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September 5, 2016

Cover letter

Report of research performed under the Florida Cattle Enhancement Fund, Application Specific Appropriation 1464

To the Florida Cattlemen Association and the Florida Department of Agriculture and Consumer Services:

The accompanying report is submitted to the Florida Cattlemen Association (FCA) and Florida Department of Agriculture and Consumer Services in accordance with the terms of the grant provided under the Specific Appropriation 1464 to "conduct programs designed to expand uses of beef and beef products and strengthen the market position of Florida's cattle industry in the state and in the Nation".

This is a report of the study: **Assessment of 'Early' Calf Loss in Florida Beef Cattle Populations**. The report addresses a problem that falls under the FCA's research priority for calf loss and animal health.

This work is of significant interest and importance to the Florida cattle industry. We are pleased to present our findings within the frame work of this project, and we look forward to continued funding to advance our work and understanding of fetal loss in our Florida cattle herds.

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Sincerely,

D. Owen Rae, DVM, MPVM

Report: Florida Cattle Enhancement Fund, Application Specific Appropriation 1464

Title:
Assessment of Calf Loss in Florida Beef Cattle Populations

D.O. Rae and M. Irsik

Notes:

1. The deliverable product for this study is the 30 day synchronized pregnancy percentage among studied cows and the losses occurring in study animals over the study period.

2. Because of the funding cycle and deliverable date (June 30th) this study only addresses a portion of the gestation of study cows. Although funding has (or will) conclude soon, the cows are continuing to be monitored to assess further losses.

3. It is hoped that further funding will be available in the coming year: to continue the study and expand the study to additional herds and cattle numbers.

Introduction:
The Florida Cattlemen have recognized 'calf loss' as a priority concern for the Florida beef industry. This loss is recognized as being non-specific. It is not clear where the loss is occurring. It is recognized that at weaning the percentage of calves is less than desired and is, on average, 9-10% less than the national average. A clear determination of where loss is occurring and what level of loss is occurring is an important start to addressing this research priority. The level of loss can be determined at important points of calf development, e.g., conception, pregnancy maintenance, calf delivery and calf survival, then further study will be possible to decrease losses and thus, increase that final crop outcome, specifically, an increased weaned calf crop. This initial study evaluates two herds of cows through the processes of breeding, conception and pregnancy maintenance to the date of August 4th of 2016. [If later, multi-year funding is available then additional herds and duration of evaluation, i.e. pregnancy, calving, calf development and weaning will be followed]. The study is observational and quantitative in determining points in fetal development that losses occur.

Significance:
The Florida cattle industry is predominantly a cow-calf industry. Producers expect a commercial beef cow to: get pregnant each and every year, deliver a live calf and raise that calf to weaning with and expectation of attaining a weaning weight of at least 40% of the maternal mature weight. The Florida calf crop (the proportion of calves weaned from cows originally exposed to a bull by natural or artificial insemination) is 9-10% less than the national average. This calf 'loss' has been a point of question for many decades. The loss may occur as 1) a failure to conceive, 2) a failure to maintain a pregnancy at any point during gestation, 3) failure to deliver a live calf or 4) failure of the calf to survive to weaning. With calf prices in the recent past at or near a $1000 per weaned calf, the economic loss to the state's beef cattle industry is substantial. Assessment of loss and degree of loss at points from conception to weaning would provide

information for reducing these losses and increasing income to beef cattle producers and the state of Florida.

Background information:
The literature reports beef cows have a fetal loss rate of up to 11%. Losses are likely largest during the first 42 days of pregnancy, during the embryonic phase. The causes of these losses are largely unexplained. Losses later in pregnancy are often related to trauma or infection. In either case, monitoring of gestational progress is important to determining when, how and why losses occur. Pregnancy evaluation can take place by a number of methods: manual palpation, ultrasonography or blood test, by pregnancy-associated glycoproteins (PAGs) detection. Regular diagnostic assessment offers an opportunity to determine the prevalence of loss by critical factors associated with the herd, animals, time of gestation and other measurable factors.

