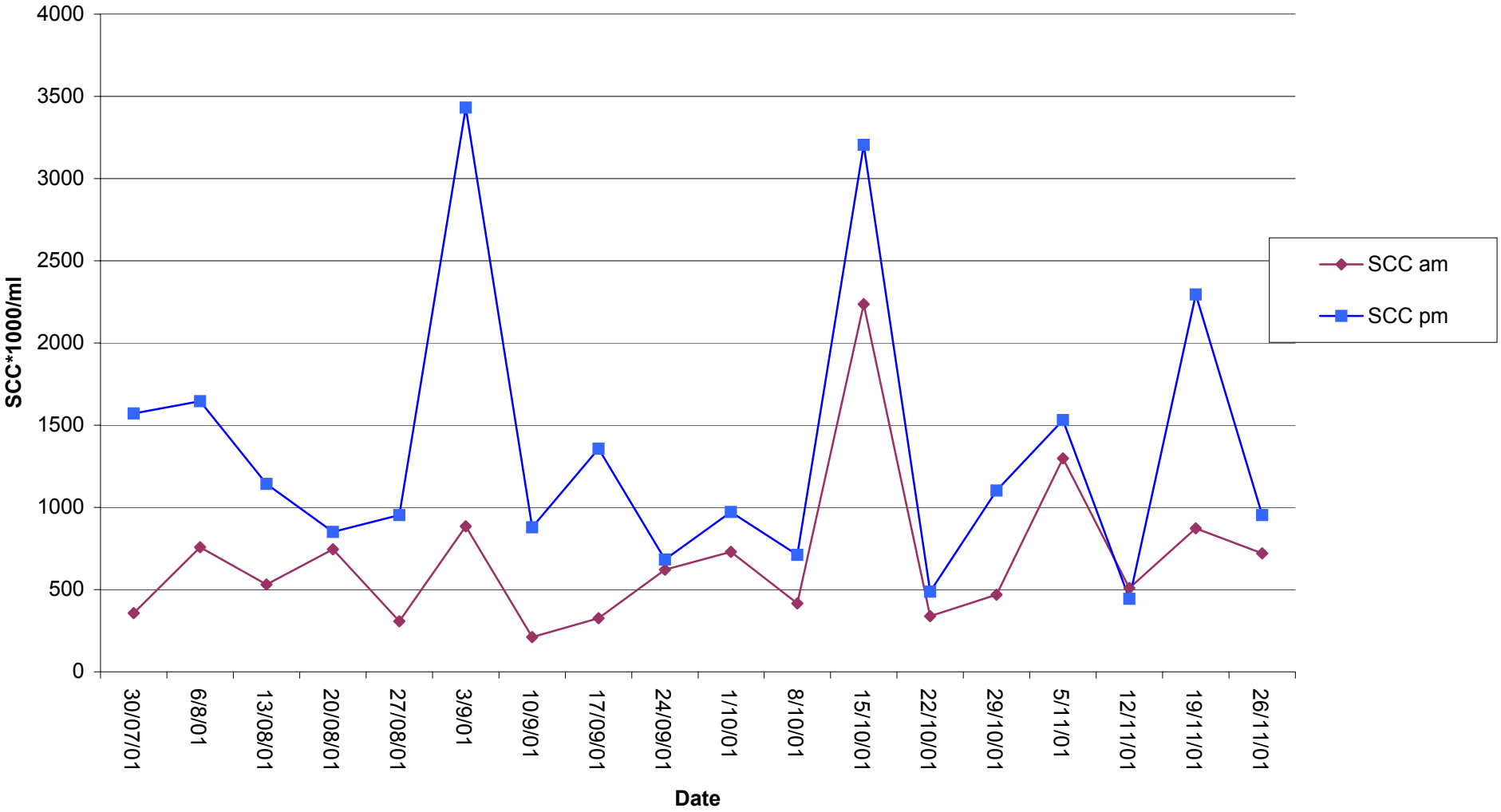


Fig 6i) Somatic Cell Count Goat 890

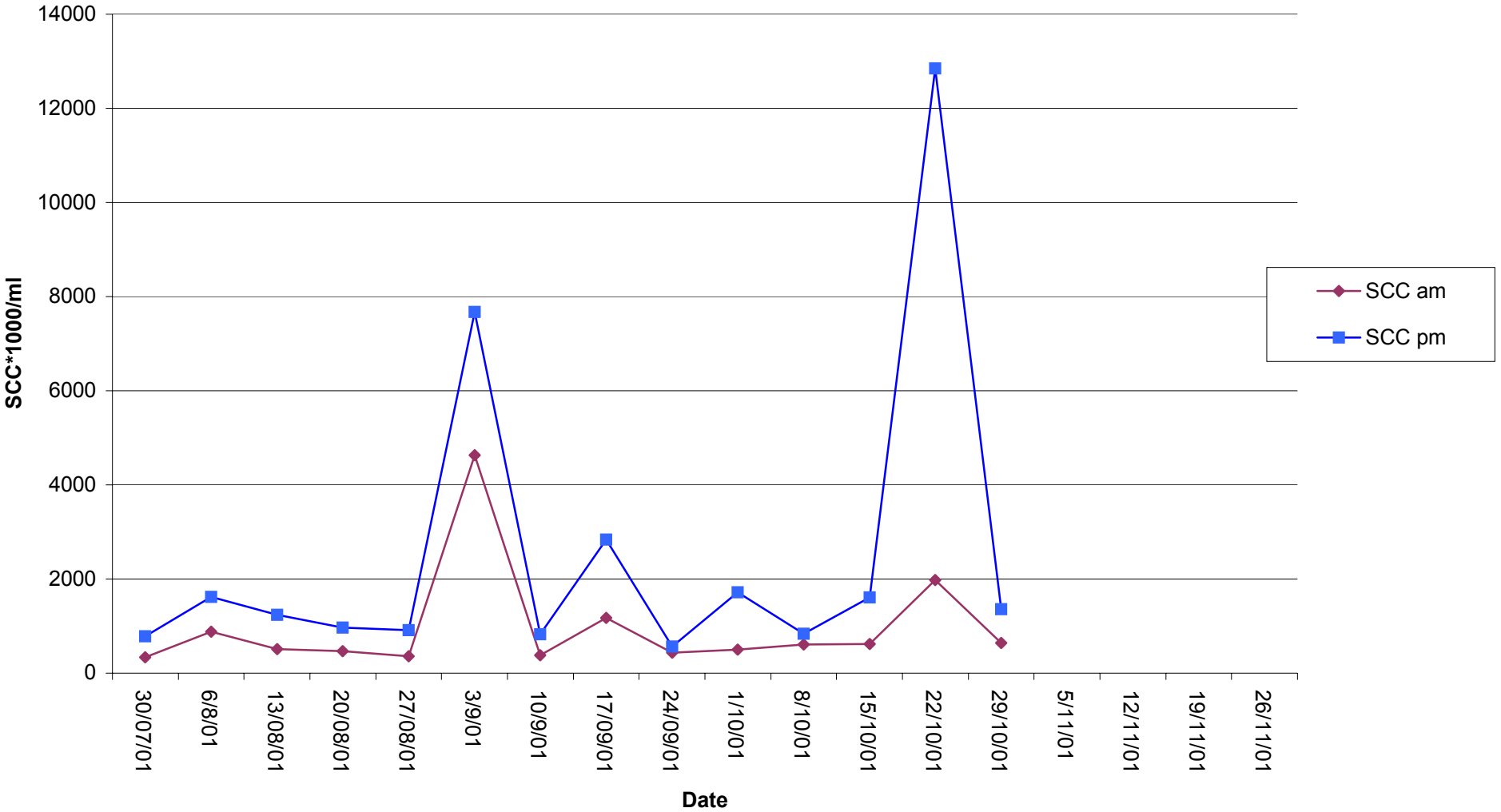


Fig 6j) Somatic Cell Count Goat 891

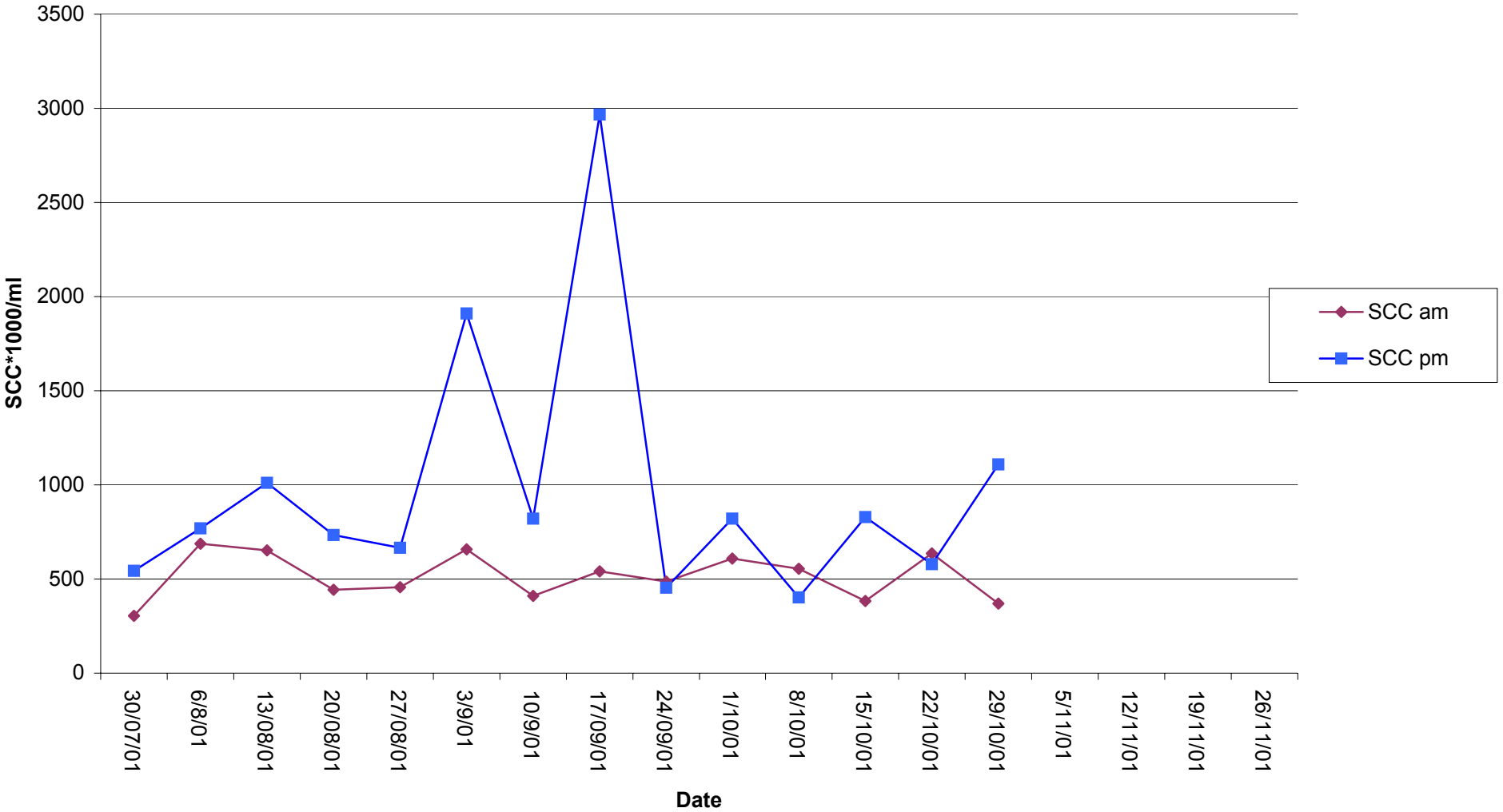


Fig 6k) Somatic Cell Count Goat 725

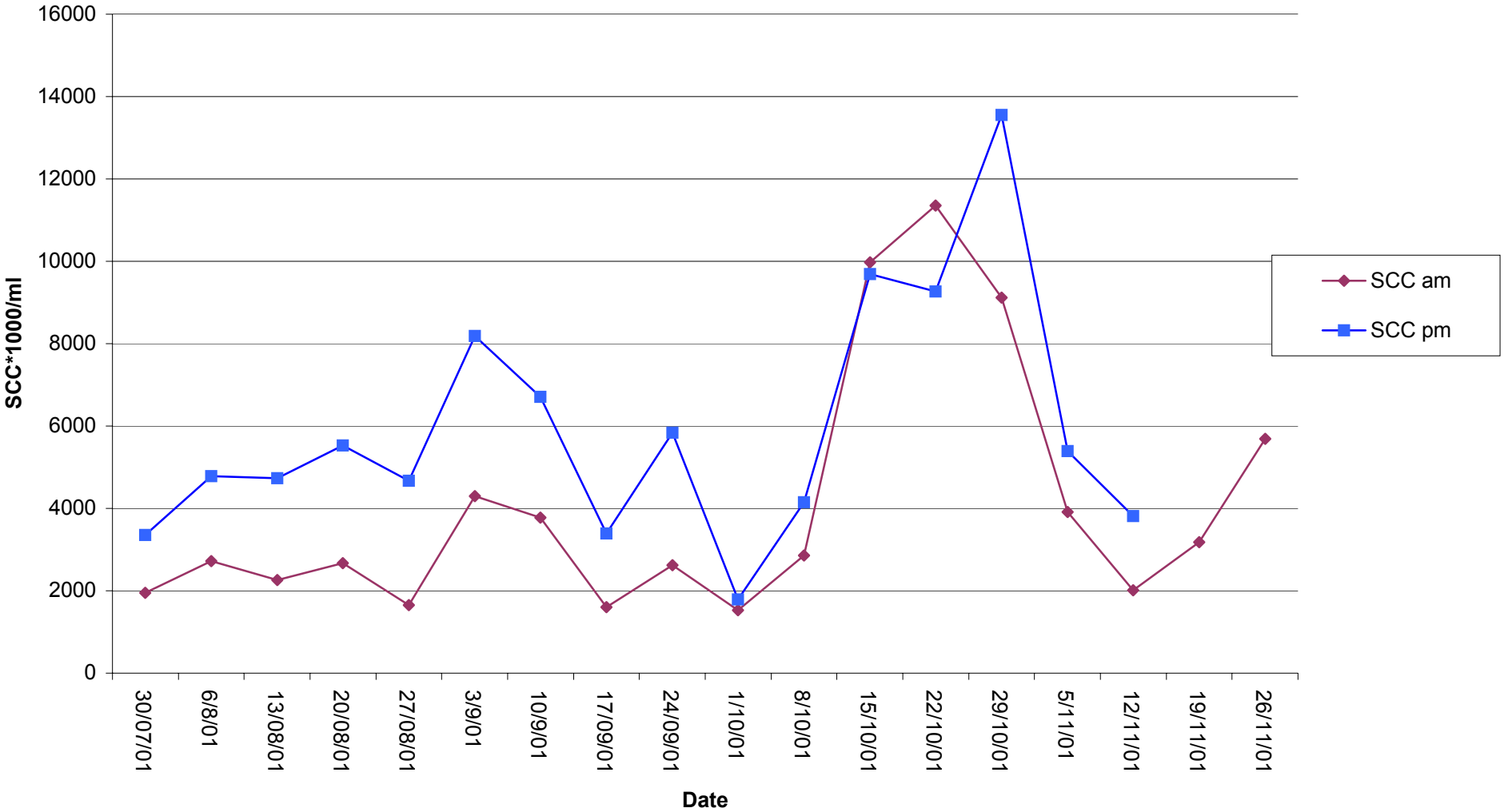


Fig 6l) Somatic Cell Count Goat 809

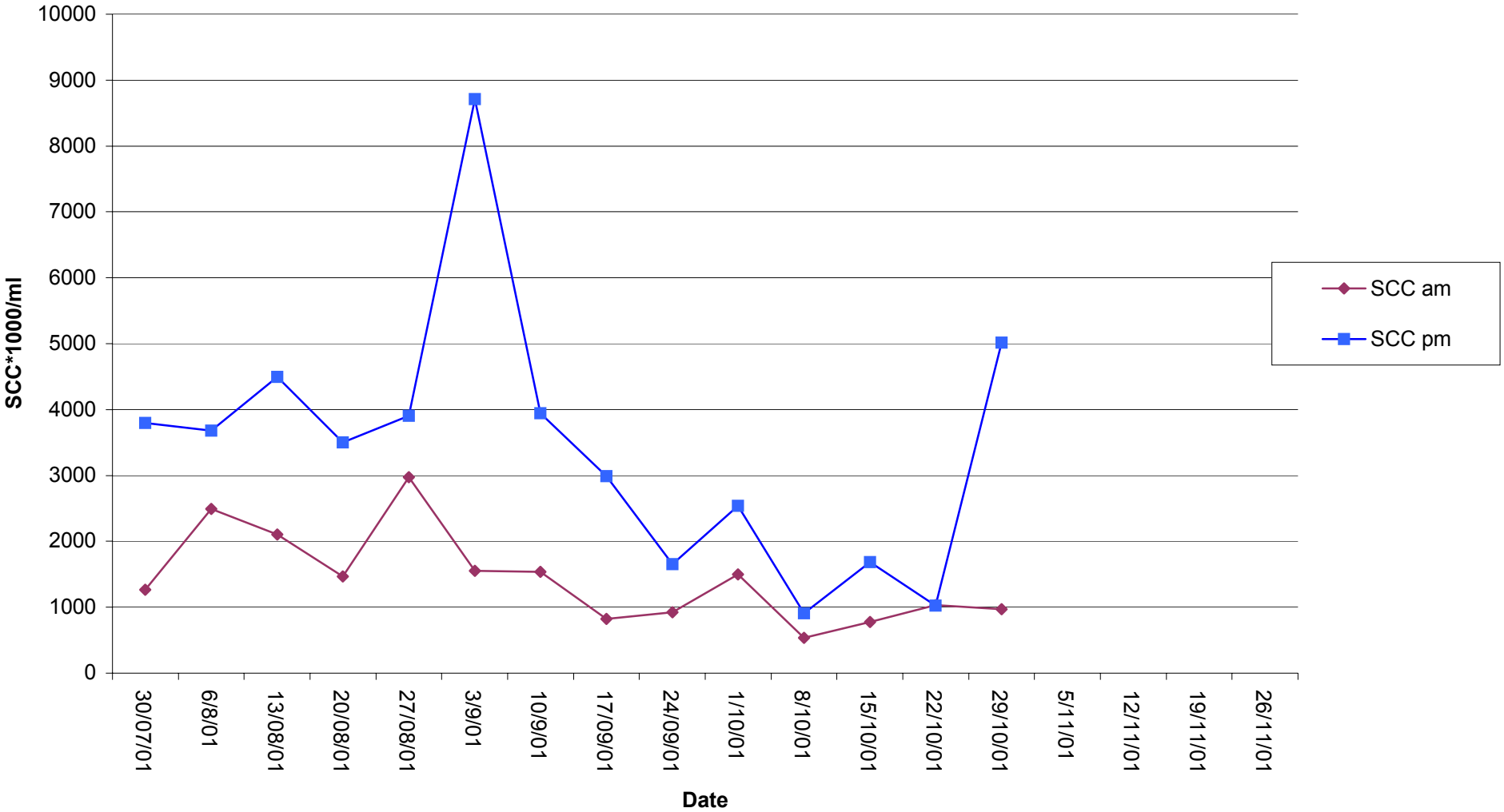


Fig 6m) Somatic Cell Count Goat 640

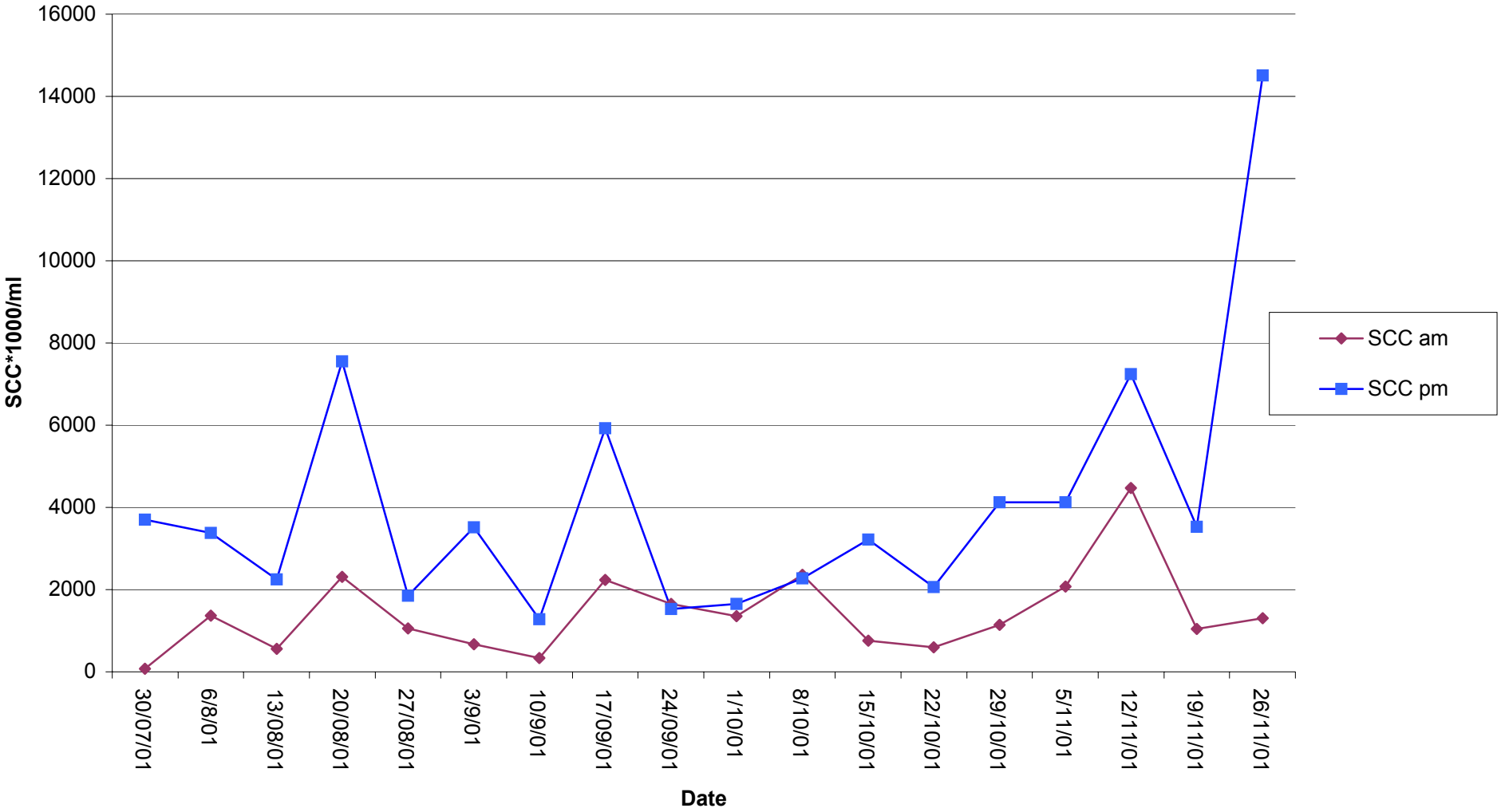


Fig 6n) Somatic Cell Count Goat 605

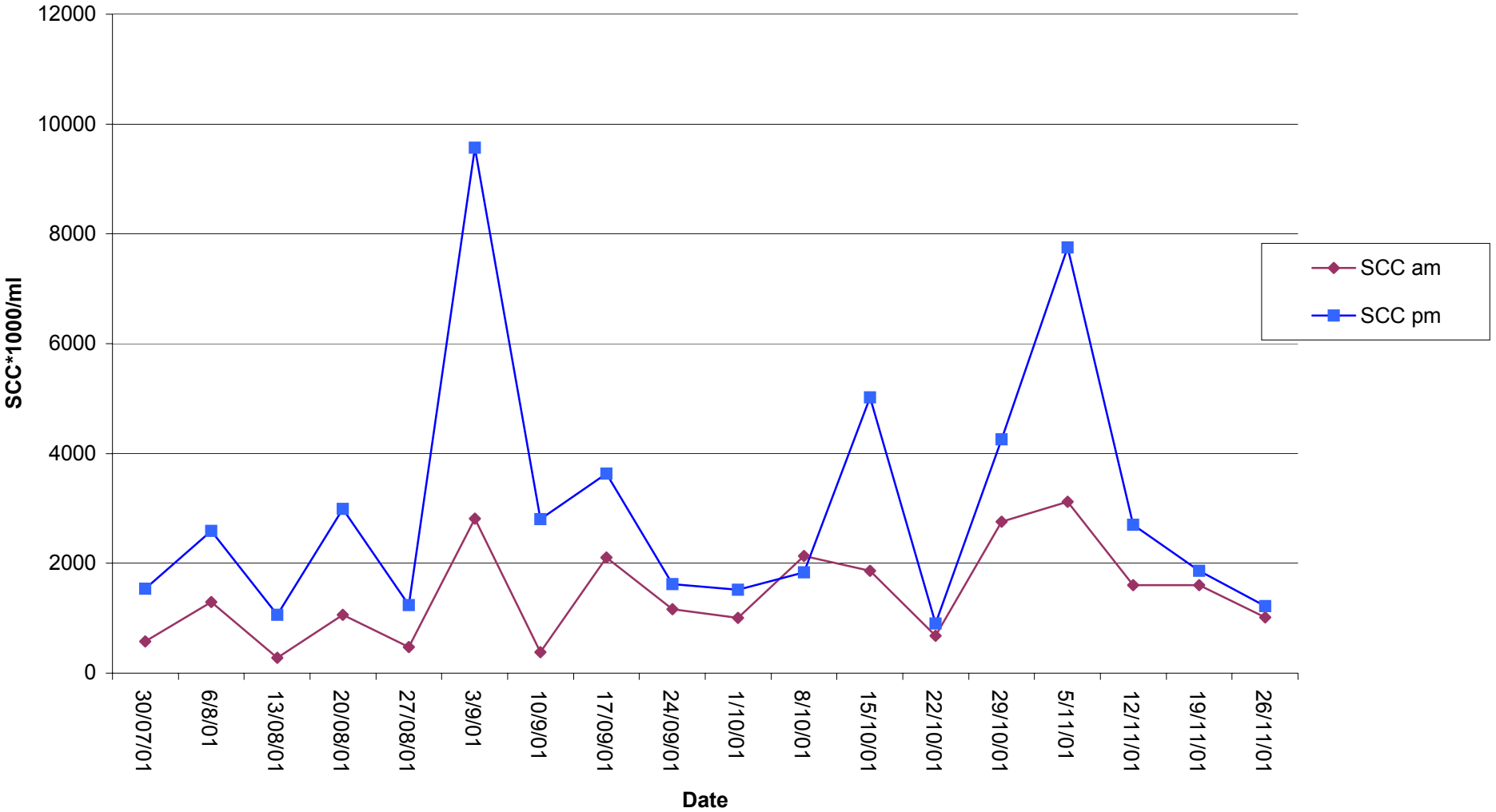


Fig 6o) Somatic Cell Count Goat 637

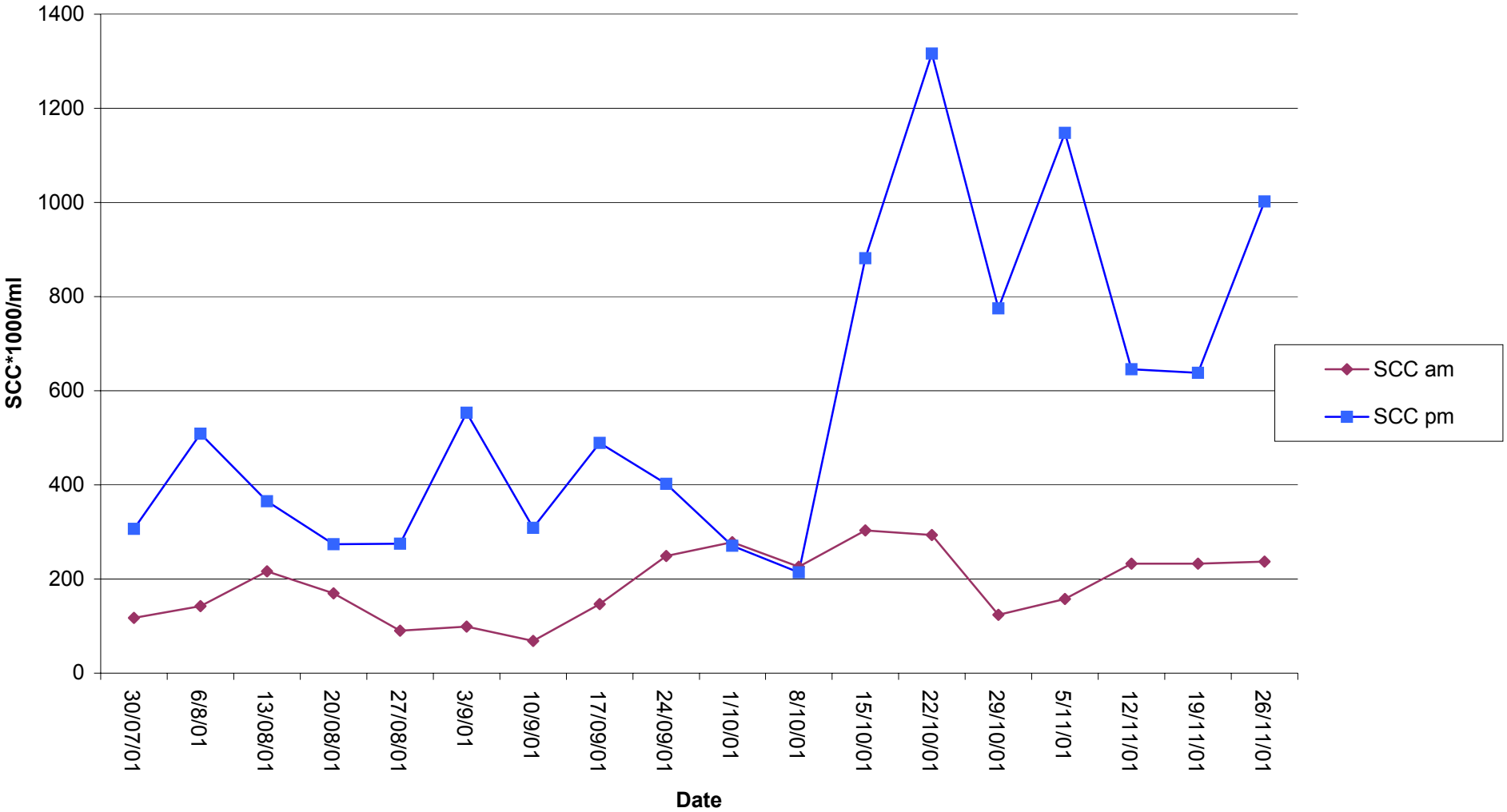


Fig 6p) Somatic Cell Count Goat 705

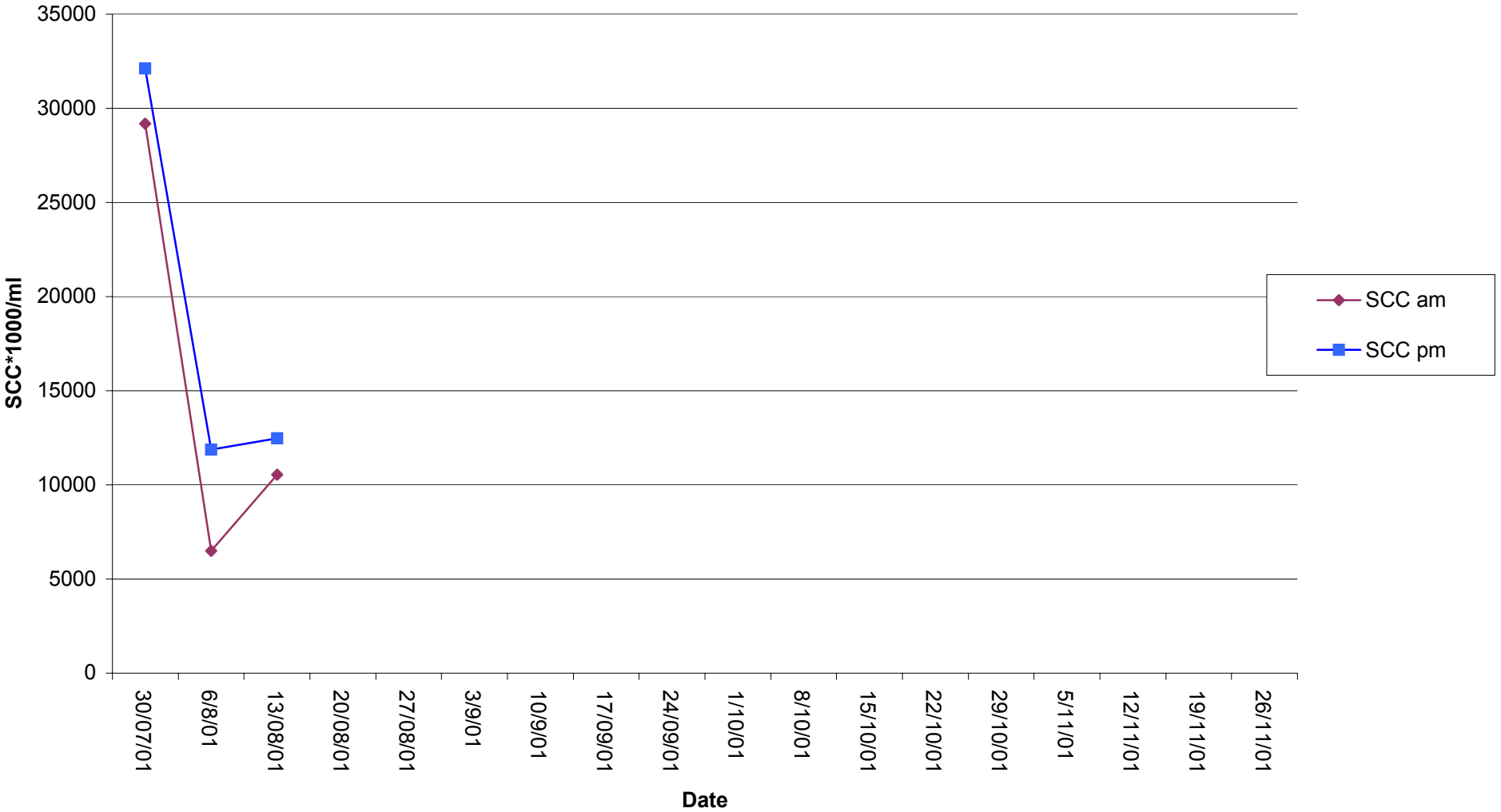
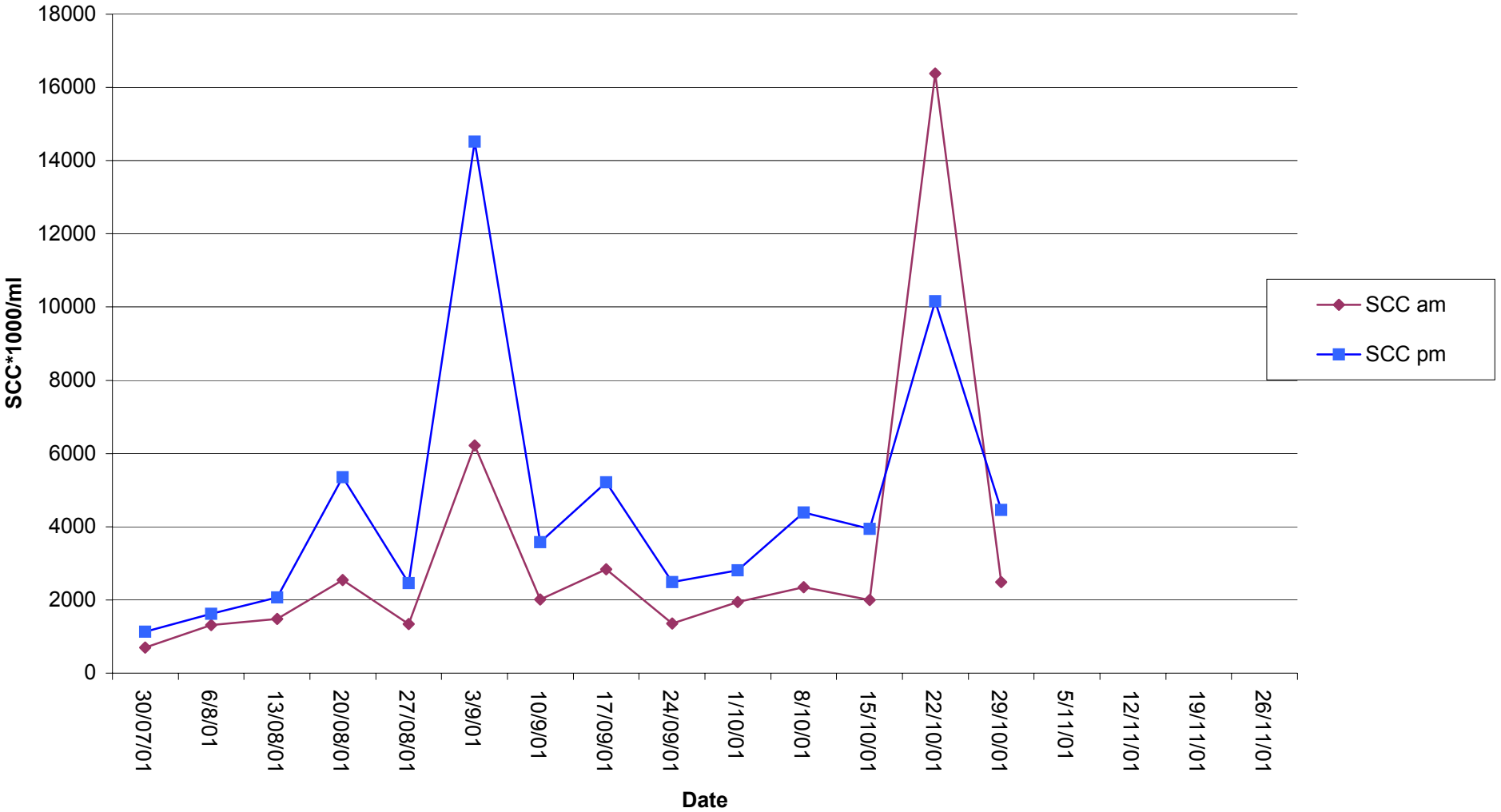


Fig 6q) Somatic Cell CountGoat 899



Statistical Analysis of Lactational and Heat Stability Data

INTRODUCTION

Statistical analysis of lactational data was undertaken in order to understand further the determinants of ALP concentration in goats' milk and how heat treatment affected ALP in these samples.

Study Design

Seventeen goats from the Hannah herd were observed during lactation between May 2001 and early February 2002. Goats were milked twice daily and a total of 8470 milk samples were collected. A subset of 1998 milk samples was tested for ALP concentration. ALP assessments were available for 1413 untreated samples. A further 17 samples were only tested for ALP after heat treatment at 95^{o}C. The remaining 568 samples were split into sub-samples. This enabled 517 samples to be tested for ALP untreated and after heat treatment at both 63^{o}C and 95^{o}C. 17 samples were tested untreated and after 63^{o}C heat treatment and 34 samples untreated and after 95^{o}C heat treatment. For each goat data were available on its age, lactation number, number of kids and casein genotype. Throughout the study its milk yield, stage of lactation and pregnancy status were available. Milk compositional analysis providing protein, fat and total solids percentages and somatic cell counts was available for a subset of the milk samples collected between 30 July and 17 December inclusive. These variables and untreated ALP concentrations were all available for a subset of 625 milk samples. These were approximately equally split between the morning and afternoon milkings. If the dataset is further restricted to those samples where morning and the corresponding afternoon milkings are both present and goat 705 is excluded (due to health problems) then the subset is reduced slightly to 600 samples. Of these, 316 samples (all from morning milkings) were split into sub-samples and tested for ALP without heat treatment and after heat treatment at 63^{o}C and 95^{o}C.

