An Exploration of relationships between three main characteristics of Equine Metabolic Syndrome, obesity, insulin resistance, and laminitis

by

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Abstract

Understanding the relationship between three main characteristics of Equine Metabolic Syndrome, obesity, insulin resistance, and laminitis, can provide us an insight into diagnosis and prevention of this disease. We analyzed data collected by Equine Genetics and Genomics Laboratory at the University of Minnesota and built two random effects models on two responses we chose, insulin level before and after glucose tolerance test, to explore the relationships between three symptoms and also other interesting covariates, such as exercise and diet. We also compared the predicted insulin values with true insulin values for both healthy horses and EMS horses, and described how EMS horses' performance differed from healthy horses' performance in these two models.

Keywords: Equine Metabolic Syndrome, obesity, laminitis, insulin resistance, glucose tolerance test, random effects model

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Chapter1 Introduction and Background

1.1 Introduction to Equine Metabolic Syndrome (EMS)

Horse owners have always worried about their horses' health. Obesity, including regional adiposity, insulin resistance, and laminitis are some common threats. These three characteristics are believed to be correlated with each other, and together contribute to a newly-defined disease, called Equine Metabolic Syndrome (EMS) (Johnson P. J., 2002). Though the study of EMS is still immature, there are already a few findings regarding the definition, diagnosis, and management of EMS (Frank, N., Geor, R. J., Bailey, S. R., Durham, A. E., & Johnson, P. J., 2010). However, further research is needed to improve our understanding of EMS.

At this time, the link between insulin resistance, obesity, and laminitis and their contribution to EMS are not completely understood. The purpose of our study is to analyze how these three main characteristics are related and how they work together to cause EMS. We want to be able to detect horses susceptible to EMS before they develop this disease.

1.2 Definition of Equine Metabolic Syndrome

As a relatively fresh discovery, many aspects of Equine Metabolic Syndrome are still unexplored. One of them is to find a suitable name for this disorder. Due to its similar symptoms with Cushing's disease, it was once known as peripheral Cushing's syndrome. However, with more and more studies on EMS, the diagnosis of this disease is becoming mature, and EMS is now distinguished from Cushing's by the horse's age and some clinical signs exceptional for Cushing's, such as delayed shedding of the winter coat and increased drinking (Equine Metabolic Syndrome (EMS), 2013). Later, because of its similarities with metabolic syndrome in humans, it was given an analogous name, but unique to equids, Equine Metabolic Syndrome.

The syndrome consists of three main symptoms: obesity, insulin resistance (IR), and laminitis. Equines with this syndrome are sometimes called "easy keepers" because they can easily get fat even with a decreased diet and increased exercise.

1.2.1 Insulin Resistance

Insulin resistance is a determining symptom for EMS. Horses with this condition tend to have a high insulin level in the blood. This is caused either by the low rate of glucose uptake into tissues, or by diminished quantity of insulin released by the pancreas. Both may result in a high glucose level, which in turn prompts the release of insulin into the blood and leads to a high insulin level in the blood.

In our study, we focus on insulin and glucose levels, though we also analyze for other related measures, such as ACTH and triglyceride. Before taking the blood sample, horses were removed from pasture and fed only grass hay and water for 12 hours. The horses also took an oral glucose tolerance test, which provides a good estimate of insulin resistance, based on the measurements of insulin and glucose before and after the glucose is given.

1.2.2 Obesity

Obesity and regional adiposity are important components of EMS, which may be induced by exercise level, diet or genetic factors. Worth mentioning, we are not only interested in obese horses, but also in horses having accumulation of fat in certain areas with overall normal bodyweight. We believe that regionally adipose horses tend to have a crest neck (high neck to height ratio) and high girth to height ratio.

1.2.3 Laminitis

Laminitis is also called “founder” in EMS since the development of laminitis encourages horse owners to test whether their horses have EMS or not. Laminitis usually appears in ungulates, including horses, where it is a disease in the feet. Regardless of the cause of this painful disease, some studies think obesity and insulin resistance are associated with laminitis, though a definite theory is still not established.

1.3 Management

1.3.1 Medical Management

EMS is currently treated by addressing the symptoms. Most horses and ponies with EMS can be effectively managed with changes in diet and exercise. However, sometimes medical treatment may also be needed, and for EMS, till now, only levo-thyroxine sodium is considered to be effective in some circumstances.

1.3.2 Dietary Management

The fundamental aspect in maintaining weight is to reduce the amount of total calories in the diet and to lower the Non-structural carbohydrate (NSC) content of the diet to reduce the glyceric response. It is also important to reduce pasture grass, which is easily ignored in calculating the total calorie consumption. If a horse is detected to be obese, it is provided with a low NSC grass hay diet with mineral/vitamin supplementation. It is also prevented from pasture until the insulin sensitivity improves since grass consumed on pasture may trigger laminitis in these horses. (Frank, N., Geor, R. J., Bailey, S. R., Durham, A. E., & Johnson, P. J., 2010)

1.3.3 Exercise

An increase in physical activity improves insulin sensitivity, however, foot pain caused by laminitis may make this difficult. A general recommendation is approximately 200 minutes per week of moderate intensity exercise (Houmard, J. A., Tanner, C. J., & Slentz, C. A., et al, 2004, Bajpeyi, S., Tanner, C. J., &Slentz, C.A., et al, 2009).

Chapter 2 Data

2.1 Data Collection

The data was collected by the Equine Genetics and Genomics Laboratory from the University of Minnesota through an online survey and from farms directly. For the 236 horses selected via online survey, three steps were taken to complete the survey. First, the participants were asked to provide the general information about the horses and also specific information related to EMS which helped to roughly prescreen ideal candidates for the study. If they proceeded to the second step, they would be asked to provide additional information on a pair of horses, one suspected to have EMS and one control. If the paired horses were determined to be likely candidates, they would need to provide a blood sample for analysis of glucose and insulin. One problem in this data collection procedure is that the horses are not randomly selected from each farm and this may lead us to find a relationship that is caused by the sampling.