Project Objectives:
The aim of this study was to evaluate the

1. Factors associated with successful establishment of pregnancy.
2. Loss of pregnancy as to prevalence by time, events, factors and/or diagnostic causes.
3. Role of Ovarian Follicular Dysplasia (OFD) in 'calf loss' (This deliverable report is prepared and submitted separately).

Hypothesis:
Losses in Florida beef cows are not due to a single event or cause, but a cumulative effect of multiple events along the process of pregnancy establishment, maintenance, calf delivery and survival.

Study population:
The study was performed during the spring and early summer of 2016, concluding August 4th (with a funding extension beyond our June 30th, fiscal year deadline). Included in the study were two beef cattle herds. One herd composed of multi-breed Angus and Brahman cross cows, typical to Florida herds (Brahman, Angus, Brangus and commercial crossbred animals); this herd is located in north central Florida. The second herd was composed of Angus and Brangus cattle in approximately equal numbers. The study period was from February to August 4th of 2016. The participating herds included both heifers and mature cows, n=477 animals.

Animals were evaluated about 30 days prior to the start of the breeding season. Animals were identified (by tag/brand, age, breed), given a body condition score, weight, pelvic area measurement, reproductive tract score (heifers) and ovaries assessed by ultrasound. A blood sample was taken for an endocrine profile, specifically to assess Anti-Mullerian Hormone (AMH).

Animals participated in a synchronized estrus, using a Select Synch + CIDR procedure. The early calving cows were bred on observed estrus or on a timed artificial insemination, if not observed in estrus. All cows were then exposed to bulls in single sire breed groups. A small number of late calving cows were immediately exposed to bulls for natural service following calving. Bulls used in the study were assessed for breeding soundness 30 to 60 days prior to the start of the breeding season. Bulls were with the herd for 55 to 60 days at a ratio of 1 bull per 25 cows. Note: heifers in the second herd were just exposed to the bull.

Pregnancy establishment and maintenance were followed by sequential evaluation at 30, 60, 90 and some at 120 days post initial breeding/exposure. At each of the evaluation times, animals were assessed by ultrasonography (Aloka SSD-500, with 5 MHz probe) as to their pregnancy status and stage of pregnancy. Fetal size was measured until gestational age/size exceeded an ease of measurement. Animals that were found to be non-pregnant were assessed by ultrasonography to detect uterine or ovarian abnormalities. Blood samples were taken from all animals to assess pregnancy associated glycoprotein (PAG) values. This provided additional assessment of fetal vitality.

Evaluation and monitoring of these herds continues but our deliverable results are provided through to the assessment made on August 4th. Pregnancy loss or maintenance information is reported. Subsequent information will be added to our data set for these cows, with anticipation of continued funding in the next granting period.

Diagnostic procedures:
Blood samples were taken for analysis of Anti-Mullerian Hormone (AMH, an indicator of follicle reserves in the ovary). The test was performed in accordance to the kit instructions. Serum PAG concentrations were determined by ELISA test using a bovine PAG test kit (IDEXX Laboratory Inc., Westbrook, ME). To ensure accuracy, the test was duplicated for each sample. The test was performed according to the ELISA test kit instructions, with minor modifications.

Selection of cases:
Animals which failed to become pregnant or were identified as pregnant but failed to maintain a pregnancy were selected as cases and assessed as to reasons contributing to a failure to become pregnant or maintain a pregnancy (i.e., fetal crown rump length, AMH or PAG value, cow breed, body condition, etc.).

Selection of controls:
A control was defined as a heifer or cow that became pregnant and remained pregnant during the time frame of the study.

Data Analysis:
All data, including animal identification numbers, pregnancy diagnoses, and PAG levels, were organized in a spreadsheet (Excel, Microsoft, Redmond WA). Descriptive statistics were performed using Excel and the statistical analysis software SAS 9.4 (SAS Institute Inc., Cary, NC).
The average PAG values for cattle that maintained their pregnancy was calculated and compared to the average PAG values for the cattle that experienced embryonic mortality. To generate the predictive probability of embryonic mortality based on day 30 PAG values, the LOGISTICS procedure of SAS 9.4 was used.