Statistical Analysis

The study generated a complex and rich data set requiring a range of different models to be fitted. The range of untreated ALP concentrations (505-1554125) and somatic cell counts (68-36978) necessitated a log transformation of these variables prior to further investigation as values differed by many orders of magnitude. A constant of one was added to all ALP concentrations prior to log transformation as ALP was not detected in some of the samples heat treated at 95^{o}C.

As already stated, the study investigated both the determinants of ALP concentration prior to any heat treatment and also the effect of heat treatment. As only a subset of samples was heat treated and these were all confined to morning milkings, two separate sets of analyses have been undertaken. The first investigated the determinants of untreated ALP. The second investigated the effects of heat treatments (no heat treatment, 63^{o}C and 95^{o}C) and whether the magnitude of these effects was related to other recorded covariates.

a) Untreated ALP

The first stage was the application of a range of exploratory data analysis techniques to the data. Boxplots classified by goat and morning/afternoon milking were produced for each variable associated with milk (yield, compositional analysis components, ALP concentration and somatic cell counts) in order to identify potential outliers in the dataset. This is a univariate check which considers each variable in isolation from other variables. Clearly, however, if covariates are related then unusual values in one covariate may in fact be explainable in terms of unusually high or low values of another covariate. (For example, high ALP concentrations tended to be associated with high somatic cell counts.) Consequently,

samples with particularly high or low values were checked to see if they had unusual values for more than one covariate. Bivariate scatterplots for key covariates were also inspected.

A preliminary investigation of whether ALP concentration in untreated milk differed between morning and afternoon milkings was undertaken by plotting afternoon concentrations against the corresponding morning samples. Similar plots were obtained for total ALP (i.e. after taking into account the lower milk yield in the afternoon which might influence concentration), somatic cell count concentration and total somatic cell count. (There were a total of 736 morning samples with corresponding afternoon ALP records.) Differences were compared formally by paired t-test.

For the subset of 600 samples with all variables recorded and matching AM and PM data, three types of correlation matrices investigating linear relationships between all pairwise comparisons of variables were produced and interesting relationships with ALP explored graphically. Firstly, correlations between the variables using the raw data (after transformation where appropriate) were computed. However, this fails to separate out relationships between variables at the "between-goat" and "within-goat" level. It is questionable to assume *a priori* that relationships at these two levels are the same. For example, it could be that although goats with higher average values for one variable also had higher values for a second variable, within animals there was no relationship between the two variables. In order to investigate this matter further two additional correlation matrices were produced. Correlations between variables using goat means enabled linear relationships at the "between goat" level to be investigated. Within-goat linear relationships were studied by first subtracting from each variable the respective individual goat means. Consequently, values for each variable then represented deviations from the corresponding animal's mean. Correlations between these deviations (standardised variables) reflected the strength of within-goat linear relationships.

These methods were intended to give a feel for the data and also some preliminary indication of whether relationships existed between the same pairs of variables at both levels. Its usefulness as a general technique is not only in identifying which variables and factors were likely to be most promising when trying to predict ALP but also identifying whether there were alternative sets of variables and factors which were likely to give models with similar predictive power.

As has already been indicated, observed relationships between pairs of variables might not *a priori* be the same at both the "between-goat" and "within-goat" levels. It was therefore important to model these two levels separately in order to check not only whether any relationship between a pair of variables existed at both levels but also whether any such relationship was similar. If there are different relationships at the two levels, this is informative and suggests that the relationship may be spurious.

At both levels statistical modelling was carried out using stepwise regression techniques. This is an iterative technique which forms a final model by successively adding or removing terms from the model. Removal of a term already in the model or addition of a new term to the model is determined by which change will produce the largest drop in unexplained variation which is also statistically significant. The process stops when removing any more terms would reduce the fit of the model significantly and the inclusion of any more terms would not significantly improve the model. For within-goat modelling it is necessary to fit individual goat effects first before starting the stepwise procedure. Caution is required when using such techniques as model determination is totally data driven. There is the danger that spurious terms may be added to the model. When datasets are large (as is the case here at the within-

goat level), the addition of a term may prove to be statistically significant but provide little improvement in the fit. Indeed there is a risk that small improvements from adding an extra term may not be reproducible if more modelling was repeated on a separate dataset. It is known that, in general, models fit better to the data used to generate them than to predict responses from a new dataset's explanatory variables. Additionally, since the technique adds and removes terms from a model following pre-specified rules, it does not consider all possible models that could be fitted. Indeed, given the number of variables recorded in this study, it would not be possible to consider all possible combinations of variables and factors which might be included in a model. There is therefore no absolute guarantee that the model fitted is the best possible model. In practice, we are likely to obtain a model which is close to the optimum but it should be remembered that there may well be other models that would perform equally well. Just because certain variables have been selected, it does not mean that other variables could not have served instead.

Univariate regressions between ALP and individual explanatory variables were computed at both levels to check that the relationships were indeed similar both between and within animals. However, direct comparison of regression coefficients between two models when both have more than one explanatory variable in them is not usually possible as explanatory variables tend to be correlated with each other.

There is also confounding and partial confounding in the dataset. This is possibly easiest seen in the context of the inter-relationship of goat effects, stage of lactation and sampling date. If a model includes differences between goats and also sampling date, the stage of lactation cannot be added to the model. This is because the difference between sampling date and the stage of lactation is a constant over time for any given individual animal. Other explanatory variables such as milk yield and compositional data are correlated (i.e. confounded) with each other to a greater or lesser extent. This means, for example, that variable A may be an important predictor before adjustment for variable B but not after adjustment for variable B.

For each model fitted the percentage variation explained has been quoted. This is commonly known as the "adjusted R^2". This is the percentage variation in the response variable explained by the model after adjusting for the number of predictors included in the model. An adjustment is needed as the inclusion of further terms would inevitably increase the percentage variation accounted for although this might not be reproducible.

b) Comparison of untreated and heat treated ALP

There were a total of 316 morning milk samples which were split into triplicate sub-samples and tested without heat treatment and after heat treatment at 63^{o}C and 95^{o}C. By restricting attention to only milk samples tested under all heat conditions, this ensures that differences observed between heat treatments are not due to differences between milk samples. These 316 morning milk samples were approximately equally distributed across all the goats. Each goat provided between 16 and 21 samples towards the total of 316. Various graphical inspections of the data have been undertaken. Log transformed ALP concentrations for the three treatment group sub-samples were plotted against the rank of the corresponding untreated ALP concentration. This enabled a visual comparison of how heat treatment at 63^{o}C and 95^{o}C changed the concentration relative to each other and also relative to untreated ALP as untreated ALP concentration increased. Additionally, the three treatment sub-samples were plotted against their milk yield, protein, fat and total solid percentages.

In order to formally test for overall effects of heat treatments, the three groups were compared by two-way analysis of variance using the 316 milk collection samples as a blocking factor. In order to investigate whether the extent of treatment effects was related to any of the covariates

collected, regression analyses were necessary. The same requirement to study both "between-goat" and "within-goat" relationships was necessary here as for when studying the determinants of untreated ALP. In order to study "between-goat" effects, a table of goat X treatment means for log transformed ALP and goat means for each covariate were calculated. Regression models were fitted to the log transformed ALP means to assess the improvement from fitting a common covariate slope or separate slopes for each treatment after allowing for separate intercepts. Each potential covariate was considered in turn. Models including more than one covariate were considered. Stepwise regression modelling was also investigated.

At the within-goat level, goat means for each covariate were subtracted and similar regression approaches adopted. For each of the three treatment groups, separate correlation matrices for "between-goat" and "within-goat" linear relationships were constructed. Similarly, correlation matrices between the raw covariates were also produced which do not distinguish between relationships at the two levels.

RESULTS

a) Untreated ALP

Morning and corresponding afternoon ALP concentrations have been plotted against each other in Figure 1. There were two very low morning ALP concentrations for Goats 601 (623) and 610 on 19 June (530). The corresponding afternoon ALP concentrations were 37085 and 12550 respectively. These two values have been checked and no error was found in data entry. Nevertheless, the values do look very suspicious indeed. For neither of these observations were compositional analysis or somatic cell counts available and so these data-points were automatically excluded when subsequently modelling ALP as a function of somatic cell counts. A line at 45^{o} through the origin has been drawn on the graph. If there was no consistent difference between AM and PM ALP concentrations then points ought to be randomly scattered around the line. In practice, it can be seen that observations are almost entirely above the line. A formal statistical test by paired t-test showed that the afternoon ALP concentration was significantly higher ($p<0.001$) than the morning. The 95% confidence interval for the mean difference on the log scale between afternoon and morning was (0.3322, 0.3827). This corresponds very approximately on the back-transformed scale to a confidence interval for the ratio of PM:AM ALP concentrations of (1.39, 1.47). Milk yield was known to be lower ($p<0.001$) in the afternoon than the morning with a 95% confidence interval for the mean difference of (0.51,0.55) litres. Hence, as an aside, there was interest in whether total ALP differed between morning and afternoon collections. A similar plot for total ALP (Figure 2) showed that, in contrast, total ALP was higher in the morning than the afternoon. Formally, a paired t-test showed that the total ALP in the afternoon was significantly ($p<0.001$) lower than the morning with a 95% confidence interval for the mean difference between afternoon and morning of (-0.4249, -0.3679). This corresponds very approximately on the back-transformed scale to a confidence interval for the ratio of PM:AM total ALP of (0.65,0.69).
Similar plots for somatic cell count concentration and total count are shown in Figures 3 & 4 respectively. Goat 640 had an exceptionally low somatic cell count (73) on the morning of 30 July. The corresponding afternoon count was 3705. Again, somatic cell count concentrations were significantly higher ($p<0.001$) in the afternoon than the morning with a 95% confidence interval on the log scale of (0.6437,0.7743). This corresponds very approximately on the back-transformed scale to a confidence interval for the ratio of PM:AM somatic cell concentrations of (1.90, 2.17). However, for total somatic cell count visual inspection did not indicate such a consistent pattern although the mean was significantly higher in the morning ($p=0.008$) than the afternoon with a 95% confidence interval on the log scale of (0.0242, 0.1581). This corresponds very approximately on the back-transformed scale to a confidence interval for the ratio of PM:AM total somatic cell count of (1.02, 1.17).

Figure 1 : Comparison of AM & PM ALP concentrations

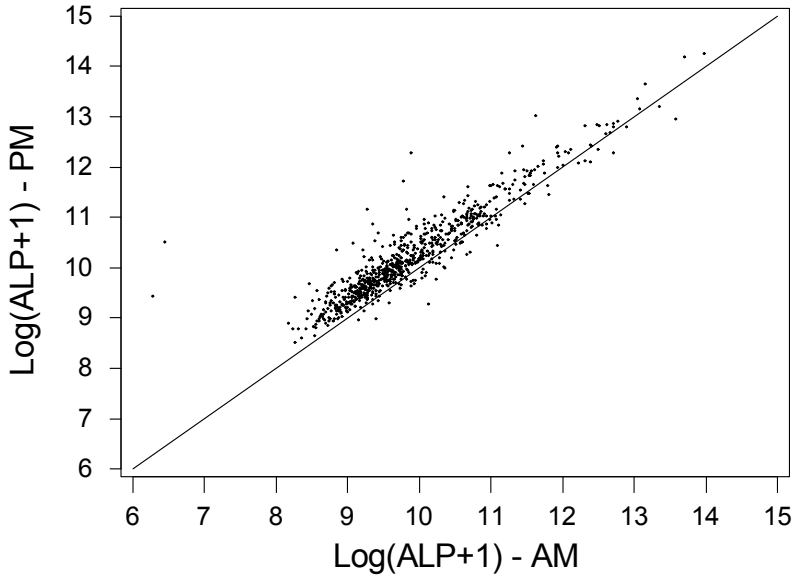


Figure 2 : Comparison of AM & PM Total ALP

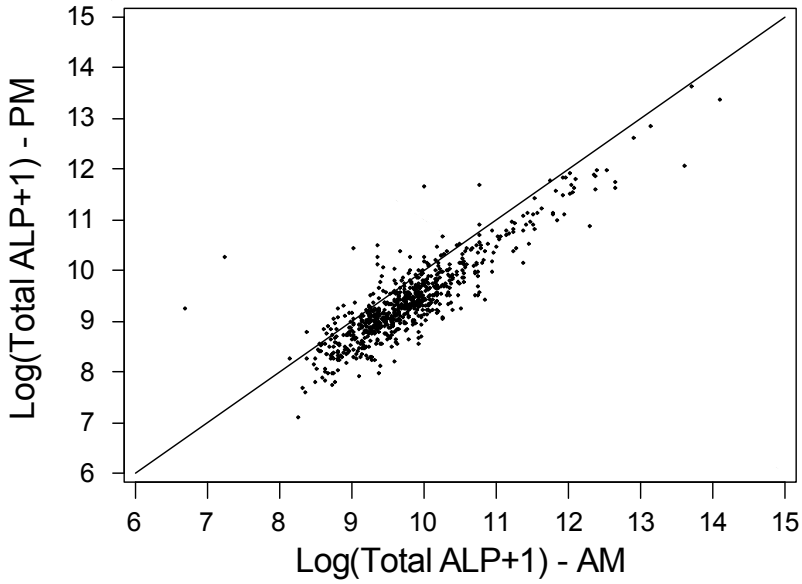


Figure 3 : Comparison of AM & PM ALP somatic cell counts

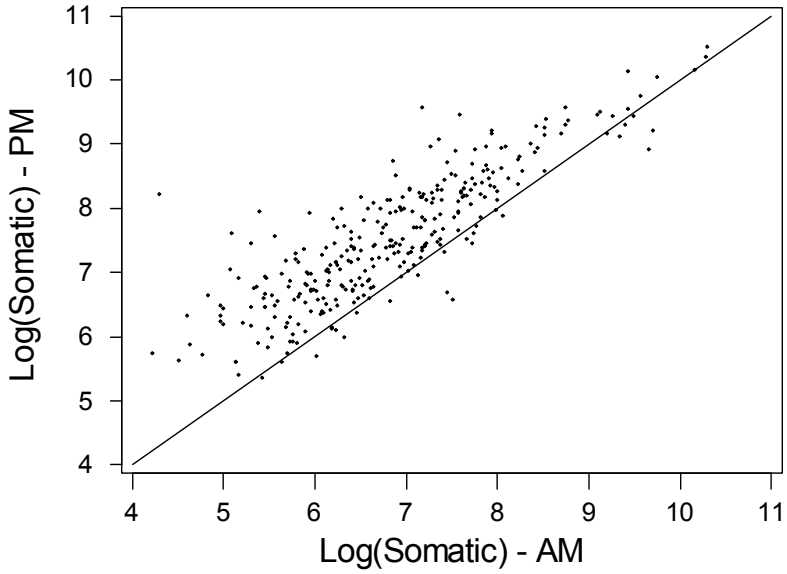
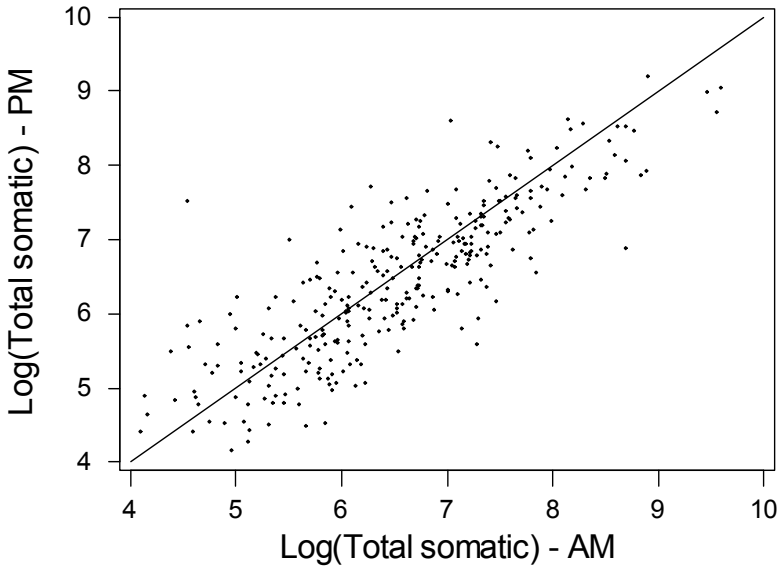


Figure 4: AM & PM ALP Total somatic cell count comparison



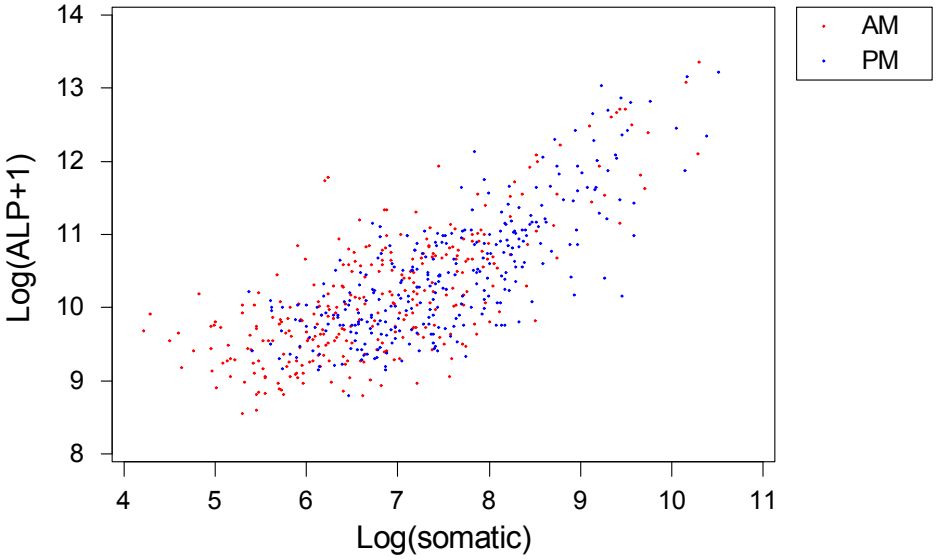
One goat (tag number 705) had considerably higher ALP concentrations than other animals. Both her kids died at birth and she herself died early in the study. Hence there is good reason to believe that she was atypical and perhaps ought to be excluded from statistical analyses. As somatic cell count and milk compositional data were collected less frequently than other variables and in any case only from 1 July onwards, the early death of this animal means that somatic cell counts are only available for her at three dates. Consequently, her subsequent omission from the dataset for formal analysis and correlation matrices has negligible impact on the results.

For the 600 untreated milk samples on which all variables were recorded and for which both AM and corresponding PM milk samples were available, a correlation matrix (not distinguishing between "within goat" and "between goat" relationships is shown in Table 1. Indicator variables have been included for AM/PM and pregnancy status (using the values 1 and 2 to denote AM and PM respectively and "not pregnant" and pregnant respectively).