In addition to the 236 horses, researchers also went to additional farms in person and randomly selected 399 horses regardless of whether they have EMS or not.

2.2 Data Description

The data set consists of 635 observations and 107 variables. We divided the variables into five groups, which were general information, laminitis and EMS diagnosis, overweight, exercise, diet and genes. This analysis did not study the genes, so in the following analysis, those variables are not included.

Variable	Description	Value
Owner	Farm where horse is from	UVM-Morgans, Van_Westen_Betsy, etc
Breed5	Five types of horse breed	Arab, Morgan, QH, TW, Welsh
AGE	Age of horse	2-33, NA
SEX	Sex of horse	Stallion, mare, gelding
LAM	If horse has had laminitis ever	n, y
EMS	If owner thinks horse has EMS	n, y
BCS	Body condition score	2.5-9, NA
NH	Neck/height ratio	0.4958-0.8333, NA
GH	Girth/height ratio	0.7742-1.495, NA
GLU	Glucose(sugar) level in the blood before oral glucose tolerance test	32.8-161, NA
GLU_OGT	Glucose(sugar) level in the blood after oral glucose tolerance test	40.7-203, NA
INS	Insulin level in the blood before oral glucose tolerance test	0-632, NA
INS_OGT	Insulin level in the blood after oral glucose tolerance test	0-783.6, NA
Hrs_exercise_week	Exercise per week in hours	0-20, NA
Hrs_exercise_day	Exercise per day in hours	0-2.857, NA
Hrs_turnout	Pasture time in hours	-0.5714-24, NA
Total_exercise	(Hrs_exercise_day*Exercise_ intensity) +Hrs_turnout	0-26.93, NA
Mcal	Calories consumed per bodyweight(kg)	0.006389- 0.1039, NA
WSC	Soluble Carbs	0.07245- 5.464, NA
Starch	Starch	0.01945- 5.175, NA
NSC	Non-structural carbohydrate	0.2842- 7.043, NA
thinkEMS	If we think horse has EMS based on strict criteria from researchers	FALSE, TRUE

Table 1: Variable Description.

2.3 Diagnosis

The EMS is currently diagnosed based on history, physical characteristics, results of glucose-tolerance testing and elimination of similar conditions, such as Cushing’s syndrome. Our researchers developed a system of criteria especially for our study based on several measurements (Equine Metabolic Syndrome (EMS), 2013).

(a) Insulin > 20 μU/mL provides evidence of insulin resistance (IR).

(b) A neck/height ratio greater than 0.63 in horses and greater than 0.68 in ponies provides evidence of having a crest neck.

(c) A girth/height ratio greater than 1.26 in horses, and greater than 1.33 in ponies provides evidence of being overweight.

(d) Horses with laminitis history are considered to be more susceptible to EMS.

Based on the above criteria, we divided the data set into two groups, one which we think has EMS, while the other we think does not. The new grouping is used in data exploration, model fitting and model validation, and is included in the data set as thinkEMS.

2.4 Data Exploration

We began with exploring relationships between variables within each group. For the exercise group, we found that several are exactly linearly related, by definition: Minutes_exercise_week, Hrs_exercise_week, and Hrs_exercise_day. For this analysis, Hrs_exercise_day was used.

Total_exercise is a summary variable which combines information from exercise variables. Many horses are pastured all the time; these horses have a value of 24 for Hrs_turnout. So this variable contributes a large proportion to Total_exercise variable, and we observed a strong linear relationship between Hrs_turnout and Total_exercise in Figure 1.

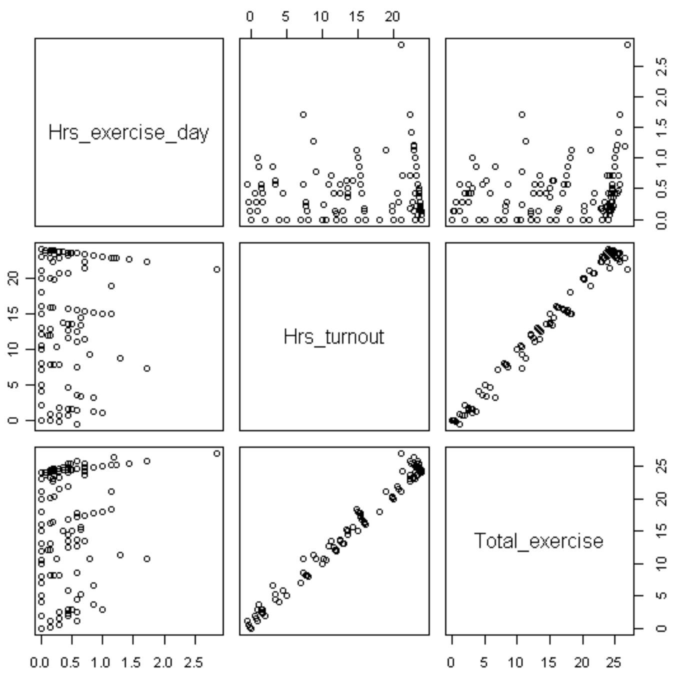


Figure 1: Hrs_exercise_day vs Hrs_turnout vs Total_exercise.

In the diet group, we also found linear relationships between variables. Since WSC (soluble carbs) and Starch are main source of sugar calories, and Mcal is calories consumed per kg bodyweight, we took these three variables as representatives for the diet group. Also, from Figure 2, we found that log transformation may be needed for Starch.