Facilities for the project:
Animal handling and evaluation took place on the two participating cattle units following management practices consistent with their standard operating procedure but also consistent with the study expectations for herd assessment. Samples from animals were handled in labs at the College of Veterinary Medicine (CVM), at the University of Florida (LACS, under Dr Rae's supervision).

Limitations of this study:
The main limitation of the present study is: Large numbers of herds and animals may be required to determine the multiple causes associated with 'calf loss'. Multiple breeds and different herds in different locations of the State will likely be required in order to best meet the aims of the study. This study provides baseline information and provides a pilot for further studies in this area.

Results:
The study followed 477 heifers and cows from 2 herds comprising cattle of Angus, Brahman and Angus – Brahman crosses. Data are summarized in Table 1.

The first herd was comprised of 205 animals, 33 heifers and 172 cows of multi-breed composition, including 46 Angus, 21 Brahman, 38 Brangus and 100 Angus – Brahman crosses. Among that group of cows, there were 6 animals that lost a pregnancy (2.9%), during the observation time period of February 1st and July 14th. The proportion of cows pregnant by 30-50 days following bull removal was 90.7% (186 of 205), ranging from 83.9% to 94.1% by single sire breed groups. Differences seem to be associated with the single sire bull breeding groups, i.e. differences in bull fertility or libido. Of the 19 non-pregnant cows, 8 were in the 3-year old cows, 2 each in the heifers and 4-year old cows.

The second herd was comprised of 272 animals, 69 heifers and 203 cows of Angus (128) and Brangus (144) breeding. Among this group of cows, there were 9 animals that lost a pregnancy (3.3%) during the observation period of March 23rd and August 4th. The proportion of cows pregnant by 30-50 days following bull removal was 91.5% (Angus cows 89.8% [115 of 128] and Brangus cows 93.1% [134 of 144]). Of the 23 non-pregnant animals, 14 were in the group of 69 yearling heifers and 4 in the group of 58, 2-year old cows. Others were distributed among other age groups. In both herds, pregnant cows tended to weigh more and have slightly higher body conditions.

Of the 15 cows that lost a pregnancy, the average age was 3.3 years. Two cows were 9 and 12 years of age; all others were 4 years of age or younger. The distribution of loss by breed group was relatively uniform, ranging from 1.6 to 4.8% loss. The body weight of cows losing a pregnancy tended to be lighter (1013 vs 1077 lb.); body condition scores were similar between the two groups. Pregnancies were lost on average at 53 days of gestation. Eight (8) of the losses occurred at 29 to 39 days of gestation. The latest loss observed in these animals, in our limited observation period, was 100 days of gestation. An estimation of point in time that the loss occurred placed our losses between May 8th and July 6th, with early June being a common estimated time of loss for these animals. These estimates were determined based on a review of the repeated ultrasound observations and the pregnancy associated glycoproteins (PAGs) measured.

The mean serum concentration of PAGs for all pregnant animals at day 30 of gestation was 2.89 ± 0.79 ng/mL (mean ± SD; range 1.09 to 3.99 ng/mL; n =230). Cattle in this subset of study animals were determined to be pregnant on day 30 of gestation by transrectal ultrasonography. At 60 days of gestation, six animals (2.6%) experienced embryonic mortality (EM), again determined by ultrasonography. Mean serum concentrations of PAGs at day 30 of gestation were significantly higher for animals that maintained their pregnancy through day 60 (3.12 ± 0.05 ng/mL; mean ± SEM; $P < 0.05$; Figure 1) as compared to animals that experienced EM between day 30 and 60 of gestation (1.65 ± 0.14 ng/mL; mean ± SEM). A logistic regression curve of EM between day 30 and 60 of gestation was formed using 30 day circulating PAG concentrations as a continuous variable. This analysis shows that as the 30-day PAG concentrations decreases below 2.0, the likelihood of EM occurring before day 60 increases ($P < 0.05$; Figure 1).

We also used fetal measurements via ultrasonography to assess fetal viability and age. For those with known AI breeding dates, the fetal measurements were used to assess viability of the fetus. In this subset of the study population, we were unable to make an assessment of fetal size and viability due to the limited number of cows with EM that also had AI breeding dates to evaluate. What was observed was a difference in fetal size relative to breed of cow. This will be important in our future assessment. Breed of cow will need to be a part of our determinant as to fetal viability.