Table 1: Combined within & between correlation matrix based on 600 milk samples with complete data

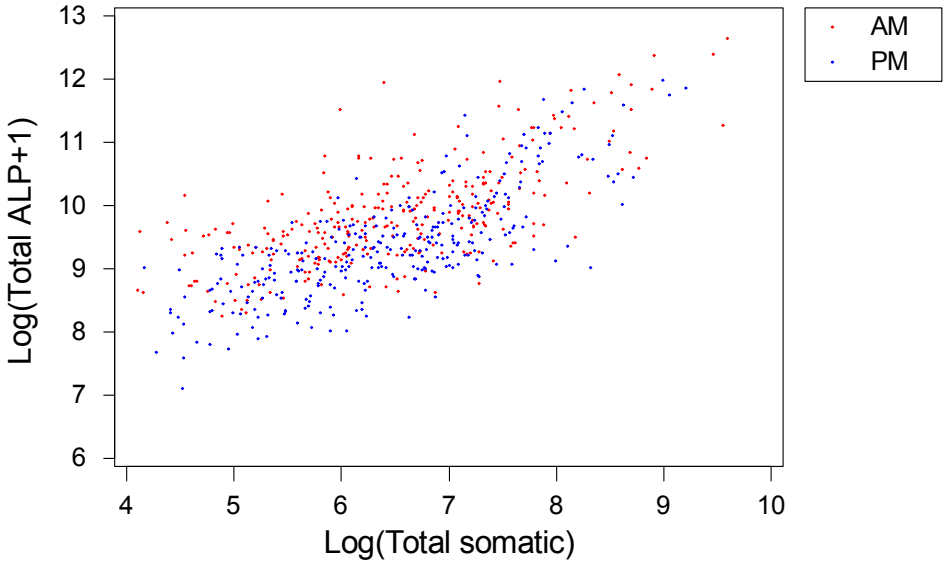
Log (ALP+1)	1.000											
Log (somatic)	0.744	1.000										
[Log(somat)]^2	0.762	0.995	1.000									
Milk yield	-0.304	-0.359	-0.359	1.000								
Log(Milk yld)	-0.361	-0.397	-0.398	0.941	1.000							
Protein	0.529	0.491	0.514	-0.563	-0.579	1.000						
Fat	0.191	0.272	0.264	-0.566	-0.564	0.270	1.000					
Total solids	0.310	0.366	0.365	-0.686	-0.691	0.566	0.917	1.000				
Collection date	0.158	0.125	0.138	-0.360	-0.352	0.613	0.173	0.351	1.000			
Lactation days	0.090	0.093	0.103	-0.300	-0.294	0.521	0.152	0.303	0.945	1.000		
Pregnant	0.019	0.037	0.046	-0.190	-0.173	0.322	-0.052	0.073	0.748	0.710	1.000	
AM/PM	0.181	0.319	0.305	-0.675	-0.674	0.108	0.595	0.545	0.000	0.000	0.000	1.000
	Log ALP	Log Somat	[Log somat]^2	Milk yield	Log Milk	Protein	Fat	Total solids	Coll date	Lact days	Preg status	AM / PM

Figure 5 : Plot of ALP and somatic cell concentrations



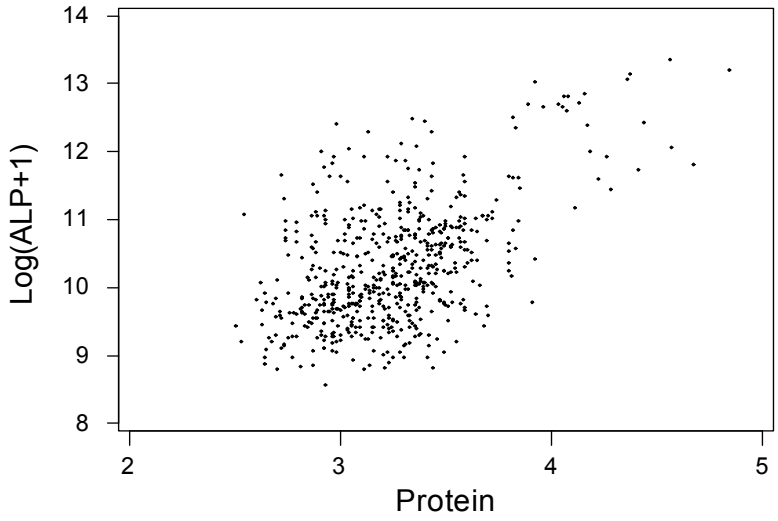
In Figure 5 all untreated ALP observations are plotted against their corresponding somatic cell count, from which a relationship between ALP and somatic cell concentrations is indicated. Hence some of the high ALP values can be seen to be associated with the corresponding high somatic cell counts. (Figure 6 shows total ALP plotted against total somatic cell count.)

Figure 6 : Plot of Total ALP and somatic cells



Due to the slight curvature evident in the figure, it was decided to include both the log of the somatic cell count and the square of the log as potential explanatory variables in all the correlation matrices and also in the stepwise regression procedures. Indeed, correlations were slighter stronger if the logged somatic cell count was squared than not as the former dealt with the slight curvature in the relationship between ALP and somatic cell count. Additionally, correlation matrices include the log transformed milk yield as this puts milk yield on a comparable scale to log transformed ALP. Figure 7 shows ALP plotted against percentage protein composition.

Figure 7: Plot of ALP concentration and %protein in milk



Within-goat and between-goat correlation matrices are shown in Tables 2 & 3 respectively. It is evident from the between and within goat correlation matrices that the highest correlations at both levels with log(ALP+1) were for functions of logged somatic cell counts but protein was also correlated. Other variables such as milk yield, total solids and fat were correlated at the within-goat but not at the between-goat level. These correlations were investigated graphically. Within-goat scatterplots for ALP with squared log somatic cell concentrations, protein and milk yield are shown in Figures 8-10 respectively. Scatterplots for other covariates with ALP are included in appendix A.

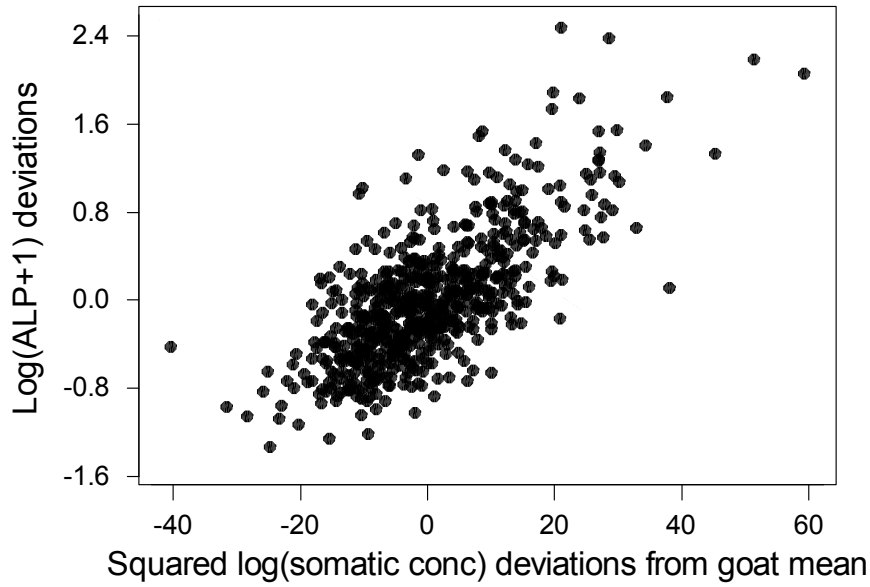
Table 2: Within-goat correlation matrix based on 600 milk samples with complete data

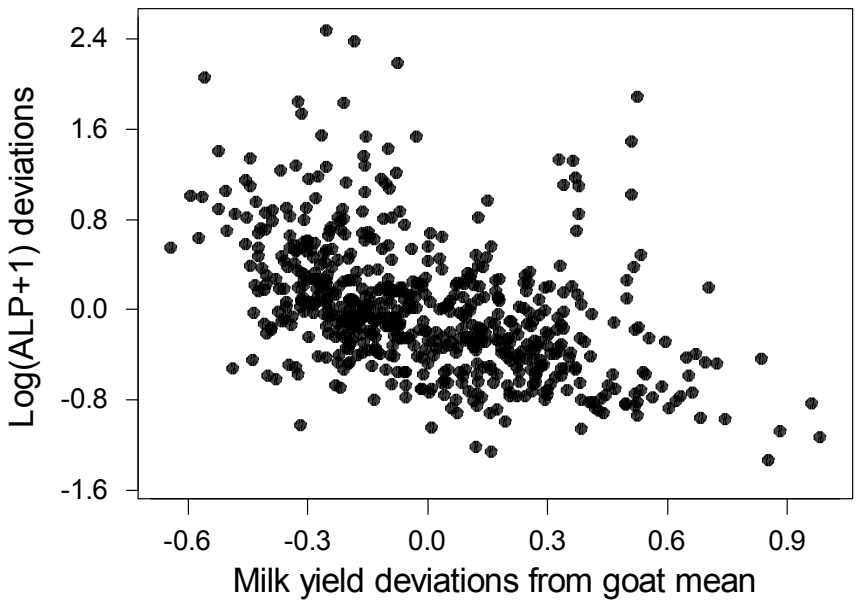
Log (ALP+1)	1.000											
Log (somatic)	0.697	1.000										
[Log(somat)]^2	0.716	0.992	1.000									
Milk yield	-0.462	-0.551	-0.548	1.000								
Log (Milk yld)	-0.478	-0.548	-0.545	0.935	1.000							
Protein	0.525	0.438	0.470	-0.566	-0.576	1.000						
Fat	0.389	0.522	0.506	-0.598	-0.613	0.297	1.000					
Total solids	0.495	0.587	0.584	-0.699	-0.724	0.569	0.927	1.000				
Collection date	0.294	0.222	0.234	-0.469	-0.477	0.740	0.204	0.415	1.000			
Lactation days	0.294	0.222	0.234	-0.469	-0.477	0.740	0.204	0.415	1.000	1.000		
Pregnant	0.118	0.129	0.135	-0.304	-0.303	0.427	-0.029	0.122	0.740	0.740	1.000	
AM/PM	0.269	0.445	0.421	-0.751	-0.764	0.123	0.629	0.593	0.000	0.000	0.000	1.000
	Log ALP	Log Somat	[Log somat] ^2	Milk yield	Log Milk	Protein	Fat	Total solids	Coll date	Lact days	Preg status	AM / PM

Table 3: Between-goat correlation matrix based on 600 milk samples with complete data

Log (ALP+1)	1.000													
Log (somatic)	0.798	1.000												
[Log(somat)]^2	0.817	0.998	1.000											
Milk yield	-0.146	-0.072	-0.063	1.000										
Log (Milk yld)	-0.286	-0.203	-0.200	0.973	1.000									
Protein	0.646	0.663	0.667	-0.569	-0.610	1.000								
Fat	-0.138	-0.273	-0.284	-0.423	-0.379	0.226	1.000							
Total solids	0.104	0.008	-0.002	-0.634	-0.583	0.616	0.874	1.000						
Collection date	-0.325	-0.285	-0.266	0.747	0.776	-0.325	-0.347	-0.393	1.000					
Lactation days	-0.360	-0.243	-0.243	0.556	0.549	-0.450	-0.252	-0.371	0.578	1.000				
Age	0.194	0.268	0.252	-0.439	-0.514	0.045	0.098	0.069	-0.764	-0.341	1.000			
Kids DOB	0.262	0.141	0.151	-0.273	-0.249	0.370	0.119	0.239	-0.165	-0.900	0.004	1.000		
No. of kids	-0.096	-0.143	-0.129	0.405	0.400	-0.212	0.155	0.012	0.278	0.140	-0.442	-0.021	1.000	
Lactation no.	0.263	0.273	0.257	-0.419	-0.510	0.046	0.205	0.116	-0.810	-0.322	0.965	-0.043	-0.379	1.000
	Log ALP	Log Somat	[Log somat] ^2	Milk yield	Log Milk	Protein	Fat	Total solids	Coll date	Lact days	Age	Kids DOB	No. of kids	Lact no.

Figure 8:Within goat ALP and somatic cell concentrations variation





The corresponding between-goat scatterplots for ALP with squared log somatic cell concentrations, protein and milk yield are shown in Figures 11-13 respectively. Scatterplots for other covariates with ALP are included in appendix B.

Figure 11:Goat means for ALP concentration & somatic cells

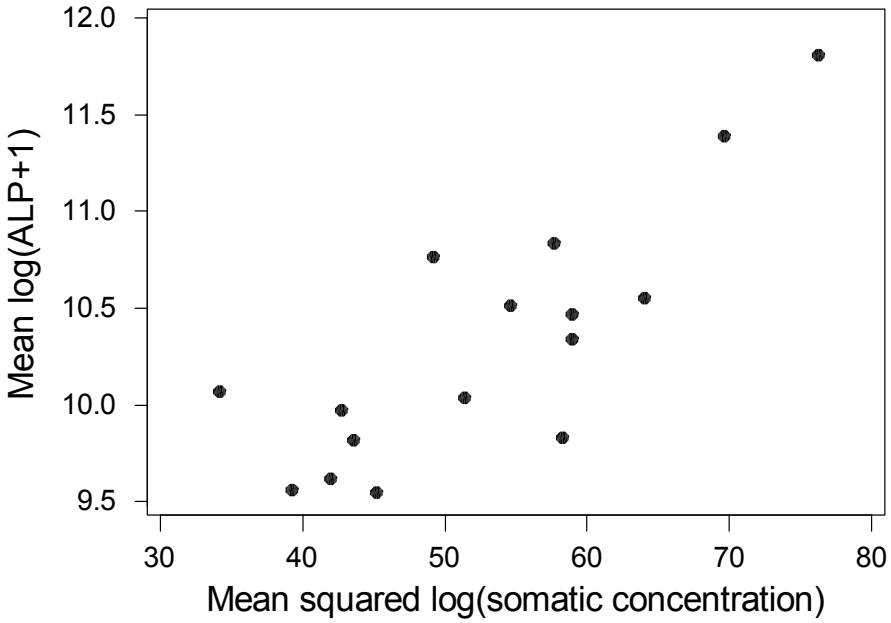


Figure 12: Goat means for ALP concentration & Protein

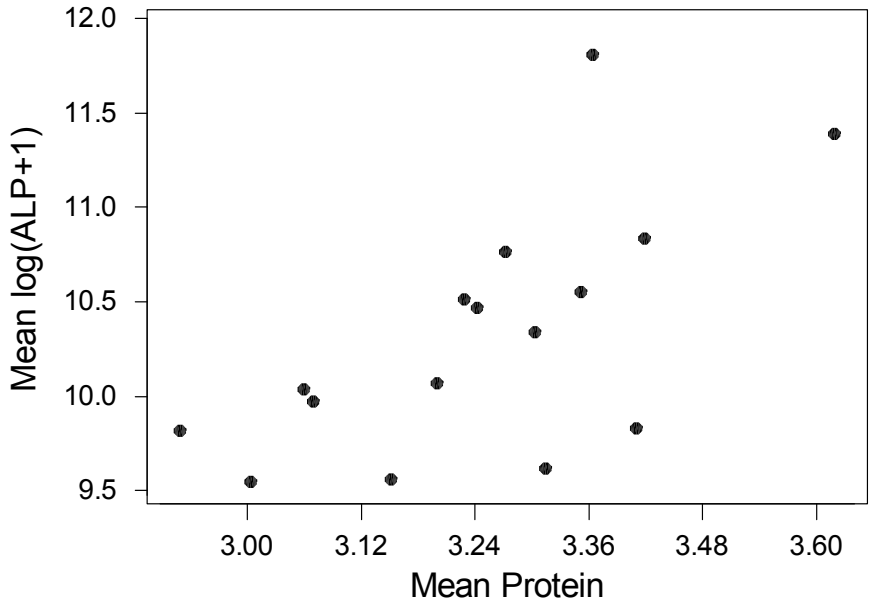
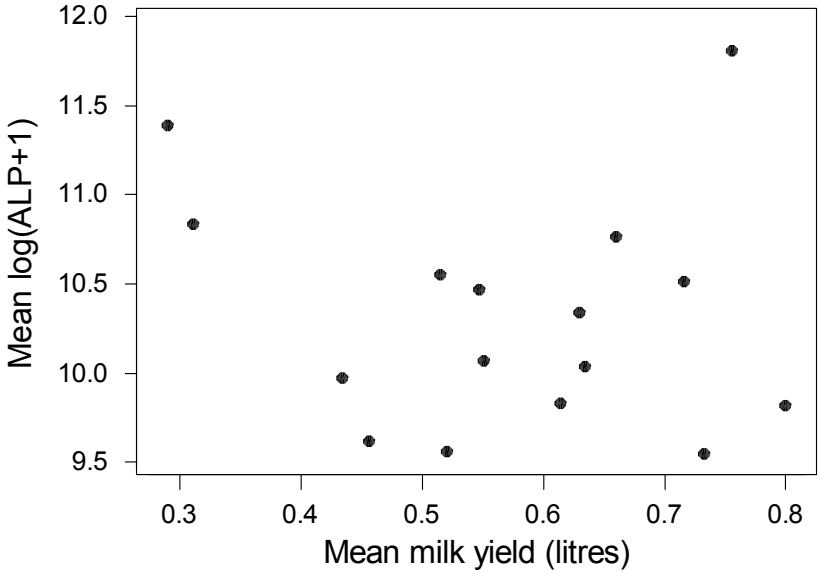


Figure 13:Goat means for ALP concentration & milk yield



The graphs show that at both levels, logged ALP increased as squared log somatic cell concentration increased. Similar patterns were observed (although less strongly) as percentage protein increased. There was the suggestion that ALP concentration decreased as milk yield increased at the within-goat level. This seemed more apparent at very low milk yields. However, at the between-goat level such a relationship was not clear.

53.6% of the variance in the ALP data could be attributed to differences between goats. The dominant term identified at the within-goat level was the squared logged somatic cell count. When added to a model already including goat, this increased the percentage variation accounted for up to 77.3% and explained 51.2% of the within-goat variation. Adding protein concentration to the model increased the percentage variation accounted for slightly to 79.4% and explained 55.7% of the within-goat variation. (Finally adding pregnancy status caused a negligible increase to 79.7% of variation explained with 56.1% of within-goat variation explained.)

The first two stepwise models were :-

$$\text{Log(ALP+1)}=\text{Goat}+0.03506*[\text{log(somatic)}]^2$$
$$(0.00142)$$

$$\text{Log(ALP+1)}=\text{Goat}+0.02948*[\text{log(somatic)}]^2+0.4678*\text{Protein}$$
$$(0.153) \qquad (0.0602)$$

Alternatively, if protein was included in the model after goat but somatic cell count was not, then the total variance accounted for was 66.4% of which only 27.5% of the within-goat variation was explained.

Log(ALP+1)=Goat+1.0132*Protein
(0.0680)

At the between-goat level, stepwise regression only selected the squared logged somatic cell count and this explained 64.3% of the variation at this level. (In comparison to the within-goat level, the statistical power at the between-goat level was low.) None of the factors and variates which differed solely at the between-goat level (age differences, lactation number, genotype, number of kids or date of giving birth) were found to be related to between-goat ALP levels. It should be noted that the numbers of goats on each genotype were low as the sixteen animals covered five genotypes. Indeed there was only a single animal on each of B2E and AbB2 genotypes. There were four, five and five goats belonging to B2E, EE and FF genotypes respectively. These between-goat factors and variates were to a greater or lesser extent confounded with each other.

The fitted model at the between-goat level was :-

$$\text{Log(ALP+1)}=7.888 + 0.04599*[\text{log(somatic)}]^2$$
(0.469) (0.00869)

The regression coefficient for squared logged somatic cell count estimated at the between and within levels were consistent with each other. This would add weight to the justification for using somatic cell count as a predictor of ALP concentration

Unlike at the within-goat level, stepwise regression did not include protein with somatic cell concentration in the model at the between-goat level. Indeed, if it was forced into the model then the percentage variance accounted for (adjusted R^2) actually fell to 63.7%, indicating that it provided no additional predictive power over that already afforded by the squared log somatic cell concentration. If protein was included in the between level model when somatic cell count was excluded, then the total variance accounted for was only 37.5% and the fitted model was :-

Log(ALP+1)=2.61 + 2.376*Protein
(2.44) (0.751)

Although the estimate of the coefficient for protein was larger at the between level than the within level, they were not statistically significantly different. very different at the two levels of modelling. However, the fact that protein did not improve a model at the between-goat level already including somatic cell count would cast some doubt on the usefulness of protein as a predictor. It would suggest that the overall model combining both levels should include somatic cell count but not protein with it.

The proposed model combining both within-goat and between-goat variation in terms of explanatory variables is:-

$$\text{Log(ALP+1)}=8.1854 + 0.04022 * [\text{log(somatic)}]^2$$
(0.0770) (0.00140)

This explained a total of 58% of the variation in the data.

If only protein was included then only 27.8% of the variation is accounted for in the overall model.

Log(ALP+1)=5.998 + 1.3272*Protein
(0.284)(0.0872)

As already explained, the predictive ability of protein is debatable. Even if a combined model including both somatic cell count and protein was fitted, it would only explain 60.5% of the variation which is a very small improvement over a model only including somatic cell count (58%).

