**USDA Milk Study: Calf Growth Analysis**

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March 10, 2022

**Statistical Model:**

To analyze the association between milkAUC and the cow/calf performance measures, a linear mixed model with repeated measures was fitted. When we have repeated measures, our time points will usually be highly correlated. The repeated measures model accounts for the correlation between the time points, and thus provides a more accurate estimate of the association between milkAUC and calf weight. Since the time points for the calf weights are not equally spaced, I used the unstructured covariance structure for the model. I did try other covariance structures but using unstructured was best since the model had the best fit statistics when using unstructured. This covariance structure makes sure that the covariances between the time points are calculated correctly so the model is fitted properly.

In the model, I included a fixed effect of gender, a fixed effect for the day in calving season, as well as cow age in the model along with two-way interactions between these factors and “day” to better estimate the association of milkAUC and calf weight. I also included a two-way interaction between milkAUC and day to analyze the milkAUC association for each day. For the repeated measures model, the variable “day” is included that indicates the day the calf was weighed. I ran the models with gender, day, and cow age as classification variables along with cdate and milk AUC as quantitative variables. I also included a random effect for the season/year. This accounts for the variability associated with the change in season. This random effect also accounts for the fact that you sometimes had the same cows in both years. This will also help us get a better estimate of the milk AUC. I first ran these models with a quadratic effect for the days in calving season (cdate) but dropped this term from the model where this term was insignificant according to the Type I Test of Fixed Effects table.

**Results:**

The SAS file “calfs\_repeated\_analysis.pdf” contains all the output from this analysis. I will walk through the most important parts of the output. I always examine the Type I test of fixed effects table first:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Type I Tests of Fixed Effects | | | | |
| Effect | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| dayn | 5 | 112 | 2946.19 | <.0001 |
| calfsex | 1 | 112 | 2.48 | 0.1183 |
| calfsex\*dayn | 5 | 112 | 2.46 | 0.0371 |
| cowagen | 2 | 112 | 1.85 | 0.1621 |
| cowagen\*dayn | 10 | 112 | 1.34 | 0.2162 |
| cdate | 1 | 112 | 1.32 | 0.2525 |
| cdate\*dayn | 5 | 112 | 8.41 | <.0001 |
| milkAUC | 1 | 112 | 24.13 | <.0001 |
| milkAUC\*dayn | 5 | 112 | 16.01 | <.0001 |

The effect of day is significant overall calf weight. I want to point out that the effect of cdate is insignificant, but the interaction of cdate and day is. MilkAUC has a significant effect on calf weight along with the interaction of day and milk AUC.

I also included the Type III test of fixed effects table in out the output and yielded the same significance conclusion as the Type I table. Just note that there is a difference in how the F-values and correspond p-values are calculated between Type I and Type III test of fixed effects. Type I fits the model “sequentially” and the terms in the model are being tested in the order they are listed in the model. I put the milkAUC terms last in the model to so that effects of the other factors are accounted for first before accounting for the effect of milkAUC. The Type III test of fixed effects table calculates the F-statistic and p-value in a “partial” manner. So, the F-statistics and p-values for all the effects in the Type III table are all calculated after accounting for the effect for the other terms in the model.

To examine the association milkAUC and calf weight, I examine the solutions for fixed effects table:

**Solutions for Fixed Effects**

| **Effect** | **calfsex** | **cowagen** | **dayn** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > |t|** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Intercept** |  |  |  | 155.30 | 14.3149 | 112 | 10.85 | <.0001 |
| **dayn** |  |  | 0 | -118.52 | 13.5075 | 112 | -8.77 | <.0001 |
| **dayn** |  |  | 30 | -85.7123 | 18.3427 | 112 | -4.67 | <.0001 |
| **dayn** |  |  | 60 | -74.4273 | 18.7467 | 112 | -3.97 | 0.0001 |
| **dayn** |  |  | 90 | -55.2032 | 17.0604 | 112 | -3.24 | 0.0016 |
| **dayn** |  |  | 120 | -28.5250 | 16.0896 | 112 | -1.77 | 0.0790 |
| **dayn** |  |  | 200 | 0 | . | . | . | . |
| **calfsex** | heifer |  |  | -9.5152 | 4.4266 | 112 | -2.15 | 0.0337 |
| **calfsex** | steer |  |  | 0 | . | . | . | . |
| **calfsex\*dayn** | heifer |  | 0 | 7.2246 | 4.1770 | 112 | 1.73 | 0.0865 |
| **calfsex\*dayn** | heifer |  | 30 | 8.5424 | 5.6722 | 112 | 1.51 | 0.1349 |
| **calfsex\*dayn** | heifer |  | 60 | 7.0623 | 5.7971 | 112 | 1.22 | 0.2257 |
| **calfsex\*dayn** | heifer |  | 90 | 5.3703 | 5.2756 | 112 | 1.02 | 0.3109 |
| **calfsex\*dayn** | heifer |  | 120 | 3.4170 | 4.9754 | 112 | 0.69 | 0.4936 |
| **calfsex\*dayn** | heifer |  | 200 | 0 | . | . | . | . |
| **calfsex\*dayn** | steer |  | 0 | 0 | . | . | . | . |
| **calfsex\*dayn** | steer |  | 30 | 0 | . | . | . | . |
| **calfsex\*dayn** | steer |  | 60 | 0 | . | . | . | . |
| **calfsex\*dayn** | steer |  | 90 | 0 | . | . | . | . |
| **calfsex\*dayn** | steer |  | 120 | 0 | . | . | . | . |
| **calfsex\*dayn** | steer |  | 200 | 0 | . | . | . | . |
| **cowagen** |  | 4 |  | 0.3187 | 5.5741 | 112 | 0.06 | 0.9545 |
| **cowagen** |  | 5 |  | 4.3175 | 5.3031 | 112 | 0.81 | 0.4173 |
| **cowagen** |  | 6 |  | 0 | . | . | . | . |
| **cowagen\*dayn** |  | 4 | 0 | -1.8323 | 5.2597 | 112 | -0.35 | 0.7282 |
| **cowagen\*dayn** |  | 4 | 30 | -8.2728 | 7.1424 | 112 | -1.16 | 0.2492 |
| **cowagen\*dayn** |  | 4 | 60 | -6.3339 | 7.2997 | 112 | -0.87 | 0.3874 |
| **cowagen\*dayn** |  | 4 | 90 | -6.4930 | 6.6431 | 112 | -0.98 | 0.3305 |
| **cowagen\*dayn** |  | 4 | 120 | -7.5553 | 6.2651 | 112 | -1.21 | 0.2304 |
| **cowagen\*dayn** |  | 4 | 200 | 0 | . | . | . | . |
| **cowagen\*dayn** |  | 5 | 0 | -5.0439 | 5.0040 | 112 | -1.01 | 0.3156 |
| **cowagen\*dayn** |  | 5 | 30 | -4.4618 | 6.7953 | 112 | -0.66 | 0.5128 |
| **cowagen\*dayn** |  | 5 | 60 | -2.4363 | 6.9450 | 112 | -0.35 | 0.7264 |
| **cowagen\*dayn** |  | 5 | 90 | -1.4418 | 6.3202 | 112 | -0.23 | 0.8200 |
| **cowagen\*dayn** |  | 5 | 120 | -1.0959 | 5.9606 | 112 | -0.18 | 0.8545 |
| **cowagen\*dayn** |  | 5 | 200 | 0 | . | . | . | . |
| **cowagen\*dayn** |  | 6 | 0 | 0 | . | . | . | . |
| **cowagen\*dayn** |  | 6 | 30 | 0 | . | . | . | . |
| **cowagen\*dayn** |  | 6 | 60 | 0 | . | . | . | . |
| **cowagen\*dayn** |  | 6 | 90 | 0 | . | . | . | . |
| **cowagen\*dayn** |  | 6 | 120 | 0 | . | . | . | . |
| **cowagen\*dayn** |  | 6 | 200 | 0 | . | . | . | . |
| **cdate** |  |  |  | -0.7079 | 0.3257 | 112 | -2.17 | 0.0319 |
| **cdate\*dayn** |  |  | 0 | 0.8556 | 0.3074 | 112 | 2.78 | 0.0063 |
| **cdate\*dayn** |  |  | 30 | 0.7491 | 0.4174 | 112 | 1.79 | 0.0754 |
| **cdate\*dayn** |  |  | 60 | 0.7644 | 0.4266 | 112 | 1.79 | 0.0758 |
| **cdate\*dayn** |  |  | 90 | 0.7338 | 0.3882 | 112 | 1.89 | 0.0613 |
| **cdate\*dayn** |  |  | 120 | 0.8358 | 0.3661 | 112 | 2.28 | 0.0243 |
| **cdate\*dayn** |  |  | 200 | 0 | . | . | . | . |
| **milkAUC** |  |  |  | 0.08432 | 0.01156 | 112 | 7.29 | <.0001 |
| **milkAUC\*dayn** |  |  | 0 | -0.08645 | 0.01091 | 112 | -7.93 | <.0001 |
| **milkAUC\*dayn** |  |  | 30 | -0.07691 | 0.01481 | 112 | -5.19 | <.0001 |
| **milkAUC\*dayn** |  |  | 60 | -0.06681 | 0.01514 | 112 | -4.41 | <.0001 |
| **milkAUC\*dayn** |  |  | 90 | -0.05511 | 0.01378 | 112 | -4.00 | 0.0001 |
| **milkAUC\*dayn** |  |  | 120 | -0.04496 | 0.01299 | 112 | -3.46 | 0.0008 |
| **milkAUC\*dayn** |  |  | 200 | 0 | . | . | . | . |