Supplemental reports:
Veterinary students that assisted in the research prepared individual studies of groups of the study cows. Abstracts from their study findings are included:

Title: "Pregnancy Associated Glycoprotein Levels and Embryonic Mortality in Florida Beef Cattle" *J Mariano, L Elmore, D Brown, D O Rae, July 2016,* Department of Large Animal Clinical Sciences, University of Florida College of Veterinary Medicine, Gainesville, FL, USA

This study examined circulating serum pregnancy associated glycoproteins (PAGs) as a potential predictor of embryonic mortality (EM) in study beef cattle. The main objective of the study was to identify the relationship between PAG concentrations at day 30 of gestation following timed artificial insemination and EM in beef cattle between day 30 and day 60 of gestation. A secondary objective was to identify a PAG value below which would likely predict EM. It was hypothesized that cattle with lower circulating PAG concentrations at day 30 of gestation would be more likely to experience EM than cattle with higher circulating concentrations. All of the animals received pregnancy examinations at 30 and 60 days after breeding. Pregnancy was diagnosed by the presence of a fetal heartbeat via ultrasonography during these checks. EM was said to occur if the animal was diagnosed pregnant during the 30 day check, but not pregnant (open) during the 60 day check. Only animals diagnosed as pregnant during the 30 day check were included in the analysis, pregnancy maintained, n = 224; EM, n = 6. Animals that experienced EM had significantly lower PAG values at day 30 of gestation (1.65 ± 0.14 ng/mL; mean ± SEM) than animals that maintained their pregnancy until day 60 of gestation (3.12 ± 0.05 ng/mL; mean ± SEM). The study was not able to identify a definitive PAG level below which EM would likely occur; however, a logistic regression curve was used to confirm an increasing probability of EM as PAG values fall below 2 ng/mL at day 30 of gestation. In conclusion, low circulating PAG concentrations at day 30 of gestation display a clear relationship with EM in beef cattle, but further studies with larger sample sizes are needed to definitively identify a PAG value below which would predict EM and factors that might produce these lower PAG values.