In order to determine how critical somatic cell count was to determining ALP, stepwise regression models explicitly excluding somatic cell counts were fitted at both levels. At the between-goat level protein was included in a model and the inclusion of total solids narrowly failed to give a significant improvement (p=0.065). This could either be because it is not important or due to lack of power. The inclusion of both these terms explained 48.7% of the variation at the between goat level. The between-goat model was :-

Log(ALP+1)=5.20 + 3.449*Protein –0.464*Total_solids
(2.56) (0.864) (0.230)

No other variables approached statistically significance at the between-goat level. Attempting to fit a within-goat model including both protein and total solids explained 33.1% of the within-goat variation and both terms were statistically significant. However, if such a model was fitted to both levels simultaneously, the inclusion of total solids did not provide a statistically significant improvement over only including protein in the model and the percentage variation accounted for was unchanged.

At the within-goat level, as has already been stated, somatic cell count alone explained 51.2% of the within-goat variation. In contrast, if somatic cell count was excluded, stepwise regression fitted a complex model involving protein, fat, collection date, log of milk yield and the morning/afternoon indicator variable but, even so, explained only 36.6% of the within-goat variation. (Most of these terms negligibly improved the percentage variation accounted for.) It may be noted that the inclusion of only log transformed milk yield and the morning/afternoon indicator (corresponding to different intercepts) explained 24.8% of the within-goat variation. Hence, even at the within-goat level it was evident that somatic cell count was critical. Given the critical importance of somatic cell count, there seemed little benefit from attempting to fit a model excluding compositional data to the complete set of untreated ALP samples which included samples omitted from the former models due to the lack of compositional data.

b) Comparison of untreated and heat treated ALP

Figure 14 shows the corresponding ALP concentrations for samples under the regimens against the rank of the untreated sample. From this it is evident that ALP concentration was substantially reduced by heat treatment. It is clear that ALP was generally lower after 95^{o}C heat treatment than 63^{o}C heat treatment. However, the precise pairing is difficult to see in this figure. It is clearer when considering a plot of ALP after 63^{o}C heat treatment against ALP for the corresponding sub-sample at 95^{o}C (see Figure 15). In fact 33 out of the 316 samples had higher ALP concentrations after the lower heat treatment than the higher. Table 4 shows the results of a formal test by analysis of variance with heat treatment modelled as a fixed factor and sample as a random factor. There was a highly significant ($p<0.001$) difference in ALP concentrations between each of the regimens. 95^{o}C heat treated milk had lower ALP concentrations than 63^{o}C heated treated milk which was in turn lower than untreated milk.

Table 4 : Log(ALP+1) means for regimens

Treatment	Untreated	63°C	95°C	s.e.d.	P value
Mean	10.182	4.314	2.831	0.070	P<0.001

From graphical inspection it would appear that there were two parallel bands for 63°C heat treated ALP. At the higher heat treatment (95°C) it is evident that a comparatively small number of samples (22) had no detectable ALP after heat treatment whilst the remaining 292 samples had higher ALP concentrations. The reason why these samples were so different could not be ascertained. Six of the 22 values were from the same day of milk collection. The values were not specific to only one or two goats. Figures 16-20 show ALP for the three regimens plotted against squared log transformed somatic cell count, protein, fat, total solids and milk yield. In each case the separation remained at the higher heat treatment. The cause of the seeming separation into two bands at the lower heat treatment becomes apparent when individual goat means for heat treated ALP are considered later.

Figure 14: Treated & untreated ALP for matched sub-samples

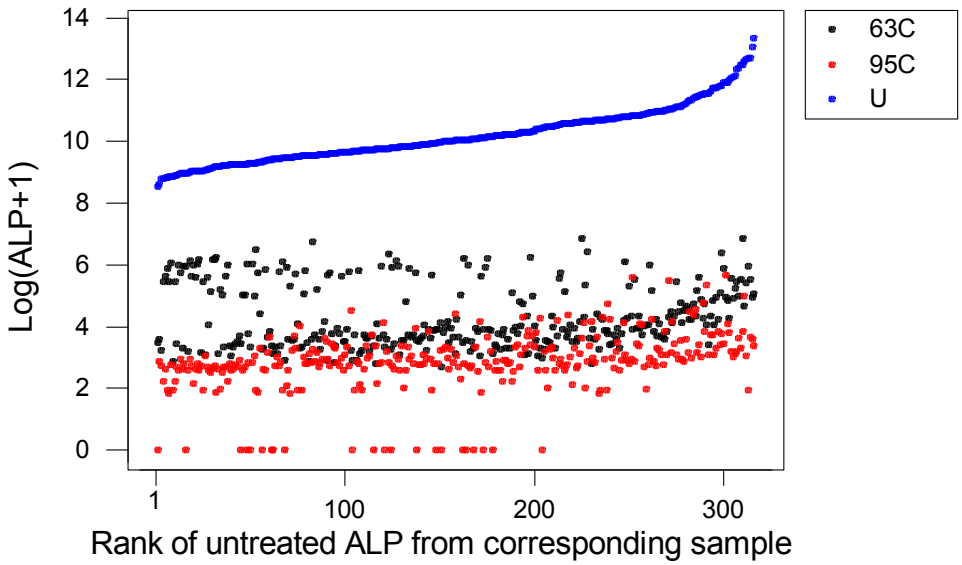


Figure 15 : ALP concentrations after 63C and 95C heat treatment

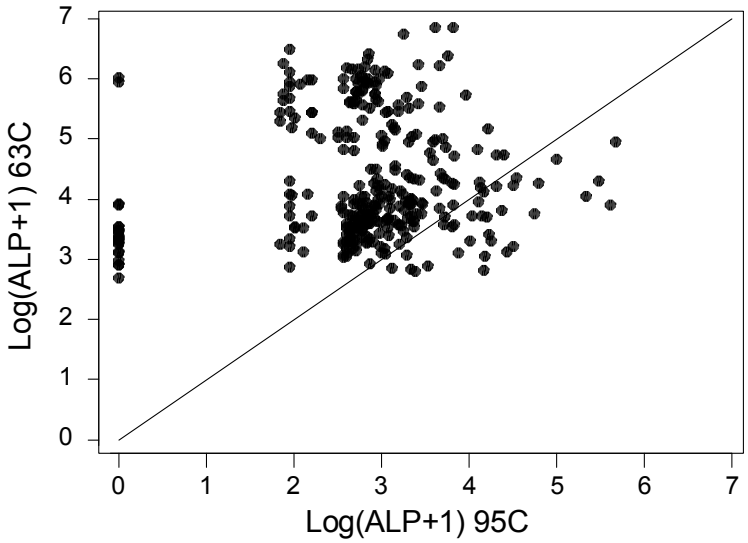


Figure 16: ALP v Squared log somatic cells

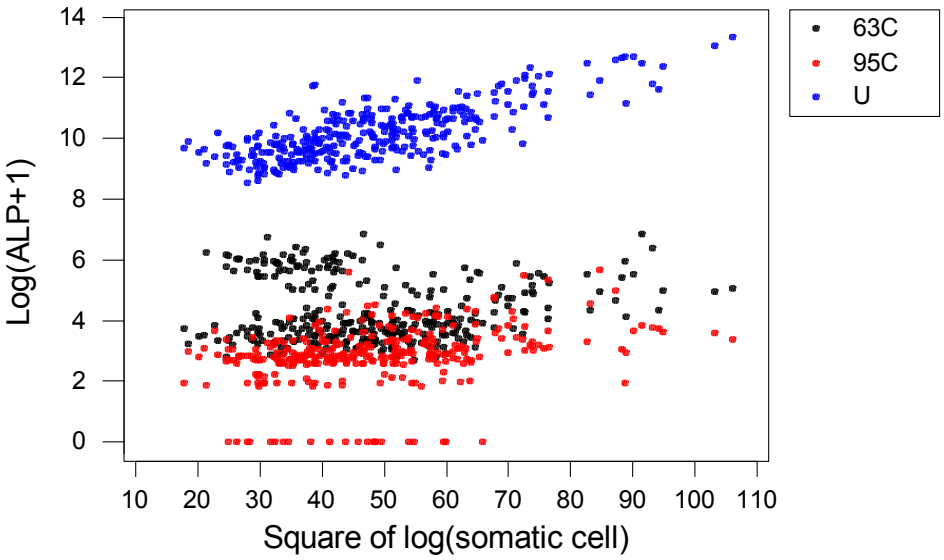


Figure 17: ALP v Protein

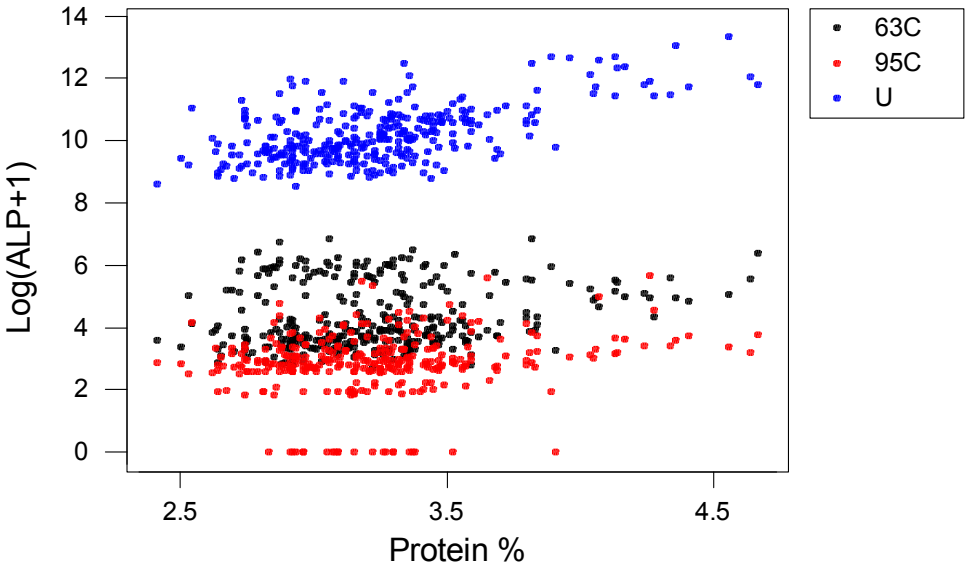


Figure 18: ALP v Fat

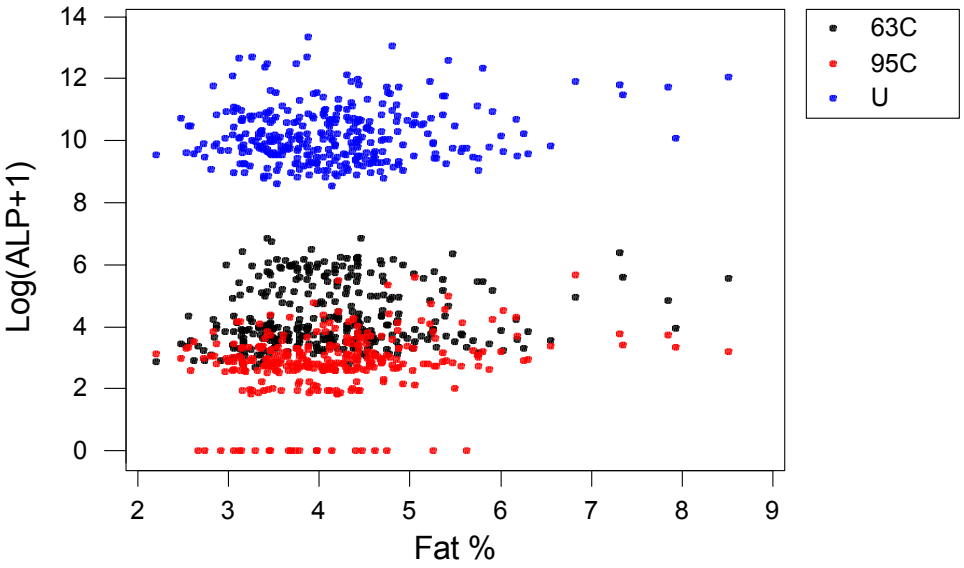


Figure 19: ALP v Total solids

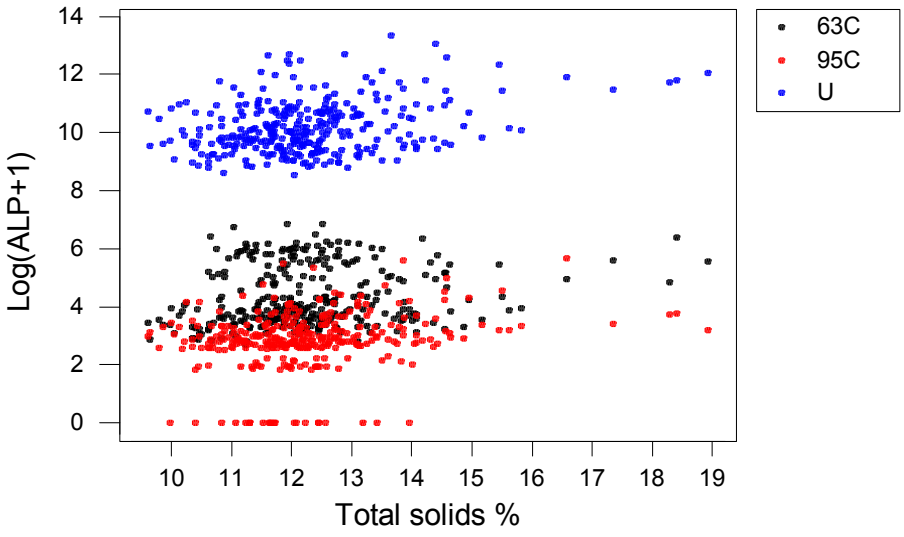
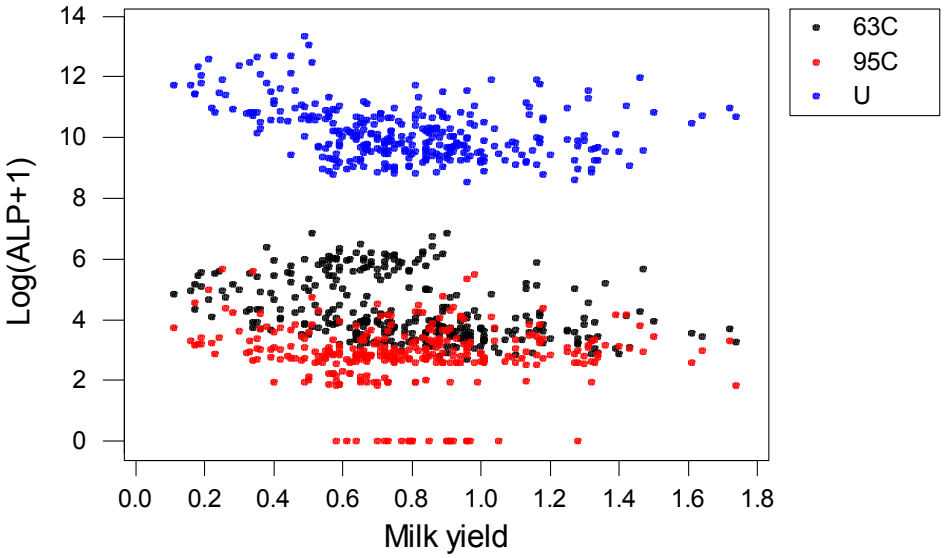


Figure 20 : ALP v Milk yield



Of course, the previous figures do not make any distinction between the within and between goat levels of variation. At the between-goat level, it is evident that the dominant factor is heat treatment and that any other covariate (either with a common or separate slope for each treatment group) would have comparatively little impact relative to the heat treatment itself. Correlation matrices at the between-goat level are displayed in Tables 6-8 for untreated, 63^{o}C and 95^{o}C heat treated sub-samples respectively. Corresponding matrices for the within-goat level are shown in Tables 9-11. It is worth noting that when comparing matrices in tables 6-8 with each other or matrices in Tables 9-11 with each other that only the first column differs from matrix to matrix within the set of three matrices. This is because only ALP concentration varies depending on treatment. The other covariates such as milk yield and protein content are from the same common samples and hence correlations within these covariates are identical from matrix to matrix.

At the between-goat level the covariate explaining the most variation was the squared log of the somatic cell count although protein and milk yield were also important. Figures 21-23 show the between goat relationships between ALP and the squared log somatic cell concentration, protein and log transformed milk yield respectively. Table 5 summarises the adjusted R^2 (percentage variance accounted for) for models where significant improvements were obtained by including a covariate with or without separate slopes for each of the three treatment groups. Stepwise regression fitted a common slope with log

(milk yield) as an explanatory variable. It should be noted that none of these models explained a large proportion of the variation left unexplained by heat treatment alone.

Table 5 : Summary of various between-goat models with significant covariates

Terms	Covariate	Slope for covariate	Adjusted R^2
Treatment group	-	-	95.4%
Treatment + covariate	Milk yield	Common slope	95.9%
Treatment + covariate	Log(Milk yield)	Common slope	96.0%
Treatment + covariate	Log(somatic conc)	Separate slope	96.5%
Treatment + covariate	Squared log(somatic)	Separate slope	96.6%

The addition of further covariates was investigated for models already including either somatic cell count or log milk yield. In both cases the inclusion of the other gave a statistically significant improvement. Separate slopes for protein were also found to give a statistically significant improvement to a model already including log(milk yield). However, these latter models should be viewed with extreme caution. A large number of possible models have been fitted and the best ones identified on the basis of their fit to data from a small number of goats and they therefore may not be reproducible. Additionally, correlation coefficients for ALP with somatic cells and protein were very different for the 63^{o}C heat treatment than the other two groups which is of concern. It is apparent from Figure 21 that this was due to four goats having high ALP after 63^{o}C heat treatment. (These were goats 617, 618, 890 and 891.)

At the within-goat level, again treatment group was the dominant factor with other variables explaining comparatively little variation. The inclusion of squared log somatic cell count with a separate slope for each group was a statistically significant improvement although it only explained approximately 10% of the remaining variation. Protein and milk yield would seem to be other alternatives although they each explained less variation than somatic cell count. Figures 24-26 show the between goat relationships between ALP and the squared log somatic cell concentration, protein and log transformed milk yield respectively. Stepwise regression fitted a complex model including treatment group effects, squared log somatic cell counts, total solids and collection date. However the adjusted R^2 value only increased from 93% to 93.8% with the addition of all these terms after treatment group and the inclusion of only a common slope for squared log somatic cell count increased it to 93.6%.

The regression model at the within-goat level including somatic cell count was :-

$\text{Log(ALP+1)} = \text{Goat effect} + 4.4067 + 0.03732 * [\text{log(somatic)}]^2$ (Untreated)
$\text{Log(ALP+1)} = \text{Goat effect} -1.4617 + 0.02169 * [\text{log(somatic)}]^2$ (63^{o}C)
$\text{Log(ALP+1)} = \text{Goat effect} -2.9450 + 0.0145 * [\text{log(somatic)}]^2$ (95^{o}C)

In all three cases the standard errors for the intercept and slope were 0.0467 and 0.00443 respectively.

The regression model at the between-goat level including somatic cell count was :-

$\text{Log(ALP+1)} = 7.976 + 0.0458 * [\log(\text{somatic})]^2$ (Untreated)
$\text{Log(ALP+1)} = 5.055 - 0.0148 * [\log(\text{somatic})]^2$ (63^oC)
$\text{Log(ALP+1)} = 1.610 + 0.0255 * [\log(\text{somatic})]^2$ (95^oC)

In all three cases the standard errors for the intercept and slope were 0.637 and 0.0128 respectively. The slopes at the between and within levels were consistent for the untreated and 95^oC heat treated ALP but the 63^oC heat treated ALP differed between levels. Indeed, the estimated slope at the between goat level was negative for 63^oC heat treated ALP in contrast to the other two groups due to four goats with high ALP levels.

The regression model combining both levels was :-

$\text{Log(ALP+1)} = 8.152 + 0.04219 * [\log(\text{somatic})]^2$ (Untreated)
$\text{Log(ALP+1)} = 4.224 + 0.00187 * [\log(\text{somatic})]^2$ (63^oC)
$\text{Log(ALP+1)} = 1.837 + 0.02064 * [\log(\text{somatic})]^2$ (95^oC)

In all three cases the standard errors for the intercept and slope were 0.156 and 0.00308 respectively.