This table gives the slope of milkAUC over all the days, along with the milkAUC slope at each day. Something I want to emphasize is that the milkAUC\*day interactions are not showing negative associations at each day. For example, to get the association at day 120, I take the overall slope coefficient for milkAUC and add it to the slope coefficient for milkAUC\*dayn for day 30: 0.08432 + (-0.04496) = 0.03936, so there is a statistically significant positive association between milkAUC and calfweight at day 120.

The plot below shows the interaction of milkAUC and day. This shows the estimated regression lines for milkAUC and calfweight for each day. The association is not significant at day 0 and day 30, but does become significantly positive after day 30. As the day increases, the more positive the association between the milkAUC and the calf weight:

Chart, line chart

Description automatically generated

I examined the calfsex\*dayn interaction to see how the heifers and the steers weight changes over time according to the model. We see that the weight does increase over the days for both the heifers and the steers according to the table below:

| **calfsex\*dayn Least Squares Means** | | | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| **calfsex** | **dayn** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > |t|** |
| heifer | 0 | 33.3717 | 0.5231 | 112 | 63.80 | <.0001 |
| heifer | 30 | 74.0118 | 1.9588 | 112 | 37.78 | <.0001 |
| heifer | 60 | 95.6841 | 2.3548 | 112 | 40.63 | <.0001 |
| heifer | 90 | 125.11 | 2.3130 | 112 | 54.09 | <.0001 |
| heifer | 120 | 161.26 | 2.4358 | 112 | 66.20 | <.0001 |
| heifer | 200 | 225.05 | 3.0976 | 112 | 72.65 | <.0001 |
| steer | 0 | 35.6623 | 0.5204 | 112 | 68.53 | <.0001 |
| steer | 30 | 74.9846 | 1.9488 | 112 | 38.48 | <.0001 |
| steer | 60 | 98.1370 | 2.3427 | 112 | 41.89 | <.0001 |
| steer | 90 | 129.26 | 2.3011 | 112 | 56.17 | <.0001 |
| steer | 120 | 167.36 | 2.4234 | 112 | 69.06 | <.0001 |
| steer | 200 | 234.57 | 3.0818 | 112 | 76.12 | <.0001 |

I also examined the cowage\*day interaction to see how the model estimates calf weight for each age and to see if their weights are increasing for each day in all age groups. The table below does confirm that the weight increases over each day as well as for each age group.

| **cowagen\*dayn Least Squares Means** | | | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| **cowagen** | **dayn** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > |t|** |
| 4 | 0 | 33.7500 | 0.6538 | 112 | 51.62 | <.0001 |
| 4 | 30 | 69.2435 | 2.4482 | 112 | 28.28 | <.0001 |
| 4 | 60 | 92.2733 | 2.9431 | 112 | 31.35 | <.0001 |
| 4 | 90 | 122.11 | 2.8908 | 112 | 42.24 | <.0001 |
| 4 | 120 | 158.41 | 3.0444 | 112 | 52.03 | <.0001 |
| 4 | 200 | 228.59 | 3.8715 | 112 | 59.04 | <.0001 |
| 5 | 0 | 34.5373 | 0.5805 | 112 | 59.50 | <.0001 |
| 5 | 30 | 77.0534 | 2.1738 | 112 | 35.45 | <.0001 |
| 5 | 60 | 100.17 | 2.6132 | 112 | 38.33 | <.0001 |
| 5 | 90 | 131.16 | 2.5668 | 112 | 51.10 | <.0001 |
| 5 | 120 | 168.87 | 2.7032 | 112 | 62.47 | <.0001 |
| 5 | 200 | 232.58 | 3.4376 | 112 | 67.66 | <.0001 |
| 6 | 0 | 35.2636 | 0.6757 | 112 | 52.19 | <.0001 |
| 6 | 30 | 77.1976 | 2.5305 | 112 | 30.51 | <.0001 |
| 6 | 60 | 98.2885 | 3.0420 | 112 | 32.31 | <.0001 |
| 6 | 90 | 128.28 | 2.9879 | 112 | 42.93 | <.0001 |
| 6 | 120 | 165.65 | 3.1466 | 112 | 52.64 | <.0001 |
| 6 | 200 | 228.27 | 4.0016 | 112 | 57.04 | <.0001 |