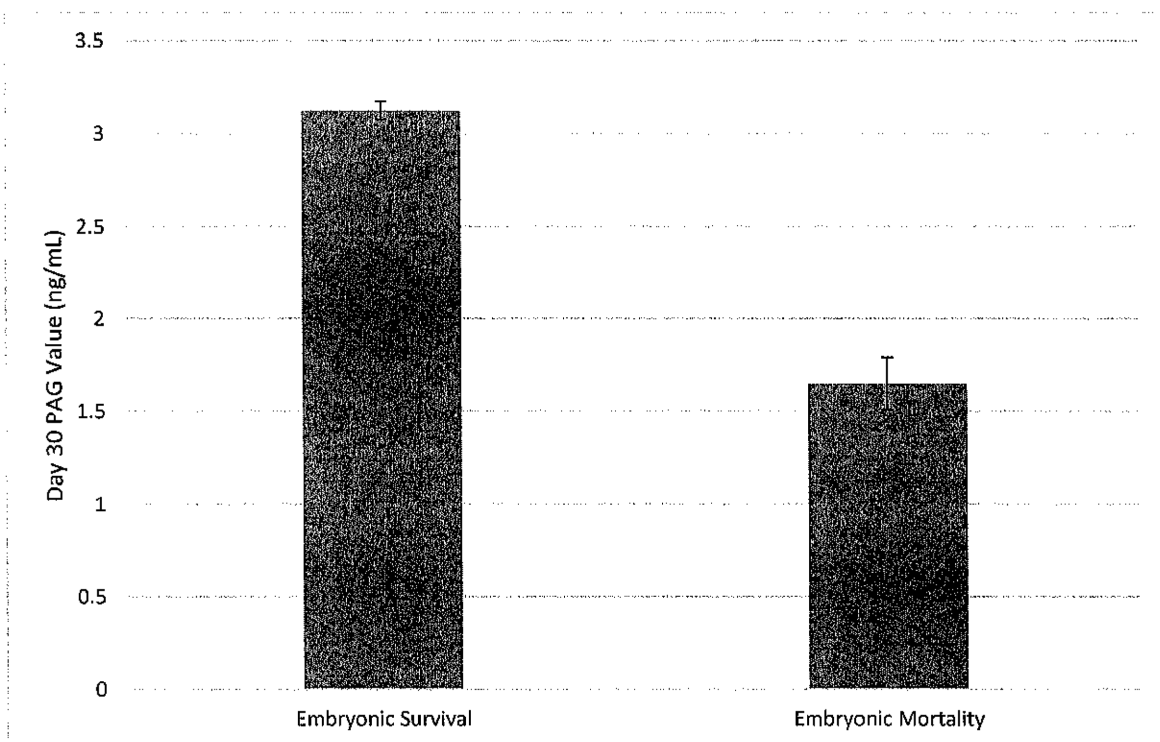

Figure 1 Mean (±SEM) serum concentrations of pregnancy associated glycoproteins (PAGs) in beef cattle that received timed artificial insemination (TAI) and had a viable embryo at day 30 of gestation (n = 230) and either maintained (embryonic survival; n = 224) or lost (embryonic mortality; n = 6) the embryo by day 60. Cattle that experienced embryonic mortality between day 30 and 60 had decreased ($P < 0.05$) PAG levels compared to cattle that maintained a viable embryo during that time.