Table 6: Between-goat correlation matrix for untreated milk based on 316 common samples

Log (ALP+1)	1.000													
Log (somatic)	0.796	1.000												
[Log(somat)]^2	0.814	0.997	1.000											
Milk yield	-0.296	-0.203	-0.200	1.000										
Log (Milk yld)	-0.463	-0.353	-0.355	0.966	1.000									
Protein	0.680	0.653	0.657	-0.733	-0.794	1.000								
Fat	-0.107	0.075	0.073	-0.388	-0.374	0.405	1.000							
Total solids	0.159	0.253	0.251	-0.664	-0.653	0.724	0.898	1.000						
Collection date	-0.188	-0.233	-0.206	0.548	0.553	-0.189	0.158	-0.008	1.000					
Lactation days	-0.242	-0.164	-0.164	0.391	0.392	-0.332	0.197	-0.034	0.493	1.000				
Age	0.244	0.340	0.326	-0.445	-0.522	0.192	0.049	0.107	-0.644	-0.184	1.000			
Kids DOB	0.212	0.110	0.119	-0.265	-0.265	0.311	-0.170	0.035	-0.238	-0.962	0.004	1.000		
No. of kids	-0.121	-0.174	-0.157	0.392	0.375	-0.242	-0.129	-0.165	0.319	0.108	-0.442	-0.021	1.000	
Lactation no.	0.322	0.346	0.333	-0.442	-0.546	0.219	0.056	0.109	-0.627	-0.137	0.965	-0.043	-0.379	1.000
	Log ALP	Log Somat	[Log somat]^2	Milk yield	Log Milk	Protein	Fat	Total solids	Coll date	Lact days	Age	Kids DOB	No. of kids	Lact no.

Table 7: Between-goat correlation matrix for 63°C heat treated milk based on 316 common samples

Log (ALP+1)	1.000													
Log (somatic)	-0.210	1.000												
[Log(somat)]^2	-0.199	0.997	1.000											
Milk yield	-0.375	-0.203	-0.200	1.000										
Log (Milk yld)	-0.313	-0.353	-0.355	0.966	1.000									
Protein	-0.016	0.653	0.657	-0.733	-0.794	1.000								
Fat	-0.012	0.075	0.073	-0.388	-0.374	0.405	1.000							
Total solids	-0.035	0.253	0.251	-0.664	-0.653	0.724	0.898	1.000						
Collection date	-0.246	-0.233	-0.206	0.548	0.553	-0.189	0.158	-0.008	1.000					
Lactation days	0.291	-0.164	-0.164	0.391	0.392	-0.332	0.197	-0.034	0.493	1.000				
Age	0.168	0.340	0.326	-0.445	-0.522	0.192	0.049	0.107	-0.664	-0.184	1.000			
Kids DOB	-0.402	0.110	0.119	-0.265	-0.265	0.311	-0.170	0.035	-0.238	-0.962	0.004	1.000		
No. of kids	-0.296	-0.174	-0.157	0.392	0.375	-0.242	-0.129	-0.165	0.319	0.108	-0.442	-0.021	1.000	
Lactation no.	0.126	0.346	0.333	-0.442	-0.546	0.219	0.056	0.109	-0.627	-0.137	0.965	-0.043	-0.379	1.00
	Log ALP	Log Somat	[Log somat]^2	Milk yield	Log Milk	Protein	Fat	Total solids	Coll date	Lact days	Age	Kids DOB	No. of kids	Lact no.

Table 8: Between-goat correlation matrix for 95°C heat treated milk based on 316 common samples

Log (ALP+1)	1.000													
Log (somatic)	0.744	1.000												
[Log(somat)]^2	0.755	0.997	1.000											
Milk yield	-0.445	-0.203	-0.200	1.000										
Log (Milk yld)	-0.575	-0.353	-0.355	0.966	1.000									
Protein	0.672	0.653	0.657	-0.733	-0.794	1.000								
Fat	0.177	0.075	0.073	-0.388	-0.374	0.405	1.000							
Total solids	0.397	0.253	0.251	-0.664	-0.653	0.724	0.898	1.000						
Collection date	-0.381	-0.233	-0.206	0.548	0.553	-0.189	0.158	-0.008	1.000					
Lactation days	-0.196	-0.164	-0.164	0.391	0.392	-0.332	0.197	-0.034	0.493	1.000				
Age	0.378	0.340	0.326	-0.445	-0.522	0.192	0.049	0.107	-0.644	-0.184	1.000			
Kids DOB	0.100	0.110	0.119	-0.265	-0.265	0.311	-0.170	0.035	-0.238	-0.962	0.004	1.000		
No. of kids	-0.020	-0.174	-0.157	0.392	0.375	-0.242	-0.129	-0.165	0.319	0.108	-0.442	-0.021	1.000	
Lactation no.	0.424	0.346	0.333	-0.442	-0.546	0.219	0.056	0.109	-0.627	-0.137	0.965	-0.043	-0.379	1.000
	Log ALP	Log Somat	[Log somat]^2	Milk yield	Log Milk	Protein	Fat	Total solids	Coll date	Lact days	Age	Kids DOB	No. of kids	Lact no.

Table 9: Within-goat correlation matrix based for untreated milk based on 316 common samples.

Log (ALP+1)	1.000									
Log (somatic)	0.499	1.000								
[Log(somat)]^2	0.517	0.991	1.000							
Milk yield	-0.342	-0.434	-0.466	1.000						
Log (Milk yld)	-0.365	-0.431	-0.466	0.935	1.000					
Protein	0.429	0.530	0.572	-0.780	-0.815	1.000				
Fat	0.247	0.366	0.372	-0.438	-0.480	0.533	1.000			
Total solids	0.351	0.469	0.492	-0.606	-0.659	0.764	0.930	1.000		
Collection date	0.252	0.317	0.328	-0.779	-0.776	0.759	0.441	0.611	1.000	
Lactation days	0.252	0.317	0.328	-0.779	-0.776	0.759	0.441	0.611	1.000	1.000
	Log ALP	Log Somat	[Log somat]^2	Milk yield	Log Milk	Protein	Fat	Total solids	Coll date	Lact days

Table 10: Within-goat correlation matrix based for 63°C heat treated milk based on 316 common samples.

Log (ALP+1)	1.000									
Log (somatic)	0.245	1.000								
[Log(somat)]^2	0.263	0.991	1.000							
Milk yield	-0.250	-0.434	-0.466	1.000						
Log (Milk yld)	-0.266	-0.431	-0.466	0.935	1.000					
Protein	0.252	0.530	0.572	-0.780	-0.815	1.000				
Fat	0.165	0.366	0.372	-0.438	-0.480	0.533	1.000			
Total solids	0.221	0.469	0.492	-0.606	-0.659	0.764	0.930	1.000		
Collection date	0.181	0.317	0.328	-0.779	-0.776	0.759	0.441	0.611	1.000	
Lactation days	0.181	0.317	0.328	-0.779	-0.776	0.759	0.441	0.611	1.000	1.000
	Log ALP	Log Somat	[Log somat]^2	Milk yield	Log Milk	Protein	Fat	Total solids	Coll date	Lact days

Table 11: Within-goat correlation matrix based for 95°C heat treated milk based on 316 common samples.

Log (ALP+1)	1.000									
Log (somatic)	0.154	1.000								
[Log(somat)]^2	0.157	0.991	1.000							
Milk yield	0.053	-0.434	-0.466	1.000						
Log (Milk yld)	0.025	-0.431	-0.466	0.935	1.000					
Protein	0.042	0.530	0.572	-0.780	-0.815	1.000				
Fat	0.228	0.366	0.372	-0.438	-0.480	0.533	1.000			
Total solids	0.178	0.469	0.492	-0.606	-0.659	0.764	0.930	1.000		
Collection date	-0.109	0.317	0.328	-0.779	-0.776	0.759	0.441	0.611	1.000	
Lactation days	-0.109	0.317	0.328	-0.779	-0.776	0.759	0.441	0.611	1.000	1.000
	Log ALP	Log Somat	[Log somat]^2	Milk yield	Log Milk	Protein	Fat	Total solids	Coll date	Lact days

Figure 21 : Between goat ALP and somatic cell variation

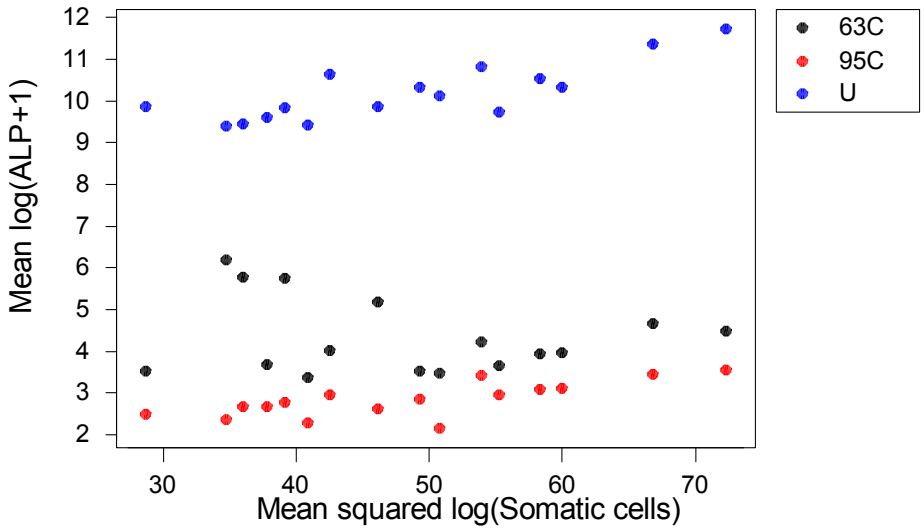


Figure 22 : Between goat ALP and protein variation

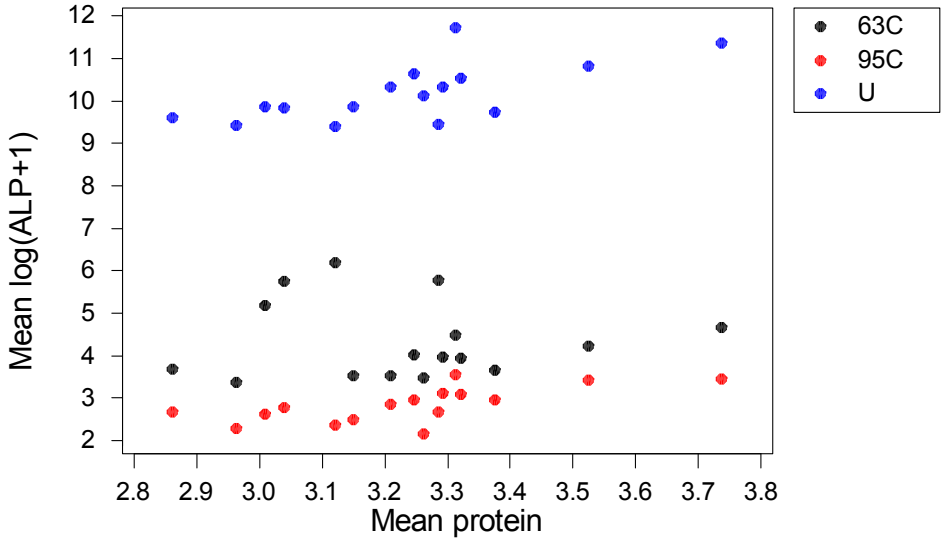


Figure 23:Between goat ALP and milk yield variation

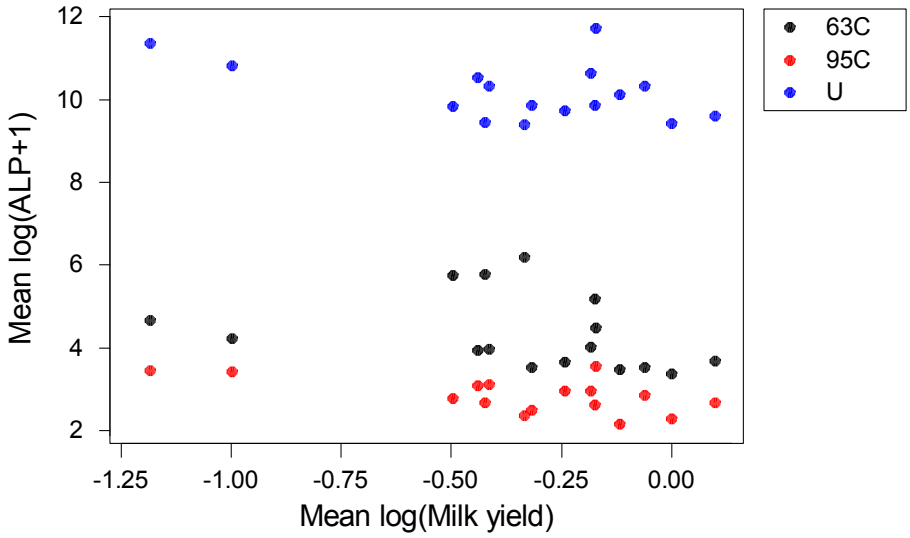


Figure 24 : Within goat ALP and somatic cell variation

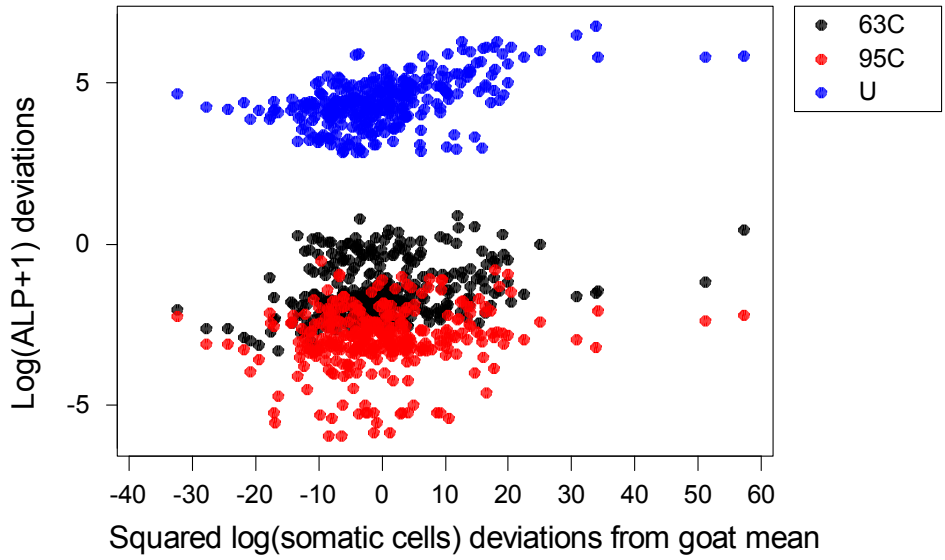


Figure 25 : Within goat ALP and Protein variation

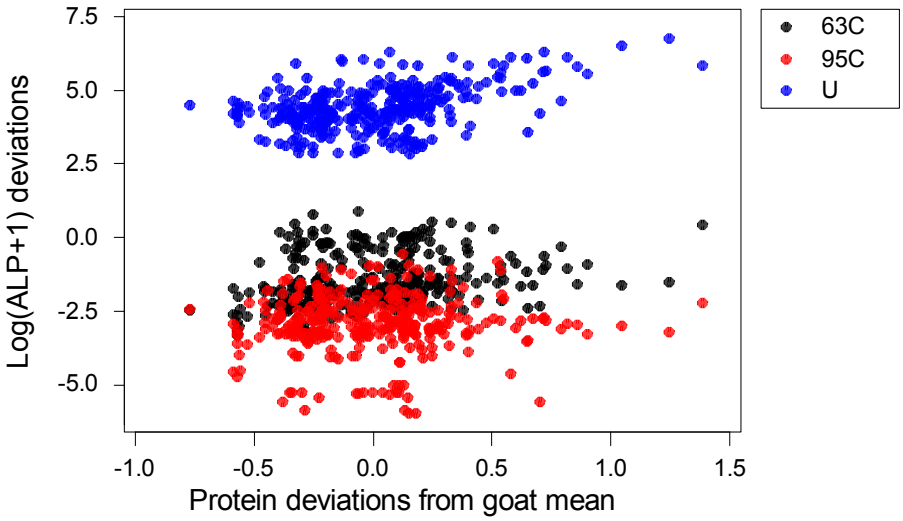
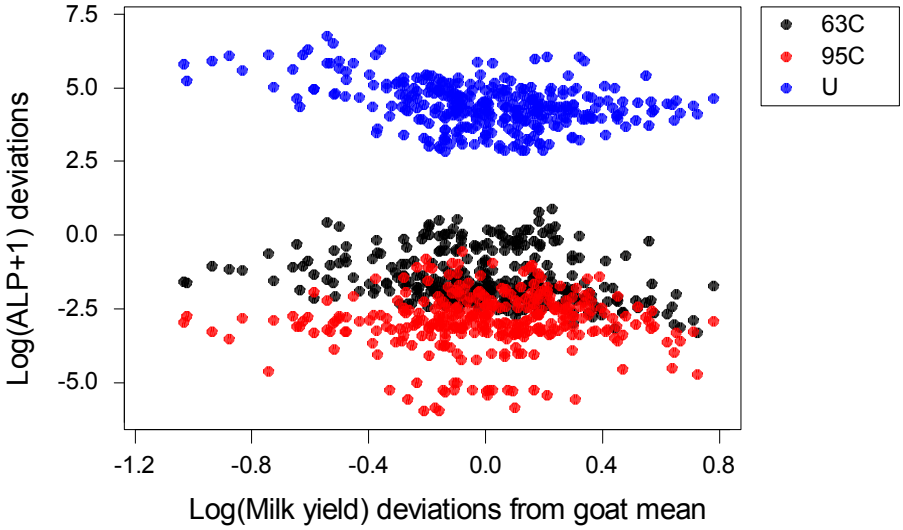


Figure 26 : Within goat ALP and Milk yield variation



CONCLUSIONS

Both ALP and somatic cell concentrations from untreated samples were higher in the afternoon than in the morning. However, milk yield was significantly lower in the afternoon than the morning. When ALP was expressed as a total rather than a concentration, total ALP was higher in the morning than the afternoon. Total somatic cell counts were on average higher in the afternoon than the morning but this was not as consistent. The most effective single explanatory variable for logged ALP concentration was the square of the logged somatic cell count. There were two reasons for this. Firstly, within both morning and afternoon datasets there was a relationship between ALP and somatic cell concentrations. Secondly, since both somatic cell count and ALP concentrations were higher in the afternoon than the morning, regressing ALP as a function of somatic cell count was able to model variation due to the time of day effect on ALP without explicitly including a term for time of day. Thus it was not necessary to include a separate intercept or slope for time of day in the model. There was an indication that the very highest ALP concentrations occurred when milk yield was very low although this was not clear at the between-animal level. The only statistically significant and consistent relationship with ALP found at both levels was for somatic cell count. It was evident that inclusion of somatic cell count in modelling was very important and that alternative models including functions of milk yield, for example, were inferior.

Heat treatment at both 63^{o}C and 95^{o}C significantly reduced ALP concentration compared to no heat treatment. On average, the higher heat treatment further reduced ALP concentration considerably more than the lower heat treatment although for 10% of samples the converse was the case. Four goats had very high ALP concentrations after heat treatment at 63^{o}C. When modelling the heat treatment data, squared log somatic cell count with a separate intercept and slope for each treatment group appeared to offer the best fit to the ALP data from the heat treatment comparison component of the study.

Ian Nevison
BioSS

Jean Banks
Donald Muir
Hannah Research Institute

Objective No.	Objective Description
02	**Comparison of effectiveness of bioluminescence, fluorescence and spectrophotometric methods in determining the efficiency of pasteurisation of goat, sheep and cow milk**

INTRODUCTION

Methods for testing residual ALP in pasteurised milk

All raw milk contains alkaline phosphatase although the amounts vary between individual species of animal, and individual animals within each species. In the 1930's it was shown that on heat treatment of milk at between 65-75°C so that at least 96% of the original alkaline phosphatase present in milk was destroyed resulted in complete destruction of *B. tuberculosis*. (Kay and Graeme 1930). In a complementary study at the same time Kay and Neave found that over all ranges of temperature and time *Mycobacterium tuberculosis* was destroyed more quickly than phosphatase. They concluded that if the temperature time combination was sufficient to destroy all of the phosphatase originally present in milk, then all common pathogenic microorganisms that may have been present would also be destroyed. These findings subsequently formed the basis of tests to determine whether or not milk had been properly pasteurised. All tests are based on the premise that in alkaline conditions alkaline phosphatase is able to hydrolyse various phosphate esters.