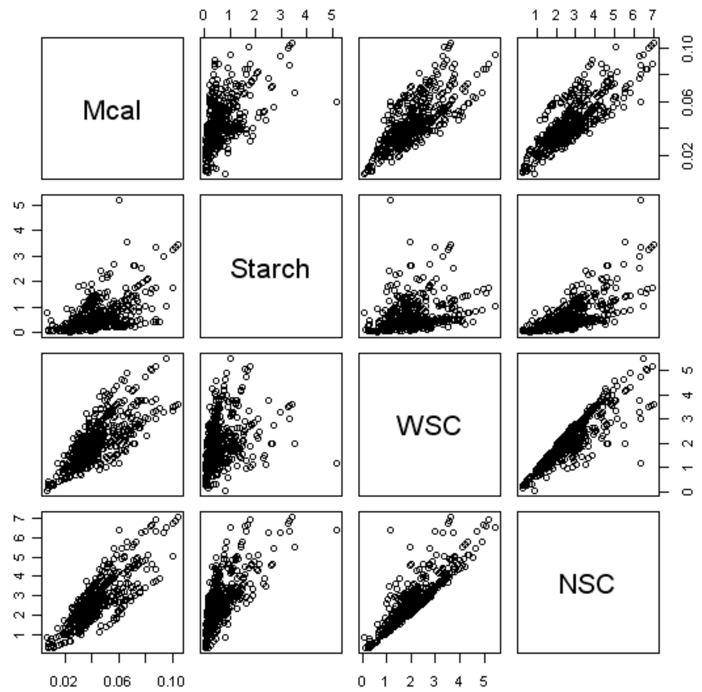


Figure 2: Mcal vs Starch vs WSC vs NSC.

In Figure 3. we find that both INS and INS_OGT are gathered around the origin, so we log-transformed both of them. The INS and INS_OGT variables in the rest study of this analysis were all log-transformed.

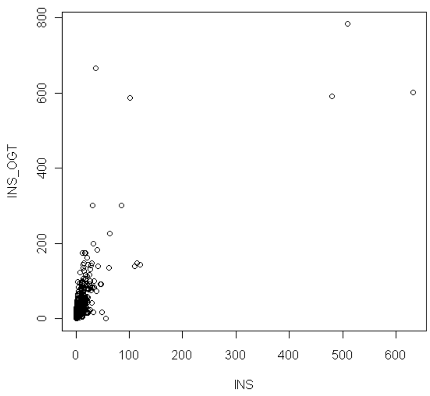


Figure 3: INS vs INS_OGT.

We also tried to explore the relationship between groups. We found that LAM and BCS (Figure 4), LAM and INS/ INS_OGT (Figure 5), BCS and INS/ INS_OGT (Figure 6) were correlated, about which it was hard to make any conclusion, since it could be induced by the sampling.

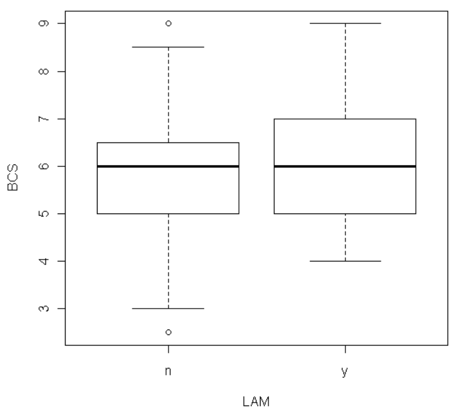


Figure 4: LAM vs BCS.

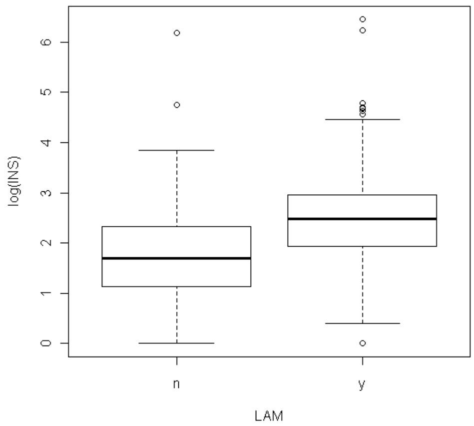


Figure 5 (a): LAM vs log(INS).

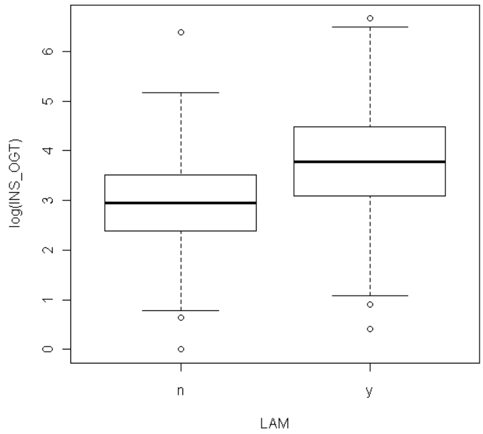


Figure 5 (b): LAM vs log(INS_OGT).

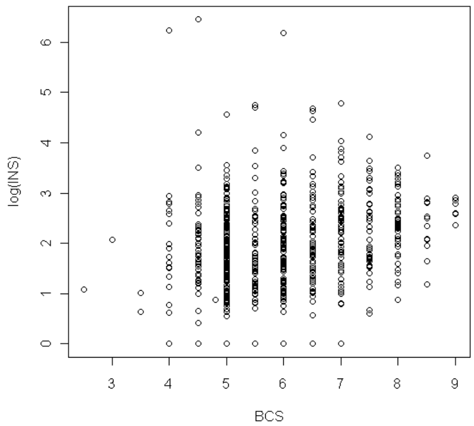


Figure 6 (a): BCS vs log(INS).