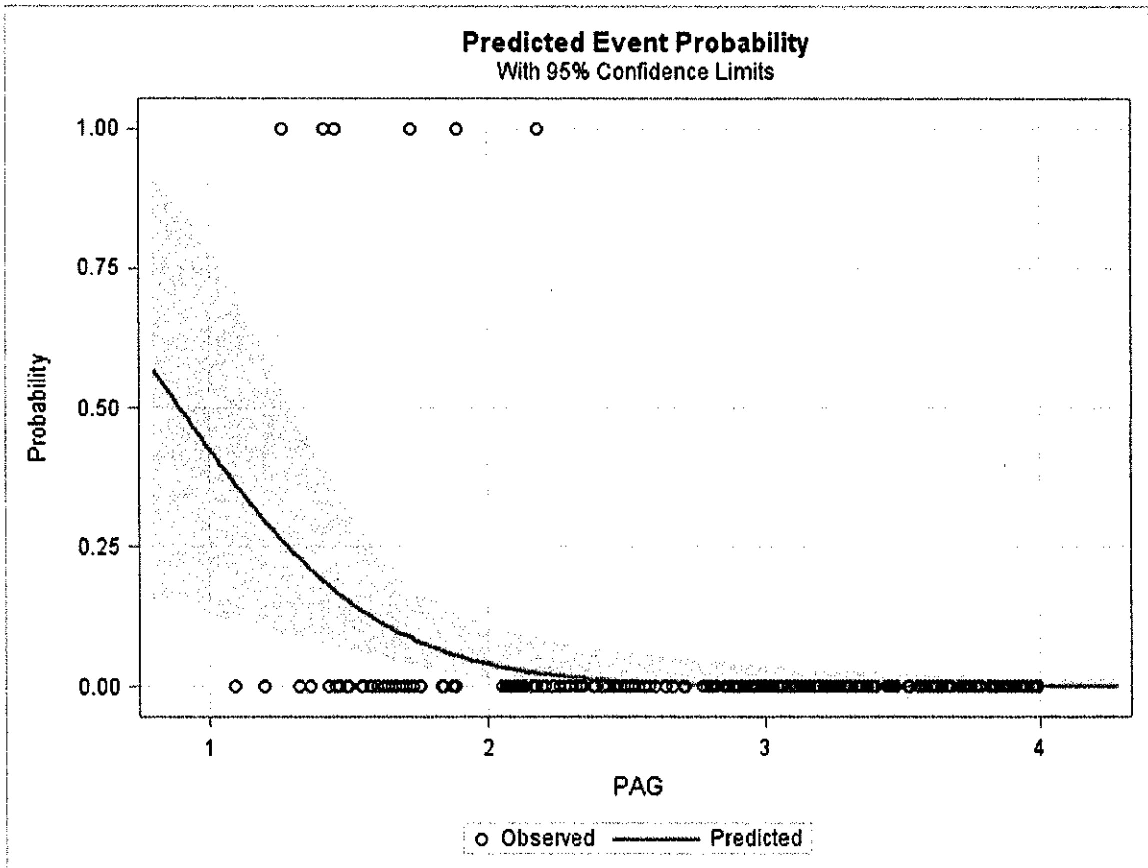

Figure 2. Probability of embryonic mortality between day 30 and 60 of gestation following TAI based on 30 day serum concentrations of PAGs (n = 230). Decreased PAG values significantly ($P < 0.05$) increased the probability of embryonic mortality in beef cattle following TAI.

Title: Comparison of fetal crown rump lengths in multiple breeds of Florida beef cattle via ultrasound diagnostics. *L H Elmore and D O Rae, July 2016,* Department of Large Animal Clinical Sciences, University of Florida College of Veterinary Medicine, Gainesville, FL, USA

The beef cattle industry relies on a successful and thriving weaned calf crop each year. With a host of variables, it is difficult to determine what may cause a cow to lose a pregnancy and fail to wean a calf. The purpose of this study was to evaluate/compare fetal crown rump (FeCR) lengths via transrectal ultrasound to gestational age (days pregnant, DPg) across different breeds prominent in the Florida beef cattle herds. Measuring FeCR length provides a fairly accurate indicator of pregnancy development and, as hypothesized in this study, can be used to monitor a potentially unsuccessful pregnancy based on the fetal size at measurement in early gestation. For study cows that had a known AI breeding date and a pregnancy examination that provided a FeCR length measurement, the FeCR was compared across six Angus x Brahman breed crosses to determine if a size difference in developing fetuses by breed was observed. Results were displayed in scatter plots for all studied pregnancies and by multi-breed categories (6), showing that a distinct size pattern by breed is evident, based on the first 60 days of pregnancy. In this group of cows, only one early embryonic loss was observed, it was, therefore, not possible to assess FECR in cows that lost pregnancy or maintained pregnancy. We were able to see that breed may influence our assessment of gestational age of the developing fetus.

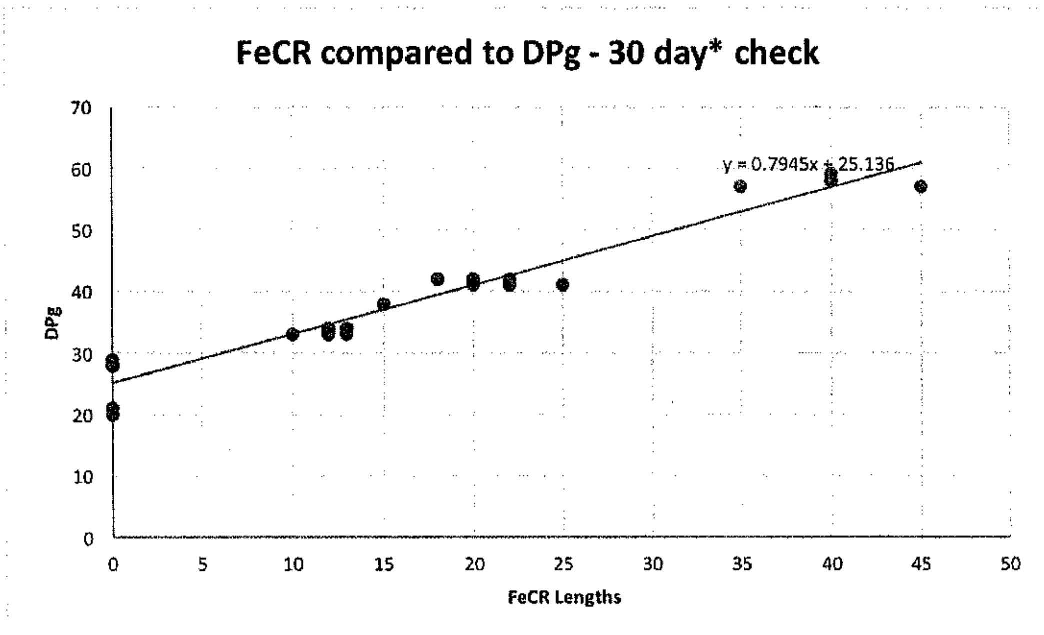

Figure 3. FeCR = fetal crown rump lengths (in mm), DPg = days pregnant

Table 1. Summary of data for 477 heifers and cows evaluated for pregnancy status (pregnant or non-pregnant) and state of pregnancy (pregnancy maintained or lost) by breed and variables of interest.