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In the UK, the now obsolete Milk Special Designation Regulations required that pasteurised milk should satisfy a test for residual phosphatase. The test specified was the Aschaffenberg and Mullen test, under which milk is satisfactorily pasteurised if it produces no more than 10ug of *p*-nitrophenol during a two hour incubation period. These regulations were superseded by the EC Official method (Commission Decision 91/180/EEC). Pasteurised milk is still required to satisfy a phosphatase test but this is based on the Sanders and Sager method (1946). Milk that is satisfactorily pasteurised should liberate no more than 4 μg phenol during a one hour incubation period. This test is not more sensitive than the Aschaffenburg and Mullen test and the limiting values in each test represent equivalent phosphatase activities since incubation times during testing differ between methods (Bruce, 2001). Both methods are set to the same cut off point which is equivalent to a 0.1% contamination with raw milk.

A number of variants of the test are used based on the fact that alkaline phosphatase will liberate phenol from disodium phenyl phosphate, p-nitrophenol from p-nitrophenyl

phosphate or phenolphthalein from phenolphthalein monophosphate, provided pH and temperature conditions are correct.

The amount of phenol, p-nitrophenol or phenolphthalein released from the substrate is proportional to the activity of any phosphatase remaining in the heat treated milk. Any phosphatase activity in milk is expressed in terms of µg phenol per ml of milk
In 1990 the USA-based Advanced Instruments developed a new substrate for use in the detection of alkaline phosphatase activity in milk. The substrate was known as Fluorophos, a non fluorescent monoester of orthophosphoric acid and an aromatic compound. When acted upon by alkaline phosphatase, the phosphate radical was cleaved from the Fluorophos molecule to produce a highly fluorescent molecule called fluoroyellow. Since the reaction product was fluorescent it was possible to determine the phosphatase activity of a milk sample in three minutes rather than the two hours required for the Aschaffenburg and Mullen method. A method for the determination of the phosphatase activity of milk and milk based drinks using a fluorometric method has been published as a dual British and ISO Standard (1997).

When the Fluorophos method was introduced it was claimed that it was possible to detect the presence of as little as 0.006% raw milk in pasteurized milk. The limit for the Fluorophos was set at 500mU per litre, this value corresponding to the phosphatase activity of pasteurized milk containing approximately 0.1% raw milk. In practice the majority of commercially produced samples with values of less than 50mU per litre.

Therefore if routine testing reveals a sudden increase in phosphatase activity to levels above those normally found this should act as a trigger to investigate the cause. This is the case, even if the elevated levels are still well below the generally recognized maximum limit of 500mU per litre.

The Charm Pas Lite system was also introduced in the 1990's. This system is produced by Charm Sciences and is distributed by Foss in the UK.

In the Charm Paslite system the substrate for alkaline phosphatase is a phosphorylated luminescent molecule. Hydrolysis of the substrate by alkaline phosphatase releases the phosphate radical, which allows the luminescent moiety to emit light. The method is rapid, producing a result for a single sample in 4 minutes.

The pass level currently used for the Charm PasLite system in liquid milk is 350mU of phosphatase activity per litre of product, which equates to 0.1% raw milk in pasteurised milk.

Application of tests to milk of different species
Recent reports in the literature highlight differences between species in the level of alkaline phosphatase in raw milk. The pool of ALP in goats milk has been shown to be approximately 20% of that in cows milk, while sheep milk contains 5-fold more alkaline phosphatase than cows milk (Assis *et al.*, 2000).

The statutory maximum level of residual alkaline phosphatase in correctly pasteurised sheep and goat milk is currently the same as for cows milk (500Mu per litre of milk). Because of the variation in the initial pool of indigenous alkaline phosphatase in milk, this leads to different amounts of raw milk being allowed in the pasteurised milk product at the statutory pass level.

Lactational study on goat milk

Bulk goat milk samples were collected during early, mid and late lactation and were pasteurised using the HRI pilot plant pasteuriser. The pasteurised milk samples were then contaminated with raw milk at levels ranging from 0.01 to 0.1%. In preliminary experiments this level of contamination was found to be too low for experiments with goat milk and none of the phosphatase methods for validating effectiveness of pasteurisation produced failures. The level of contamination for goat milk was therefore raised to between 0.1 and 1.0% for the lactation study.

Goat milk samples

Morning milk from 14 individual goats was collected and bulked at intervals of one week for a four week period in early, mid and late lactation. The bulked milk was pasteurised at 73±1°C for 16s using an APV pilot plant pasteuriser. The pasteurised milk was then re-contaminated with raw goat milk at levels of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0%.

Methodology

Phosphatase levels in milk were measured using the Fluorophos, Charm, and Sanders and Sager methods**.]**

(F-Fluorophos, Fail > 500 mU/L; C-Charm, Fail > 350mU/L; Phenol-Sanders and Sager method, Fail > 4μg/ml Phenol)

Results

Results for the Fluorophos, Charm and Sanders and Sager methods for phosphatase on goat milk are shown in Tables 1 to 3. In early lactation milk, none of the test methods produced failures on contamination of pasteurised milk with raw milk at a level of 1% (Table 1, August samples). The ALP activity in raw milk was between 19010 and 20077 mU/L as measured by the Fluorophos method. Measurements on raw milk ALP activity with the Charm instrument were ten fold lower than those obtained with Fluorophos. However values for contaminated milk using both methods were comparable.

Results from mid lactation studies are shown in Table 2. In mid lactation the higher base level of phosphatase in goat milk resulted in some positive results with the Fluorophos and the Sanders and Sager methods. The effectiveness of methods in detection of raw milk contamination was related to the initial level of phosphatase in the raw goat milk. The Sanderson and Sagers method produced failures on four occasions and the level of contamination at which failure was detected was related to the initial phosphatase level in the milk.

At an initial level of 46983 mU/L ALP in raw milk, neither Fluorophos or Charm produced failures at 1% contamination of pasteurised milk with raw milk. However the Sanders and Sager method produced a positive phosphatase result at 0.9% contamination. At the highest initial level of 82750mU/L ALP in raw milk the Fluorophos and the Sanders and Sager methods produced positive results at levels of 0.7% and 0.4% respectively. The Charm test did not give a positive result at 1% contamination of pasteurised milk with raw milk in any of the mid lactation samples.

For late lactation samples results are shown in Table 3. A limited number of goats were available and the samples taken had a low initial level of phosphatase in raw milk. Contamination of pasteurised milk with raw milk was therefore not detected in any of the samples examined.

Bovine milk samples – seasonal stud

Samples of bulk milk were taken from the HRI bulk tank at weekly intervals from August 2001 until January 2002. The milk was pasteurised at 73±1°C for 16s using an APV pilot plant pasteuriser.

Pasteurised milk was contaminated with a sample of raw bulk milk at levels of 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.11, and 0.12%. Phosphatase levels in contaminated milks, pasteurised milk and raw milk were measured using Fluorophos, Charm and the Sanders and Sager method. Results to date include those from 22 Fluorophos tests, 18 Charm tests and 7 tests using the Sanders and Sager method.

Results are shown in Tables 4-8. Levels of phosphatase in raw cow milk are approximately ten-fold higher than those seen in goat milk. 78% of pasteurised samples had ALP levels of less than 40mU/L while 43% had levels less than 30mU/L. Of the 22 samples tested with Fluorophos 27% gave a positive result at 0.1%. Of the 18 samples tested by the Charm method, 33% gave a positive result at 0.1% contamination. Using the method of Sanders and Sager 85% of the 7 samples produced a positive result at 0.1% contamination.

Table 1. Contamination of pasteurised goat milk with raw goat milk (full goat herd excluding 617, 618, 890 and 705)

Phosphatase Test failure highlighted in red

	Goat milk early lactation										
	1/8/01			8/8/01			15/8/01			22/8/01	
% RAW	F(mU/L ALP)	C(mU/L ALP)	Phenol ug/ml	F(mU/L ALP)	C(mU/L ALP)	Phenol ug/ml	F(mU/L ALP)	C(mU/L ALP)	Phenol ug/ml	F(mU/L ALP)	C(mU/L ALP)
0	16.3	14.5	0	23.9	11	0.14	22.05	8	0.05	30.1	14.5
0.1	30.1	36.5	0	27.6	28.5	0.18	41.15	24.5	0.32	49.65	35.5
0.2	54.25	29.5	0	48.7	48.5	0.22	55.4	39.5	0.32	60.9	38
0.3	63.7	47.5	0	68.7	60.5	0.18	67.8	56	0.41	95.6	53.5
0.4	75.85	68	0	91.9	73.5	0.41	86.65	77	0.86	98.15	67.5
0.5	87.6	78.5	0	109.6	91	0.58	104.8	104	0.91	131.7	76
0.6	109.2	90	0	119.5	97	0.46	126.4	109.5	0.73	144.8	96.5
0.7	118.4	97.5	0.29	145.7	128	0.82	128.75	115.5	0.73	159.05	102.5
0.8	138.4	105.5	0.96	147.35	145.5	0.62	148.25	112	0.82	184.6	120.5
0.9	159.3	120	1.73	172.85	109	0.912	160.9	127.5	0.91	211.7	132.5
1	153.1	129	1.92	185.95	141.5	1.032	168.7	136	0.91	228.25	135
100	20077.5	1475	112	22675	1950	98	19010	1125	113	21410	975

Table 2. Contamination of pasteurised goat milk with raw goat milk. (Full goat herd excluding 617, 618, 890 and 705)

Phosphatase Test failure highlighted in red

	Goat milk mid lactation											
	17/10/01			24/10/01			31/10/01			7/11/01		
% RAW	F(mU/L ALP)	C(mU/L ALP)	Phenol ug/ml	F(mU/L ALP)	C(mU/L ALP)	Phenol ug/ml	F(mU/L ALP)	C(mU/L ALP)	Phenol ug/ml	F(mU/L ALP)	C(mU/L ALP)	Phenol ug/ml
0	38.85	6.5	2.5	44.6	6.5	2.04	28.5	7	0.36	133.75	18.5	2.26
0.1	88.95	23.5	2.66	98.15	34.5	2.66	91.7	30.5	1.37	145.7	39	3.14
0.2	133.3	64	2.5	168.25	58	2.83	151.5	46	2.3	188	58	4.03
0.3	170.75	66.5	2.5	231	100	3.19	201.35	81	2.35	295.35	67.5	3.73
0.4	203.85	80.5	2.66	300.4	112.5	4.44	239.95	90.5	3.46	337.4	97.5	4.09
0.5	253.95	110	2.83	345	155.5	4.61	301.8	118	3.91	371.45	113	4.7
0.6	301.8	134.5	3.19	407.95	208	4.97	424.8	199	3.6	430.5	119.5	5.16
0.7	353.95	136	3.19	542.2	240	6.04	405.25	185.5	4.49	539.25	179.5	6.31
0.8	395.15	167	3.74	654.85	347	6.93	465.9	184.5	4.54	456.75	161.5	6.17
0.9	454.65	203	4.27	607	308	7.2	525.45	212.5	5.59	521.1	197.5	6.72
1	471.65	205	4.8	463.85	292.5	7.29	573.5	313.5	6.22	629.55	225.5	7.37
100	46982.5	3825	197	82780	6525	310	68242.5	2725	247	51315	2175	314

Table 3. Contamination of pasteurised goat milk with raw goat milk. (Full remaining goat herd)

Phosphatase Test failure highlighted in red

	Goat milk late lactation				
	9/1/02	16/1/02		23/1/02	
% RAW	F(mU/L ALP)	F(mU/L ALP)	C(mU/L ALP)	F(mU/L ALP)	C(mU/L ALP)
0	94.95	138.8	39.5	146.65	41
0.1	137.7	174.7	62	170.1	61.5
0.2	172.85	210.55	90.5	198.8	92.5
0.3	197.7	237.85	109.5	221.6	110.5
0.4	233.5	252.15	136	257	130.5
0.5	259.95	308.9	171.5	262.25	163
0.6	304.3	334.45	179	299.5	188
0.7	344.8	365.45	185.5	344.1	207.5
0.8	350.95	412.8	210	352.35	236
0.9	399.25	434.4	219	411.9	305.5
1	394.45	409.85	233.5	406.6	283
100	34592.5	34512.5	2350	26090	2025

Table 4. Contamination of pasteurised bulk cow milk with raw cow milk (HRI bulk tank-August)

Phosphatase Test failure highlighted in red

Cows milk	2/8/01			9/8/01			16/8/01		
% RAW	F(mU/L ALP)	C(mU/L ALP)	Phenol ug/ml	F(mU/L ALP)	C(mU/L ALP)	Phenol ug/ml	F(mU/L ALP)	C(mU/L ALP)	Phenol ug/ml
0	28.3	39.5	0	40.45	28.5	1.99	155.85	115.5	0
0.01	63.2	69	0	75.6	59	2.67	87.1	55	0
0.02	92.9	99	0.91	114.5	108.5	3.12	132.85	63	0
0.03	120.45	137.5	1.36	152.2	127	3.98	178.8	102.5	0.22
0.04	153.1	170.5	2.44	182.5	150.5	4.26	287.05	147	0
0.05	183.4	186.5	2.81	212.15	189	4.57	251.2	136	0
0.06	196.05	210	2.99	249.6	198	4.57	154.25	113	0.18
0.07	228.95	262.5	3.17	300.85	222.5	5.03	343.6	188	1.8
0.08	284.1	296.5	3.44	322.25	238	5.34	374.2	209	2.3
0.09	313.3	324	3.89	322.25	284.5	5.39	409.35	230.5	2.4
0.1	350.5	334.5	4.26	402.45	292.5	5.21	459	291.5	3.07
0.11	436.3	513	5.07	428.45	356.5	5.93	510	271.5	2.81
0.12	485.7	427	5.43	478.8	379	6.25	553	334.5	5.98
100	574175	28000	1242	409350	12500	2465	550950	19750	1592

Table 5. Contamination of pasteurised bulk cow milk with raw cow milk (HRI bulk tank -August-September)

Phosphatase Test failure highlighted in red

	23/8/01		30/8/01		5/9/01		12/9/01		19/9/01	
% RAW	F(mU/L ALP)	C(mU/L ALP)	F(mU/L ALP)	C(mU/L ALP)	F(mU/L ALP)	C(mU/L ALP)	F(mU/L ALP)	C(mU/L ALP)	F(mU/L ALP)	C(mU/L ALP)
0	20.7	12.5	21.4	8.5	28.3	20	32.2	26	30.1	30
0.01	61.1	38.5	65.05	40	77.9	50.5	72.85	62	81.15	66.5
0.02	109.85	70	115.6	52.5	134.9	85	123.65	98.5	129.2	91.5
0.03	149.15	104	150.55	92.5	181.8	124	162.3	183	178.6	143.5
0.04	203.45	126.5	188.25	103.5	233.55	162.5	205.95	159	211.7	162
0.05	258.55	157	246.2	153.5	296.75	195	263.4	238.5	259.75	200
0.06	301.55	204.5	283.2	203	354.4	231	309.4	280	312.15	246
0.07	321.8	217	309.85	164.5	381.05	230	340.2	287.5	340.6	246.5
0.08	372.15	258.5	354.45	241	440.4	273	374.45	380	381.8	331
0.09	405.7	269.5	405.5	265	489.35	294.5	433.95	351.5	433.7	328.5
0.1	455.3	317	442	277.5	528.65	369.5	476.25	400	480.85	410.5
0.11	532.6	389	482.7	330	578.3	396.5	521.05	454	520.85	490
0.12	550.5	404.5	509.35	303	630.25	426.5	558.55	489	588.2	458.5
100	612550	26250	544650	10500	518650	24250	454400	15250	590150	19500

Table 6. Contamination of pasteurised bulk cow milk with raw cow milk (HRI bulk tank- September to October)

Phosphatase Test failure highlighted in red

	26/9/2001		3/10/01	10/10/01	18/10/01			25/10/01		
% RAW	F(mU/L ALP)	C(mU/L ALP)	F(mU/L ALP)	F(mU/L ALP)	F(mU/L ALP)	C(mU/L ALP)	Phenol ug/ml	F(mU/L ALP)	C(mU/L ALP)	Phenol ug/ml
0	59.3	45	33.3	30.1	31.3	15.5	0.72	43.2	19	0.89
0.01	136.1	69	90.3	74	103.4	47	0.89	82.05	38.5	0.41
0.02	169.9	98.5	122.7	123	145.5	78.5	0.43	129.2	68.5	1.2
0.03	214.9	148	159.1	172.2	191.5	101.5	1.61	179.5	85.5	1.63
0.04	276.5	185	196.3	214.9	246.85	118	1.78	220.7	126	3.7
0.05	299.95	190.5	234.2	246.4	310.3	186	2.57	271.7	134	2.98
0.06	328.45	258.5	300.6	282	385.9	195	3.55	306.4	167.5	3.55
0.07	354	240	349.4	335.6	411.4	220	3.91	362.9	223.5	3.19
0.08	470.7	316.5	360.2	388.7	438.75	302	4.8	396.25	239.5	4.54
0.09	505.4	257	405.7	415.1	514.4	309	4.61	460.6	235	4.08
0.1	482.2	308.5	487.05	458.3	567.3	352	4.97	499.9	315	5.86
0.11	562.4	331.5	508.65	505.4	539.25	340	5.78	497.65	306.5	5.9
0.12	618.3	458.5	546.35	538.1	673	307.5	6.05	590.5	361	6.1
100	641500	44300	561850	496600	755975	18250	2201	631950	16250	1706

Table 7. Contamination of pasteurised bulk cow milk with raw cow milk (HRI bulk tank- November)

Phosphatase Test failure highlighted in red

	1/11/01			8/11/01			14/11/01		21/11/01
% RAW	F(mU/L ALP)	C(mU/L ALP)	Phenol ug/ml	F(mU/L ALP)	C(mU/L ALP)	Phenol ug/ml	F(mU/L ALP)	C(mU/L ALP)	F(mU/L ALP)
0	28.7	9	0.31	25.05	7.5	0.26	25.5	26.5	26.2
0.01	77.9	41	0.98	72.4	30	0.48	73.1	85	80.9
0.02	123.65	52.5	1.68	135.6	51	0.74	111.25	138	126
0.03	167.1	93.5	2.5	151.45	73.5	1.15	167.3	203	163.7
0.04	204.8	108.5	1.78	191	101.5	2.2	195.8	237.5	204.6
0.05	285.25	151	3.19	227.55	124	1.68	278.35	323.5	232.15
0.06	322.25	166.5	3.86	278.6	145	2.98	274	415.5	301.3
0.07	339.3	181.5	4.18	299.05	169.5	3.19	314	419.5	334.4
0.08	385.7	230	4.8	326.4	190.5	3.29	382.7	511.5	421.3
0.09	435.1	243.5	4.8	385.95	187	3.65	450.5	522	436
0.1	492.3	285	5.16	415.6	187	4.01	484.5	554.5	460.6
0.11	514.9	303.5	5.42	476.5	203	6.05	550	605.5	461.3
0.12	571.85	354	6.74	488	268	6.22	511.4	651.5	563.4
100	456375	11500	1550	707475	12000	2801	621275	25500	521650

Table 8. Contamination of pasteurised bulk cow milk with raw cow milk (HRI bulk tank- December 2001 to January 2002)

Phosphatase Test failure highlighted in red

	28/11/01		5/12/01	11/12/01		19/12/01	10/1/02		18/1/02	
% RAW	F(mU/L ALP)	C(mU/L ALP)	F(mU/L ALP)	F(mU/L ALP)	C(mU/L ALP)	F(mU/L ALP)	F(mU/L ALP)	C(mU/L ALP)	F(mU/L ALP)	C(mU/L ALP)
0	143.4	72	27.8	38.85	14.5	30.6	25.5	14.5	23.2	18
0.01	187.3	93.5	77.7	93.3	62	91.95	77.5	38.5	74.25	65.5
0.02	227.8	123.5	140.65	136.8	73.5	130.3	120	64.5	107.35	93.5
0.03	308.45	159	170.1	183	114	178.6	163.2	86	152.4	132
0.04	349.8	177.5	222.3	241.35	139.5	227.55	202.05	109.5	171.95	167
0.05	379.45	199	262.95	262.7	162.5	301.8	278.6	142	233.75	194.5
0.06	417.65	219	323.6	323.6	193.5	331.7	276.95	154	274.9	218.5
0.07	429.55	259.5	339	342.7	219.5	328.7	313.5	183	284.8	256.5
0.08	573.95	289	357.4	408.45	241.5	417.6	360.6	212.5	355.35	308.5
0.09	621.5	300.5	445.9	469.35	281.5	421.3	386.15	214	399	347.5
0.1	667.95	295.5	443.6	545.45	314	501.1	485.9	238.5	419.75	383.5
0.11	710.2	310	539	547.75	342.5	563.15	518.5	276.5	482.9	416.5
0.12	751.65	371.5	594.15	622.2	362.5	651.15	563.4	296.5	486.8	474.5
100	493025	8750	398775	556000	10000	623700	533950	11250	464875	14000

Objective No.	Objective Description
03	**Study of the origins and factors influencing the formation of heat stable alkaline phosphatases in goat milk**

Preliminary studies on ALP activity in goat milk from 12 animals in late lactation milk indicated that almost 50% of animals produced ALP which was stable to pasteurisation by heat treatment. This heat stable ALP is thought to be microbial in origin. Formation of heat stable ALP in individual goats will be studied throughout lactation. In a selection of samples in which we identify heat stable ALP, attempts will be made to separate the heat stable enzyme from bovine ALP using non denaturing PAGE. The molecular weight of components of heat stable ALP will be determined.