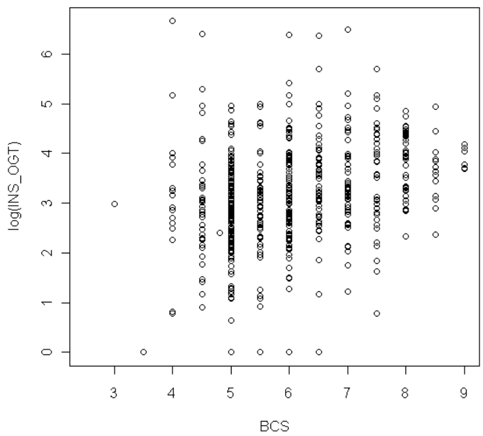
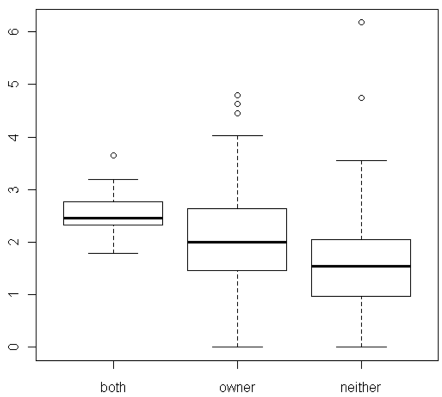


Figure 6 (b): BCS vs log(INS_OGT).

We also plotted INS, INS_OGT, BCS, and LAM vs three EMS status we defined to make some comparisons. The three EMS status were horses diagnosed to have EMS both by us and by owner (group “both”), horses diagnosed to have EMS by owner, but not by us (group “owner”), and horses diagnosed to not have EMS either by us or by owner (group “neither”).

In Figure 7, horses in group “both”, which were diagnosed to have EMS by both owner and us, had largest insulin level, before and after glucose tolerance test. Horses in group “owner” had the second largest insulin level, followed by horses in group “neither”.



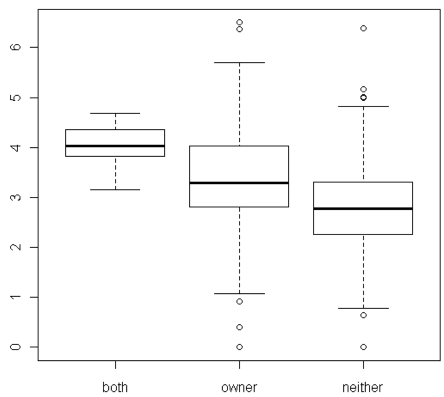


Figure 7(a): Comparison of log(INS). **Figure 7(b):** Comparison of log(INS_OGT).

In Figure 8, we also compared BCS in the three groups, and they had similar relationship as in INS and INS_OGT.

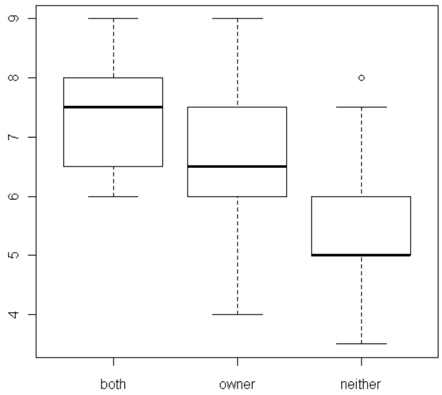


Figure 8: Comparison of BCS in three groups.

From Table 2, we found that for horses diagnosed to not have EMS either by us or by owner, no one was diagnosed to have laminitis history. For horses diagnosed to have EMS by owner, but not by us, 109 out of 192 was diagnosed to have laminitis history, while the rest was diagnosed to not have laminitis history. For horses diagnosed to have EMS by both owner and us, all 27 horses were diagnosed to have laminitis history.

		EMS (by owner)			
		n		y	
		thinkEMS (by us)		thinkEMS (by us)	
		n	y	n	y
LAM	n	231	0	109	0
	y	0	0	83	27

Table 2: LAM vs thinkEMS vs EMS.

Chapter 3 Methods

3.1 Concerns

In this study, we wanted to explore the relationship between the three main characteristics: obesity, insulin resistance, and laminitis, with a secondary question of possibly predicting EMS status. We hoped to fit the model connecting these three variables and other interesting covariates. However, the researcher asked horse owners to diagnose whether their horses have EMS or not based on their own criteria and then selected paired horses based on this sampling. Two problems arose from this data collection process.

First, the researchers were not quite sure if the diagnosis from horse owners was correct, and it was also very likely that the criteria from different farms wouldn't be consistent, so the paired horses are selected differently. To solve this problem, we chose to use the researcher's strict diagnosis guidelines, as stated in data exploration part, instead of the owner's diagnosis to alleviate this concern.

Second, this sampling method might introduce some trends into our data set, such as the correlation between three main characteristics. If we continued our analysis based on this problematical sampling, we might found some relationship we were interested, but we still could not make any conclusion since it might be caused by the sampling. In a result, we chose not to use EMS criterion in the data set in model building.

With our goal in mind, we considered several candidate responses that might arrive at valid conclusion. We first considered Bodyweight= Girth* Girth* length*(1/330) and residual of BCS~Mcal, which was the extra amount over/under expected weight given the amount they ate. We found them to be interesting candidate responses because they could be viewed as a proxy for EMS. However, these variables were used as criteria for distinguishing healthy and EMS horses in the sampling process. For example, a horse with high bodyweight was likely to be EMS. If we continued using this variable as our response for exploring the relationship between obesity and EMS, we might find a positive correlation between obesity and EMS, but it might be created by our sampling method and this conclusion would not be valid.

We then tried to find a new response that was unrelated to the poor sampling method. We were interested in INS and INS_OGT since the insulin level for EMS and healthy horses would be very different both before and after glucose tolerance test. Also, they were not known during the period of data collection, so they were not directly linked to whether or not owner thought the horse had EMS and therefore the decision of inclusion.