I also did simple effect comparisons for the calfsex\*days interaction since this effect was significant according to the Type I table:

| **Simple Effect Comparisons of calfsex\*dayn Least Squares Means By dayn** | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Simple Effect Level** | **calfsex** | **\_calfsex** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > |t|** |
| **dayn 0** | heifer | steer | -2.2906 | 0.7475 | 112 | -3.06 | 0.0027 |
| **dayn 30** | heifer | steer | -0.9728 | 2.7993 | 112 | -0.35 | 0.7289 |
| **dayn 60** | heifer | steer | -2.4529 | 3.3651 | 112 | -0.73 | 0.4676 |
| **dayn 90** | heifer | steer | -4.1448 | 3.3053 | 112 | -1.25 | 0.2125 |
| **dayn 120** | heifer | steer | -6.0981 | 3.4809 | 112 | -1.75 | 0.0825 |
| **dayn 200** | heifer | steer | -9.5152 | 4.4266 | 112 | -2.15 | 0.0337 |

We see in the table above, heifers and steers had significantly different weights at day 0 and at day 200. The table below compares the calf weights for each cow age group over each day:

| **Simple Effect Comparisons of cowagen\*dayn Least Squares Means By dayn** | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Simple Effect Level** | **cowagen** | **\_cowagen** | **Estimate** | **Standard Error** | **DF** | **t Value** | **Pr > |t|** |
| **dayn 0** | 4 | 5 | -0.7873 | 0.8845 | 112 | -0.89 | 0.3753 |
| **dayn 0** | 4 | 6 | -1.5136 | 0.9412 | 112 | -1.61 | 0.1106 |
| **dayn 0** | 5 | 6 | -0.7263 | 0.8955 | 112 | -0.81 | 0.4190 |
| **dayn 30** | 4 | 5 | -7.8099 | 3.3122 | 112 | -2.36 | 0.0201 |
| **dayn 30** | 4 | 6 | -7.9541 | 3.5249 | 112 | -2.26 | 0.0260 |
| **dayn 30** | 5 | 6 | -0.1443 | 3.3535 | 112 | -0.04 | 0.9658 |
| **dayn 60** | 4 | 5 | -7.8965 | 3.9817 | 112 | -1.98 | 0.0498 |
| **dayn 60** | 4 | 6 | -6.0152 | 4.2373 | 112 | -1.42 | 0.1585 |
| **dayn 60** | 5 | 6 | 1.8812 | 4.0314 | 112 | 0.47 | 0.6417 |
| **dayn 90** | 4 | 5 | -9.0501 | 3.9110 | 112 | -2.31 | 0.0225 |
| **dayn 90** | 4 | 6 | -6.1743 | 4.1621 | 112 | -1.48 | 0.1408 |
| **dayn 90** | 5 | 6 | 2.8758 | 3.9598 | 112 | 0.73 | 0.4692 |
| **dayn 120** | 4 | 5 | -10.4583 | 4.1187 | 112 | -2.54 | 0.0125 |
| **dayn 120** | 4 | 6 | -7.2367 | 4.3832 | 112 | -1.65 | 0.1015 |
| **dayn 120** | 5 | 6 | 3.2216 | 4.1701 | 112 | 0.77 | 0.4414 |
| **dayn 200** | 4 | 5 | -3.9989 | 5.2378 | 112 | -0.76 | 0.4468 |
| **dayn 200** | 4 | 6 | 0.3187 | 5.5741 | 112 | 0.06 | 0.9545 |
| **dayn 200** | 5 | 6 | 4.3175 | 5.3031 | 112 | 0.81 | 0.4173 |

I’ve highlighted in the table above each day where weights differ significantly among each age group. Calf weights differ for cow ages 4 and 5 across all the days (except 200) and we see that the calf weights are significantly different for cows ages 4 and 6, but only for day 30.