	Pregne▾y Status		
Variables by Breed ▾	Non-preg	Pregnant	Total
Angus			
Count	16	158	174
Percentage	9.2%	90.8%	100.0%
Av Weight (time 1)	976	1059	1052
Av BCS (time 1)	4.7	4.8	4.8
Av AMH	1177	777	815
Av PAG (time 1)	0.260	1.442	1.331
Av PAG (time 2)	0.123	1.730	1.578
Av PAG (time 3)		1.876	1.876
Av PAG (time 4)	0.036	2.115	2.050
Av PAG (time 5)	0.076	2.576	2.413
Angus 0.75 x Brahman 0.25			
Count	5	26	31
Percentage	16.1%	83.9%	100.0%
Av Weight (time 1)	1250	1187	1198
Av BCS (time 1)	4.8	4.5	4.6
Av AMH	1601	1250	1306
Av PAG (time 1)	0.069	0.203	0.181
Av PAG (time 2)	0.109	2.024	1.769
Av PAG (time 3)			
Av PAG (time 4)	0.035	2.670	2.354
Av PAG (time 5)	0.095	2.825	2.461
Brangus			
Count	13	169	182
Percentage	7.1%	92.9%	100.0%
Av Weight (time 1)	993	1070	1064
Av BCS (time 1)	5.1	4.9	4.9
Av AMH	1129	1398	1381
Av PAG (time 1)	0.182	1.245	1.167
Av PAG (time 2)	0.052	1.834	1.712
Av PAG (time 3)		2.652	2.652
Av PAG (time 4)	0.152	2.088	2.028
Av PAG (time 5)	0.148	2.756	2.550
Angus 0.5 x Brahman 0.5			
Count	3	32	35
Percentage	8.6%	91.4%	100.0%
Av Weight (time 1)	1097	1090	1091
Av BCS (time 1)	4.3	4.4	4.4
Av AMH	1535	1086	1125
Av PAG (time 1)	0.180	0.163	0.165
Av PAG (time 2)	1.500	1.768	1.745
Av PAG (time 3)	3.990	1.740	2.115
Av PAG (time 4)	0.150	2.269	2.118
Av PAG (time 5)	0.125	2.774	2.540
Angus 0.25 x Brahman 0.75			
Count	2	32	34
Percentage	5.9%	94.1%	100.0%
Av Weight (time 1)	1285	1140	1149
Av BCS (time 1)	5.0	4.9	4.9
Av AMH	1208	1165	1168
Av PAG (time 1)	0.028	0.200	0.190
Av PAG (time 2)	0.046	1.149	1.082
Av PAG (time 3)		3.375	3.375
Av PAG (time 4)		2.067	2.067
Av PAG (time 5)	0.091	2.747	2.581
Brahman			
Count	3	18	21
Percentage	14.3%	85.7%	100.0%
Av Weight (time 1)	1167	1030	1050
Av BCS (time 1)	5.7	5.2	5.3
Av AMH	779	1070	1022
Av PAG (time 1)	0.143	0.134	0.135
Av PAG (time 2)	0.050	1.162	1.003
Av PAG (time 3)	0.038	1.779	1.431
Av PAG (time 4)	0.777	2.759	2.461
Av PAG (time 5)	0.351	3.234	2.823
Total Count	**42**	**435**	**477**
Total Percentage	**8.8%**	**91.2%**	**100.0%**
Total Av Weight (time 1)	**1051**	**1078**	**1075**
Total Av BCS (time 1)	**4.9**	**4.8**	**4.8**
Total Av AMH	**1215**	**1113**	**1122**
Total Av PAG (time 1)	**0.188**	**1.047**	**0.971**
Total Av PAG (time 2)	**0.194**	**1.724**	**1.592**
Total Av PAG (time 3)	**2.014**	**2.000**	**2.001**
Total Av PAG (time 4)	**0.292**	**2.276**	**2.156**
Total Av PAG (time 5)	**0.148**	**2.772**	**2.537**