Completion of the work described in this section satisfies objectives set in **Milestones 03/01; 03/02; 03/03** and fulfil requirements for **Deliverables 03/01; 03/02; 03/03.**

Milk samples
Morning milk samples from individual goats were sampled each week from 6th August 2001 to the 21st January. ALP in raw milk was measured using the Fluorophos method. Raw milk was heat treated at 63˚C for 30 minutes or 95˚C for 2 minutes. The first treatment was used to establish if the goat ALP would survive pasteurisation, while the second treatment was to test for the presence of heat stable microbial phosphatase. The presence of heat stable ALP can result in false positives in phosphatase testing of bovine milk.

Results
Changes in heat stability throughout lactation are shown in Figs. 7a) to 7e). The graphs show the initial level of ALP in raw milk together with the residual ALP in the heat treated samples (Note these are on different scales).

Substantial levels of heat stable microbial phosphatase were identified in only two samples whereas ALP stable to pasteurisation was identified in the majority of samples.

Milk from goat 601 contained substantial levels of microbial heat stable ALP (i.e. in excess of 350 Mu/ml) in November and samples from goat 705 were high in August prior to death of the animal.

Four of the goats produced milk with ALP stable to holder pasteurisation throughout lactation. This included goats 617, 618, 806 and 891. Goats 725 and 809 produced pasteurisation stable ALP in late lactation. In the remaining animals, pasteurisation stable ALP was observed on one or two occasions, and this was generally in late lactation.

Conclusions
ALP stable to pasteurisation was found in goats milk from individual animals throughout lactation. 15 of the animals produced pasteurisation stable ALP on one or two occasions during the lactation. Factors influencing the presence of heat stable ALP are considered in the report on the statistical analysis of the lactational data (Objective 01). High somatic cell counts were associated with a high incidence of heat stable ALP.

Completion of the work described in this section satisfied objectives set in **Milestones 03/01; 03/02; 03/03** and fulfilled requirements for **Deliverables 03/01; 03/02; 03/03.**

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Fig 7a) Heat Treatment Goat 601

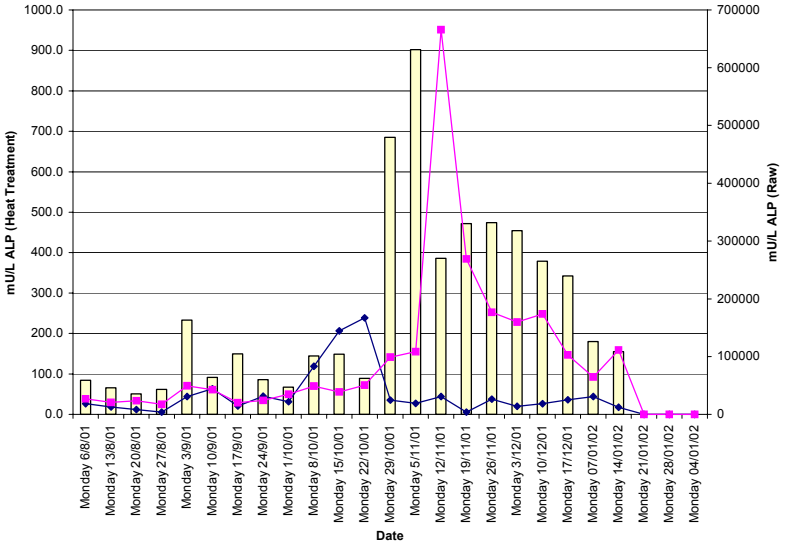


Fig 7b) Heat Treatment Goat 610

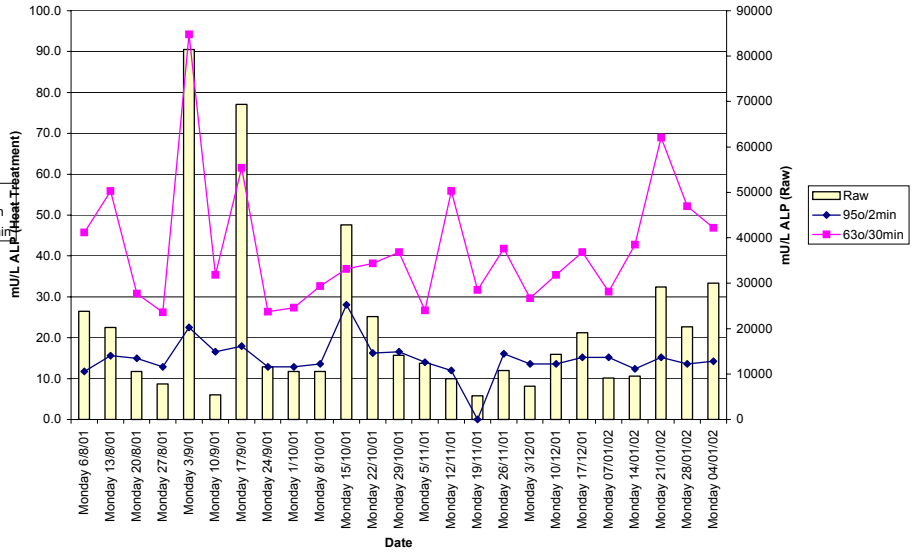


Fig 7c) Heat Treatment Goat 617

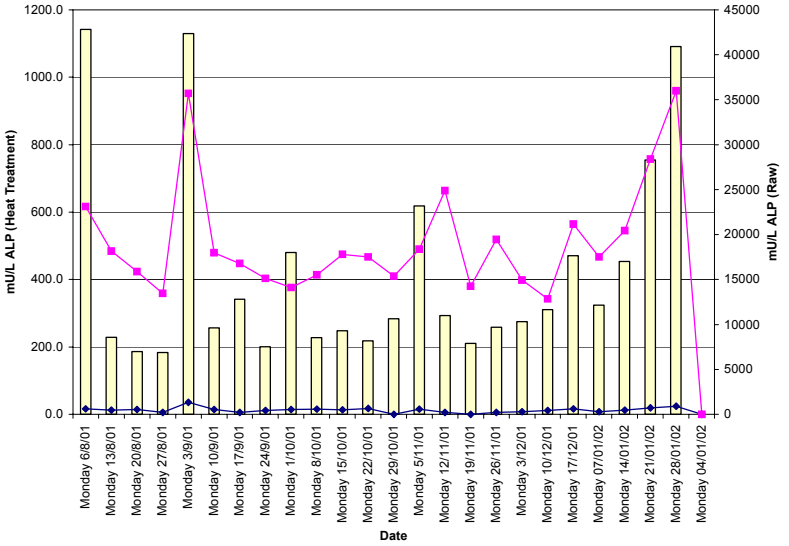


Fig 7d) Heat Treatment Goat 618

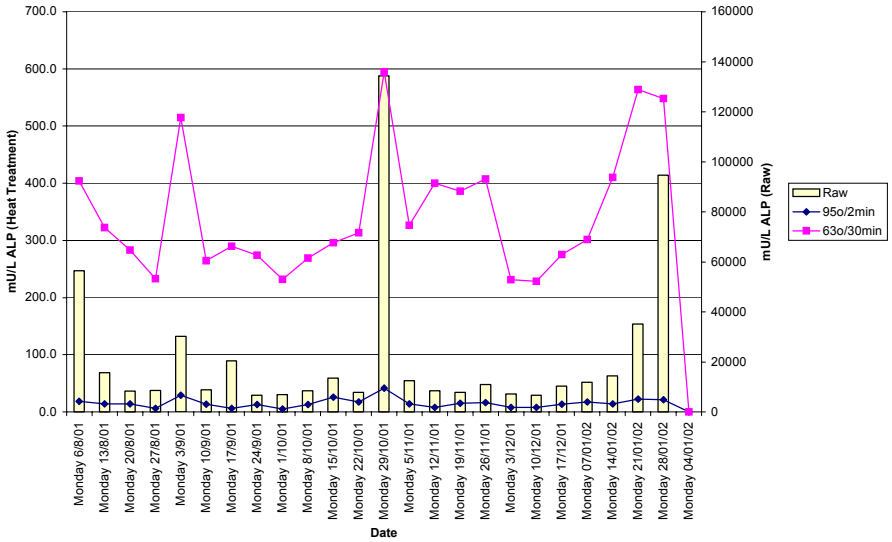
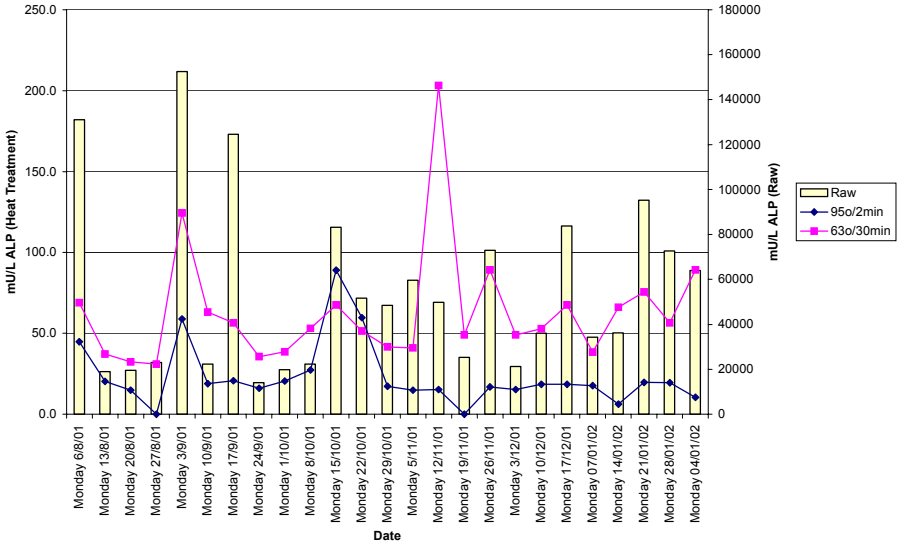


Fig 7e) Heat Treatment Goat 622



Objective No.	Objective Description
04	Survey of residual ALP activity in commercial pasteurised and unpasteurised goat and sheep milk on sale in Scotland
05	Survey of the microbiological quality of pasteurised and unpasteurised goat milk on sale in Scotland

Objective 04

Survey of residual ALP activity in commercial pasteurised and unpasteurised goat and sheep milk on sale in Scotland

Samples of commercially produced pasteurised goat milk will be collected from retail outlets in the North (Aberdeen), East (Edinburgh), South West (Ayrshire) and Central (Glasgow) at intervals of 6 weeks between February and June. Residual ALP activity in milk will be measured by the Flourophos and Charm methods.

Objective 05

Survey of the microbiological quality of pasteurised and unpasteurised goat milk on sale in Scotland

The microbiological quality of commercially produced goat milks [which have been collected from retail outlets for estimation of residual ALP (Objective 04) activity] will be assessed. Total bacterial count, total psychrotroph count, Enterobacteriaceae and Listeria counts will be included.

Completion of the work outlined above would satisfy requirements for **Milestones 04/01; 05/01** and **Deliverables 04/01; 05/01.**

Introduction

A substantial market now exists for goat milk in the UK, and a small number of large-scale producers are supplying the main retail outlets. Twenty nine goat milk samples were purchased from retail outlets in Ayr. Residual ALP activity in milk was measured by the Flourophos and Charm methods. The microbiological quality of the goat milks was assessed. Total bacterial count, total psychrotroph count, Enterobacteriaceae and Listeria counts were assessed.

Samples

Twenty nine samples of goat milk were collected from retail outlets in Ayrshire. Samples included whole milk, and semi skim milk. Samples were obtained from three supermarkets which included Tesco, Asda, and Safeway. Details of date of purchase and sell by date for each sample are shown in Table 1.

Samples purchased from Safeway were manufactured by the St Helens Farm company. Samples from Tesco and Asda were produced by the Delamere Dairy Company.

All samples were placed in insulated cool boxes immediately after purchase, and temperatures on arrival at the laboratory were monitored. Samples were stored at 2°C prior to analysis.

Although it had been intended to sample more extensively throughout Scotland it was clear that samples obtained locally were produced by the same manufacturer as those on sale in other parts of the country.

Results
Residual phosphatase levels for all milk samples as measured by Fluorophos and Charm were within acceptable ranges for pasteurised milk. Results of the microbiological analysis are shown in Table 2. Total colony counts in goats drinking milk were within the regulations for heat treated drinking milk for the majority of samples, but two samples had a total count which was high. One sample was a semi skimmed goat milk, obtained from Tesco on the 11th March 2002, which had a total count of 1.34 x 10^6 cfu/ml. The sell-by date was the 15th March 2002. The other sample was a full fat goats milk purchased in Asda on the 25th March which had a total count in excess of 1 x 10^6 cfu/ml. The sell-by date was the 29th March. Both samples were from the Delamere Dairy Company. In both samples the high total count seemed to be associated with high psychrotrophic counts and Enterobacteriaceae were isolated from the milks. Listeria was absent from all samples.

Conclusions
Two of the 29 goat milk samples studied were found to have microbiological counts which are not acceptable for pasteurised milk. Residual phosphatase in milk samples suggested that the milk had been effectively pasturised.

Completion of this work fulfils requirements for **Milestones 04/01; 05/01** and **Deliverables 04/01; 05/01.**

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Table 1. Goat milk retail samples

Date	Store	Goat/Cow(PF)	Milk Type	Use by Date	Temp (°C)
11/3/02	Safeways	Goat	Whole	16/3/02	-
		Goat	Semi Sk	15/3/02	-
	Tesco	Goat	Whole	17/3/02	-
		Goat	Semi Sk	15/3/02	-
18/3/02	Safeways	Goat	Whole	22/3/02	-
		Goat	Whole	23/3/02	-
		Goat	Semi Sk	21/3/02	-
	Asda	Goat	Whole	25/3/02	-
	Tesco	Goat	Whole	25/3/02	-
		Goat	Semi Sk	21/3/02	-
25/3/02	Safeways	Goat	Whole	29/3/02	6
		Goat	Semi Sk	26/3/02	6
		Goat	Semi Sk	27/3/02	5
	Asda	Goat	Whole	29/3/02	8
	Tesco	Goat	Whole	31/3/02	6
		Goat	Semi Sk	31/3/02	8
8/4/02	Safeways	Goat	Whole	13/4/02	7
		Goat	Semi Sk	12/4/02	6.5
	Asda	Goat	Whole	12/4/02	7
	Tesco	Goat	Whole	13/4/02	6.5
		Goat	Semi Sk	13/4/02	7
15/4/02	Asda	Goat	Whole	20/4/02	11
	Tesco	Goat	Whole	22/4/02	9
		Goat	Semi Sk	22/4/02	9
22/4/02	Safeways	Goat	Whole	29/4/02	-
		Goat	Semi Sk	29/4/02	-
	Asda	Goat	Whole	28/4/02	-
	Tesco	Goat	Whole	28/4/02	-
		Goat	Semi Sk	28/4/02	-

Table 2a)	**11/3/02**						
			Total Colony	Presumptive	Psychrotrophic	Listeria	Listeria
		SAC no.	Count cfu/ml	Enterobacteriaceae	colony count	monocytogenes	monocytogenes
			(30oC)	cfu/ml	cfu/ml @6.5oC	in 25g	cfu/ml
Asda	Whole Goats						
Safeways	Whole Goats	GM02/4	95	<1	10	Absent	<20
	Semi Skimmed Goats	GM02/2	155	<1	10	Absent	<20
Tesco	Whole Goats	GM02/1	3,050	Estimate 3	4,300	Absent	<20
	Semi-Skimmed Goats	GM02/3	1,340,000	21	2500000	Absent	<21

Table 2b)	**18/3/02**					
			Total Colony	Presumptive	Psychrotrophic	Listeria
		SAC no.	Count cfu/ml	Enterobacteriaceae	colony count	monocytogenes
			(30oC)	cfu/ml	cfu/ml @6.5oC	in 25g
Asda	Whole Goats	GM02/10	460	<1	1,015	Absent
Safeways	Whole Goats	GM02/11	1,565	<1	<10	Absent
		GM02/12	165	<1	<10	Absent
	Semi Skimmed Goats	GM02/13	135	<1	<10	Absent
Tesco	Whole Goats	GM02/8	565	<1	665	Absent
	Semi-Skimmed Goats	GM02/9	3,500	213	790	Absent

Table 2c)	**25/3/02**						
			Total Colony	Presumptive	Psychrotrophic	Listeria	Listeria
		SAC no.	Count cfu/ml	Enterobacteriaceae	colony count	monocytogenes	monocytogenes
			(30oC)	cfu/ml	cfu/ml @6.5oC	in 25g	cfu/ml
Asda	Whole Goats	GM02/22	Estimate >1,000,000	310	Estimate>1000,000	Absent	Absent
Safeways	Whole Goats	GM02/21	105	<1	<10	Absent	Absent
	Semi Skimmed Goats	GM02/19	300	Estimate 2	10	Absent	Absent
		GM02/20	180	<1	<10	Absent	Absent
Tesco	Whole Goats	GM02/14	7,860	Estimate 3	905	Absent	Absent
	Semi-Skimmed Goats	GM02/15	1,600	8	580	Absent	Absent

Table 2d)	**15/4/02**		Total Colony	Presumptive	Psychrotrophic	Salmonella ssp.	Listeria
		SAC no.	Count cfu/ml	Enterobacteriaceae	colony count	in 25g	monocytogenes
			(30oC)	cfu/ml	cfu/ml @6.5oC		in 25 g
	Whole Goats	GM02/36	1,300	<1	1,000	Absent	Absent
Asda	Whole Goats						
Safeways							
	Semi Skimmed Goats						
	Whole Goats	GM02/34	545	<1	<10	Absent	Absent
Tesco	Semi-Skimmed Goats	GM02/35	1,300	Estimate 1	410	Absent	Absent

Table 2e)	22/4/02		Total Colony	Presumptive	Psychrotrophic	Salmonella ssp.	Listeria
		SAC no.	Count cfu/ml	Enterobacteriaceae	colony count	in 25g	monocytogenes
			(30oC)	cfu/ml	cfu/ml @6.5oC		in 25 g
	Whole Goats	GM02/44	735	<1	310	Absent	Absent
Asda	Whole Goats	GM02/42	95	<1	<10	Absent	Absent
Safeways		GM02/43	105	<1	<10	Absent	Absent
	Semi Skimmed Goats						
	Whole Goats	GM02/40	585	<1	<10	Absent	Absent
Tesco	Semi-Skimmed Goats	GM02/41	765	<1	<10	Absent	Absent