3.2 Models

With insulin level as our response, we built models to explore its relationship with the other two characteristics of EMS, LAM and BCS, including other interesting covariates as predictors. Despite issues with EMS variable, we also used these models to explore relationship between INS and EMS status. We first built a model based on factors not believed to be related with EMS, such as exercise, diet, etc. We wanted to use this model to look into the relationship between insulin level and EMS status, without considering obesity or laminitis. We also built a model to describe the relationship between obesity, laminitis, and insulin, based on both factors related to EMS, such as laminitis and BCS, and unrelated factors in the first model.

With INS as our response, we first considered GLU to be in our model, since it was highly correlated with insulin level before glucose tolerance test and then a main predictor of INS. While with INS_OGT as our response, except for GLU, we also included GLU_OGT and INS in our model, since they together affected insulin level after glucose tolerance test. Because of the importance of these three predictors, we decided to always keep them in our model, even if they appeared to be insignificant in later variable selection.

In addition to these three main predictors, we also considered some other covariates that might affect insulin level, but were not related to EMS, such as Total_exercise, WSC, Starch, Mcal, AGE and Breed5. We used them to improve our prediction of our insulin level, but we were not sure of their influence. So we considered deleting them if they appeared to be insignificant in the model.

Our first model (model A) was fitted on the factors described above, with INS and INS_OGT as responses separately. Since we wanted to use this model to look into the relationship between insulin level and EMS status, we needed to know the insulin level for both healthy horses and EMS horses, with same information for all factors in the model. So we fitted our model only on healthy horses, and we predicted what the insulin level would be if a horse were healthy with same values for other factors as EMS horses. We then compared the prediction for EMS horses with their true INS and INS_OGT values. If there was no relationship between insulin level and EMS status, then the predicted insulin level for EMS horses should be similar to the true insulin level, with same information about predictors in the model. But if the true values were larger than the predicted values, this suggested a relationship between insulin level and EMS, that is, insulin level tended to be higher for EMS horses.

Since we also wanted to explore the relationship between insulin and other two characteristics, obesity and laminitis for the whole horse population, we fitted our second model (model B) on all horses in the data set, and added LAM and BCS into our model. Then we made predictions based on mean of predictors (except for LAM and BCS) and actual values of LAM and BCS, to explore the portion of insulin level that is related to BCS and LAM. We compared adjusted predictions for healthy horses with EMS horses. Since the information for unrelated terms in the model were the same, the difference in the predicted values would be only caused by difference in BCS and LAM. Also, since EMS horses we diagnosed had laminitis and higher BCS, the predicted insulin level was higher for EMS horses than healthy horses. We constructed a plot to show to what extent, they increased the insulin level.

Finally, since we had horses selected from multiple farms, we wanted to take this effect into account. In order to do this, we fitted all models with Owner (variable that indicates which farm the horse is from) as random effect.

3.3 Model Selection

With variable selection, we want to select the "best" subset of predictors, so as to explain the data in the simplest way. In order to achieve this goal, we need to remove all redundant variables which may add noise to the estimation of other quantities that we may be interested in and also waste degrees of freedom. Besides, too many unnecessary variables may also introduce multicollinearity, which severely affects our interpretation of individual predictors.

In this study, we mainly used the backward elimination to implement model selection. We started with all predictors in the model and removed the predictor with the highest p-value greater than 0.05 significance level. Variables that were considered to be important beforehand were not considered for deletion. Then we refitted the model and did the same step as above until we came up a model with only significant predictors, either statistically significant or supposed to be meaningful. We deleted one-at-a-time to avoid missing any meaningful variables and after deleting each variable, we assessed the model to see how it affected the significance of other predictors.

Chapter 4 Results

4.1 Model A, INS_OGT as response

We started with log(INS), GLU, GLU_OGT, Total_exercise, WSC, Starch, Mcal, AGE, Breed5. Applying backward elimination resulted in a model with log(INS), GLU, GLU_OGT, log(Starch), AGE, Breed5 as predictors.

	Value	Std.Error	DF	t-value	p-value
(Intercept)	0.63	0.299	314	2.1	0.0373
log(INS)	0.69	0.038	314	18.2	0.0000
GLU	-0.02	0.004	314	-3.9	0.0001
GLU_OGT	0.02	0.002	314	10.2	0.0000
log(Starch)	-0.13	0.039	314	-3.4	0.0006
AGE	0.01	0.005	314	1.7	0.0913
Breed5Morgan	-0.08	0.116	314	-0.7	0.4792
Breed5QH	-0.46	0.166	314	-2.8	0.0056
Breed5TW	0.19	0.164	314	1.2	0.2358
Breed5Welsh	-0.32	0.144	314	-2.2	0.0252

Model	df	AIC	BIC	logLik	L.Ratio	p-value
Full Model	15	855	916	-413		
Final model	12	853	901	-414	3.1	0.38

The ANOVA test showed that our final model was preferable to our full model.

Then we compared the true values with the predicted values. We found among 26 EMS horses, only 17 horses had higher true insulin value in the blood after the glucose tolerance test than the predicted value from the model. However, with only 17 horses out of 26 having higher insulin level, we were wary of making any conclusion about the relationship between EMS status and insulin level after glucose tolerance test. Also, we plotted the differences of true and predicted insulin values after glucose tolerance test in Figure 9 to have a sense of how big their differences were. We could see that the differences for healthy horses were almost evenly distributed around 0, while we could not we could not find any obvious trend that indicated the relationship between EMS status and insulin level for EMS horses.