	State of ▾egnancy		
Variables by Breed ▾	Lost Preg	Pregnant	Total
Angus			
Count	8	166	174
Percentage	4.6%	95.4%	100.0%
Av Weight (time1)	1015	1053	1052
Av BCS (time 1)	4.9	4.7	4.8
Av AMH	931	809	815
Av PAG (time 1)	1.267	1.335	1.331
Av PAG (time 2)	1.172	1.599	1.578
Av PAG (time 3)		1.876	1.876
Av PAG (time 4)	0.094	2.113	2.050
Av PAG (time 5)	0.136	2.463	2.413
Angus 0.75 x Brahman 0.25			
Count		31	31
Percentage	0.0%	100.0%	100.0%
Av Weight (time1)		1198	1198
Av BCS (time 1)		4.6	4.6
Av AMH		1306	1306
Av PAG (time 1)		0.181	0.181
Av PAG (time 2)		1.769	1.769
Av PAG (time 3)			
Av PAG (time 4)		2.354	2.354
Av PAG (time 5)		2.461	2.461
Brangus			
Count	3	179	182
Percentage	1.6%	98.4%	100.0%
Av Weight (time1)	930	1067	1064
Av BCS (time 1)	4.8	4.9	4.9
Av AMH	1465	1380	1381
Av PAG (time 1)	1.194	1.167	1.167
Av PAG (time 2)	2.457	1.699	1.712
Av PAG (time 3)		2.652	2.652
Av PAG (time 4)	1.890	2.032	2.028
Av PAG (time 5)	0.308	2.611	2.550
Angus 0.5 x Brahman 0.5			
Count	1	34	35
Percentage	2.9%	97.1%	100.0%
Av Weight (time1)	1080	1091	1091
Av BCS (time 1)	5.0	4.4	4.4
Av AMH	1608	1111	1125
Av PAG (time 1)	0.048	0.168	0.165
Av PAG (time 2)	3.244	1.699	1.745
Av PAG (time 3)	3.990	1.740	2.115
Av PAG (time 4)	0.236	2.187	2.118
Av PAG (time 5)	0.135	2.613	2.540
Angus 0.25 x Brahman 0.75			
Count	2	32	34
Percentage	5.9%	94.1%	100.0%
Av Weight (time1)	1168	1148	1149
Av BCS (time 1)	5.0	4.9	4.9
Av AMH	1417	1152	1168
Av PAG (time 1)	0.072	0.197	0.190
Av PAG (time 2)	0.056	1.149	1.082
Av PAG (time 3)		3.375	3.375
Av PAG (time 4)	1.079	2.143	2.067
Av PAG (time 5)	1.066	2.682	2.581
Brahman			
Count	1	20	21
Percentage	4.8%	95.2%	100.0%
Av Weight (time1)	865	1059	1050
Av BCS (time 1)	5.0	5.3	5.3
Av AMH	1400	1000	1022
Av PAG (time 1)	0.967	0.094	0.135
Av PAG (time 2)	0.051	1.050	1.003
Av PAG (time 3)		1.431	1.431
Av PAG (time 4)	1.408	2.517	2.461
Av PAG (time 5)	0.061	2.961	2.823
Total Count	**15**	**462**	**477**
Total Percentage	**3.1%**	**96.9%**	**100.0%**
Total Av Weight (time1)	**1013**	**1077**	**1075**
Total Av BCS (time 1)	**4.9**	**4.8**	**4.8**
Total Av AMH	**1179**	**1120**	**1122**
Total Av PAG (time 1)	**0.992**	**0.970**	**0.971**
Total Av PAG (time 2)	**1.344**	**1.600**	**1.592**
Total Av PAG (time 3)	**3.990**	**1.884**	**2.001**
Total Av PAG (time 4)	**0.964**	**2.201**	**2.156**
Total Av PAG (time 5)	**0.462**	**2.601**	**2.537**