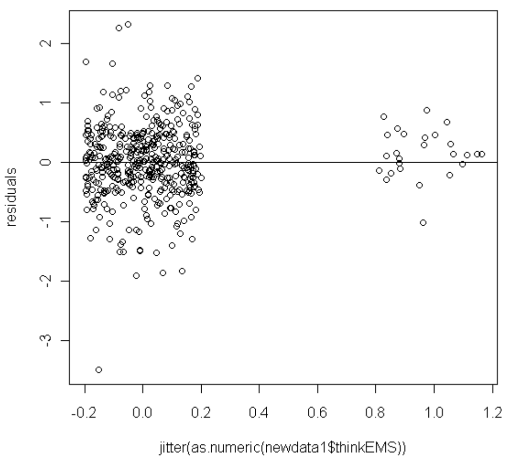


Figure 9: residuals vs thinkEMS.

4.2 Model A, INS as response

We started with GLU, Total_exercise, WSC, Starch, Mcal, AGE, Breed5. Applying backward elimination resulted in a model with GLU, Mcal, AGE, Breed5 as predictors.

	Value	Std.Error	DF	t-value	p-value
(Intercept)	-0.90	0.403	316	-2.22	0.0270
GLU	0.03	0.004	316	8.13	0.0000
Mcal	-5.61	3.062	316	-1.83	0.0678
AGE	0.03	0.006	316	4.59	0.0000
Breed5Morgan	0.22	0.191	316	1.16	0.2477
Breed5QH	-0.35	0.249	316	-1.42	0.1557
Breed5TW	0.37	0.254	316	1.45	0.1489
Breed5Welsh	0.35	0.259	316	1.34	0.1825

Model	df	AIC	BIC	logLik	L.Ratio	p-value
Full model	13	1044	1097	-509		
Final model	10	1041	1081	-510	2.94	0.401

The ANOVA test showed that our final model was preferable to our full model.

We also compared the true values and the predicted values and we found that 22 out of 25 EMS horses had higher true insulin level in the blood before glucose tolerance test than the predicted value from the model. Since this model was based on healthy horses, the predicted value should be similar to true value if horses are healthy. This implied that horses with EMS tended to have higher insulin level.

However, with our own diagnosis of EMS, there were only 27 horses in the data set that we were sure of having EMS and it was hard to make any strong conclusion with so few EMS horses. So we only claimed that this model explained their relationship to some extent.

As before, we also plotted the differences of true and predicted insulin values before glucose tolerance test in Figure 10 and we found that differences for healthy horses were also almost evenly distributed around 0. However, the differences for EMS horses were among big positive values in the distribution of differences for healthy horses. So we might conclude that EMS status made a big difference in insulin value before glucose tolerance test.

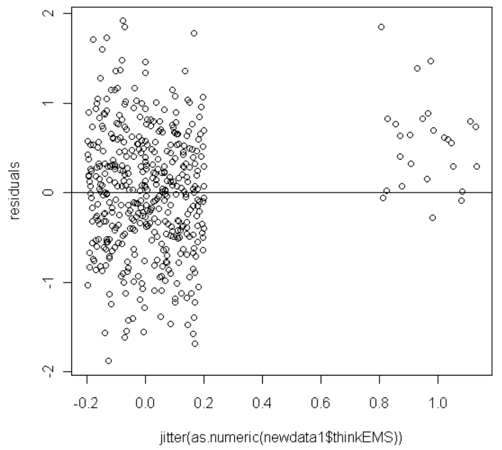


Figure 10: residuals vs thinkEMS.

4.3 Model B, INS_OGT as response

We started with log(INS), GLU, GLU_OGT, LAM, BCS, NH, GH, Total_exercise, WSC, Starch, Mcal, AGE, Breed5. Applying backward elimination resulted in a model with log(INS), GLU, GLU_OGT, LAM, BCS, log(Starch), AGE, Breed5 as predictors. Though LAM showed to be insignificant in the model, we still kept it in the model since it contained very important information that was our interest.

	Value	Std.Error	DF	t-value	p-value
(Intercept)	0.418	0.346	338	1.21	0.2286
log(INS)	0.656	0.039	338	16.64	0.0000
GLU	-0.013	0.004	338	-3.37	0.0008
GLU_OGT	0.022	0.002	338	10.08	0.0000
LAMy	0.107	0.080	338	1.33	0.1833
BCS	0.066	0.030	338	2.19	0.0289
Starch	-0.195	0.055	338	-3.54	0.0005
AGE	0.010	0.005	338	1.99	0.0470
Breed5Morgan	-0.059	0.116	338	-0.51	0.6120
Breed5QH	-0.424	0.162	338	-2.62	0.0093
Breed5TW	0.198	0.160	338	1.24	0.2164
Breed5Welsh	-0.297	0.162	338	-2.11	0.0358

Model	df	AIC	BIC	logLik	L.Ratio	p-value
Full Model	19	889	967	-426		
Final Model	14	882	940	-427	2.98	0.703

The ANOVA test showed that our final model was preferable to our full model.

The coefficient of LAM was 0.107 which indicated that the predicted insulin level after the glucose-tolerance test for horses with laminitis history was exp(0.107)=1.1129 times the ones without that history. While the coefficient of BCS, which was 0.066, meant that a unit increase in BCS multiplied the predicted insulin level after the glucose-tolerance test by exp(0.066)=1.0682. So both LAM and BCS increased the insulin level after glucose-tolerance test.

We also constructed a normal distribution using mean and standard deviation from predictions of healthy horses, and calculated in what percentile of healthy horses' population the EMS horses predicted values were.

26.0 33.3 33.3 33.3 40.3 46.2 47.4 47.7 52.8 55.2 59.3 60.3 62.5 62.5 62.5 62.5 62.5 69.4 74.2 75.7 75.7 75.7 87.7 89.1 91.9 96.0 98.9

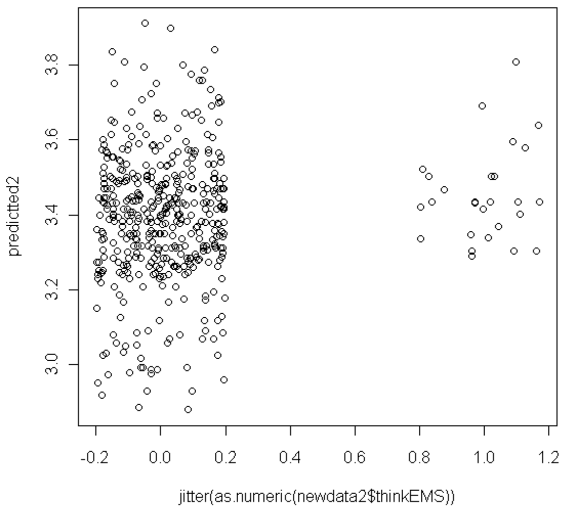


Figure 11: predicted INS_OGT vs thinkEMS.

We found among 27 EMS horses, most horses had high predicted values corresponding to high quantile values in the distribution of healthy horses' predicted insulin values, and also from Figure 11, we saw that the points for the horses that we thought having EMS fall in the higher position among the healthy horses.

4.4 Model B, INS as response

We started with GLU, LAM, BCS, NH, GH, Total_exercise, WSC, Starch, Mcal, AGE, Breed5. Applying backward elimination resulted in a model with GLU, LAM, BCS, NH, WSC, log(Starch), Mcal, AGE as predictors.

	Value	Std.Error	DF	t-value	p-value
(Intercept)	-3.66	0.59	341	-6.17	0.0000
GLU	0.03	0.00	341	9.02	0.0000
LAMy	0.52	0.09	341	5.84	0.0000
BCS	0.20	0.04	341	5.55	0.0000
NH	2.35	0.88	341	2.68	0.0078
WSC	0.19	0.11	341	1.65	0.0999
Starch	0.25	0.09	341	2.84	0.0049
Mcal	-14.24	6.10	341	-2.34	0.0201
AGE	0.03	0.01	341	4.60	0.0000

Model	df	AIC	BIC	logLik	L.Ratio	p-value
Full Model	17	1018	1088	-492		
Final Model	11	1014	1059	-496	7.28	0.296

The ANOVA test showed that our final model was preferable to our full model.

The coefficient of LAM was 0.52 which indicated that the predicted insulin level before the glucose-tolerance test for horses with laminitis history was exp(0.52)=1.682 times than the ones without that history. While the coefficient of BCS, which was 0.20, meant that a unit increase in BCS multiplied the predicted insulin level before the glucose-tolerance test by exp(0.20)=1.2214. So both LAM and BCS increased the insulin level before glucose-tolerance test.

As before, we calculated in what percentile of healthy horses' population the EMS horses predicted values were.

60.4 68.2 78.2 78.9 80.0 80.8 81.5 81.5 82.6 87.0 87.6 88.0 88.3 90.6 91.3 94.8 95.6 96.5 96.5 97.7 98.1 98.7 98.8 98.8 98.9 99.2 99.3

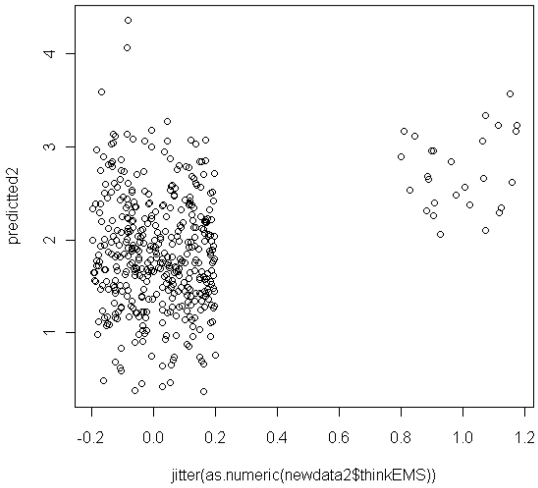


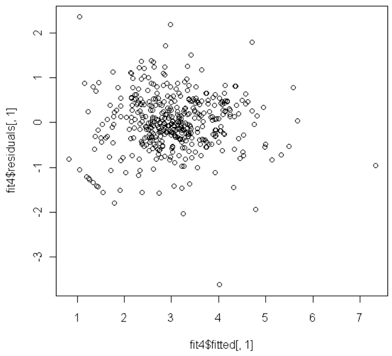
Figure 12: predicted INS vs thinkEMS.

We found among 27 EMS horses, almost all horses had high predicted values corresponding to high quantile values in the distribution of healthy horses' predicted insulin values, and also from Figure 12, we saw that the points for the horses that we thought having EMS fall in the higher position among the healthy horses.

4.5 Model Diagnosis

In our analysis, we chose to plot residuals with predicted values and also normal qq-plot to see how the model fits the data. If the residuals randomly fell in the plot around mean 0 and normal qq-plot seemed to have linearly relationship, then we might conclude that the model fitted the data pretty well.

Model A INS_OGT



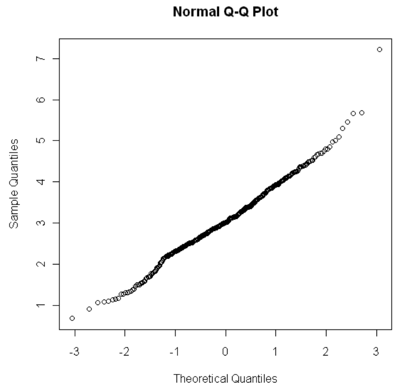
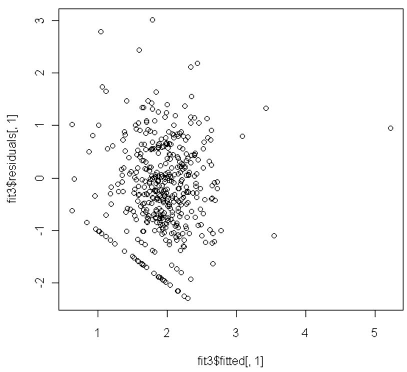


Figure 13(a): residuals vs fitted values **Figure 13(b):** qq-plot of residuals

Model A INS



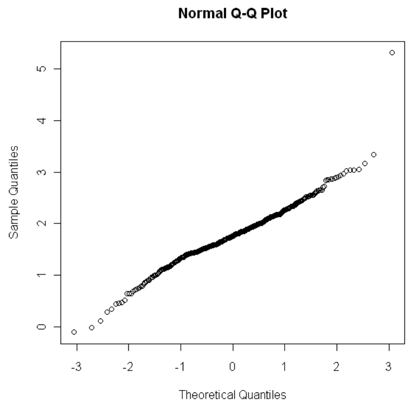
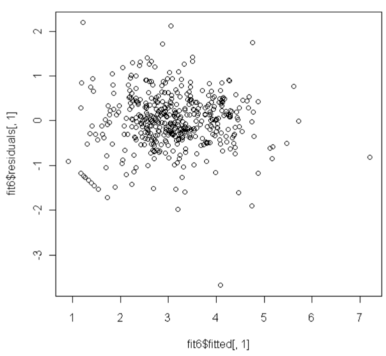


Figure 14(a): residuals vs fitted values **Figure 14(b):** qq-plot of residuals

Model B INS_OGT



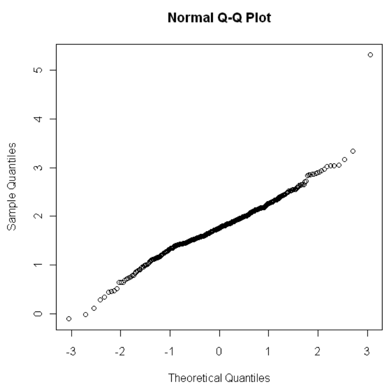
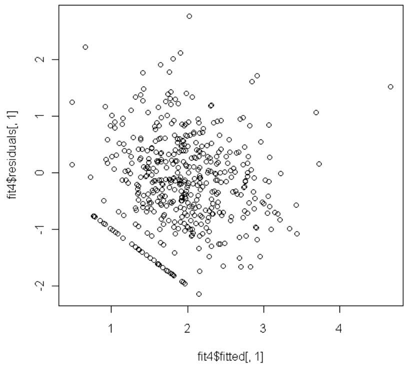


Figure 15(a): residuals vs fitted values **Figure 15(b):** qq-plot of residuals

Model B INS



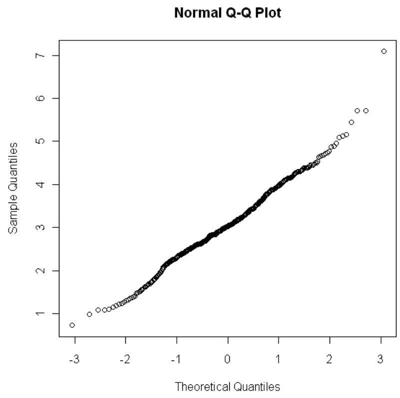


Figure 16(a): residuals vs fitted values **Figure 16(b):** qq-plot of residuals

All the diagnosis plots above looked good, which indicated that the two models for two responses fitted the data pretty well.

Also, we checked extrapolation in Model A, since we were using the values of EMS horses which were not used in model fitting. We found that Owner "Walls_William" was not in the values of Owner that we used to fit the model . So we excluded its prediction result in our conclusion for both INS and INS_OGT models. We also found that the Starch value for the horse from Taber_Amie was out of the range of the Starch value of the model. However, this horse has higher true value than the predicted value only in INS, but not in INS_OGT. So we only excluded this horse's prediction in INS, but not in INS_OGT model.

Chapter 5 Conclusions and Future Work

This study focused on two models. Model A was fitted on healthy horses, based on unrelated terms. We used this model to look into the relationship between insulin level and EMS status, since it didn't include obesity or laminitis. After backwards elimination, we compared the prediction for EMS horses with their true INS and INS_OGT values. We found that, most of the time (For INS_OGT, 17 out of 26, while for INS, 22 out of 25), the true values were larger than the predicted values for both INS and INS_OGT, and we might make a moderate conclusion that horses with EMS tended to have higher insulin level before glucose tolerance test. However, the relationship between EMS status and insulin level after glucose tolerance test is less strong. In the future, with more EMS horses, we might be able to prove that this model can be used for predicting susceptible horses.

We also fitted Model B on all horses we have, with both EMS related and unrelated terms in the model. This model helped us to understand the relationship between insulin and laminitis and obesity. Based on the model we got, we explained this relationship using their coefficients. We found that INS_OGT was increased by 11.29% with LAM positive and 6.82% for each increase in BCS, while INS was increased by 68.2% with LAM positive and 22.14% for each increase in BCS.

We also made predictions based on mean of predictors (except for LAM and BCS) and actual values of LAM and BCS, and we compared adjusted predictions for healthy horses with EMS horses to see if the three characteristics' relationship was affected by EMS status. As we expected, we obtained higher predicted insulin level for EMS horses, after adjusting for other unrelated terms. We also plotted the predicted values for both healthy horses and EMS horses to see where the values for EMS horses lay in and also calculated in what percentile of healthy horses' population the EMS horses values were. Our analysis show that the EMS horses have high percentile in the distribution of healthy horse, especially for INS.

Though we've done some exploration about EMS and make some improvements in predicting sensible horses, there are still a lot of things to do. Since this data set also contains information about genes, our researcher originally want to determine the genetic role in this disease and to find the genetic differences which are highly correlated to having EMS. Also, we can fit separate models on paired horses and randomly selected horses to explore the difference in this sampling difference. What's more, if we have more data on EMS horses, we may be able to build a more conving model to predict EMS status.

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