

Non-linear relationships of gestation length, rate of still births, early reproductive disorders and milk production in Fleckvieh cattle

Master Thesis

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Statutory declaration

I declare that I have authored this thesis independently, that I have not used other than the declared sources or resources and that I have explicitly marked all material, which has been quoted either literally or by content from the used sources.

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Abstract

Functional traits in breeding program have been the point of concern in animal breeding in recent years due to their economic importance and animal well-being considerations. The selection models are based on the additive infinitesimal model and require the genetic parameters: heritability and genetic correlations. By construction, correlations measure linear relationships. Genetic associations of traits are often curvilinear, more than at the phenotypic scale. Conclusions drawn from approaches assuming linear relationships may differ markedly from those derived from the non-linear association approach. Hence, a study was conducted to find the non-linear relationships of gestation length, rate of still births, early reproductive disorders and milk production in Fleckvieh cattle by the offspring-parent polynomial regression method of Fürst-Waltl et al. (1996). Systematic environmental effects were adjusted by calculating the residuals for different traits using linear model and treating lactation number (1-3), sex of calf (1-2), herd-year-season and calving age in months (both linear and quadratic) as the fixed effects. Then the data with standardised residuals were combined into dam daughter pairs. Quadratic regression analysis was performed on the dam-daughter pairs to compute the estimates of phenotypic regression coefficients. Genetic regression coefficients were calculated only if the quadratic phenotypic regression coefficient was found to be significant.

We found the calving traits behaving differently in different parities. Cows giving birth to male calves were found to have longer gestation length, as was evident in all parities. For milk production parameters, there was no significant effect of sex in the first parity. Yet, cows giving birth to male calves were found to produce significantly more 305 day milk ($P < 0.001$), fat (Kg) ($P < 0.01$) and protein (Kg) ($P < 0.001$) yield in second and third parities. Cows giving birth to female calves had significantly higher fat% ($P < 0.05$) and protein% ($P < 0.001$) in the milk during second and third parities. Nonlinear relationships at genetic level were found between gestation length and stillbirth (combined lactation), gestation length and early fertility disorders (second lactation), calving ease and milk yield (first and combined lactation) and calving ease and stillbirth (first and combined lactation). The transformation from phenotypic to genetic regression coefficients partly yielded results that were far from the range of expected values, particularly when the independent trait was of low heritability or was a categorical trait. Such traits should not be used as independent trait in the offspring parent regression method of polynomial degree 2.

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1. Introduction

Functional traits in breeding programs have been of substantial concern in animal breeding in recent years. Cattle breeders have been showing an increasing interest (Mark, 2004) and are motivated by their economic importance and by animal well-being considerations (de Maturana et al., 2009). These functional traits do not increase the profit by higher output of products, but rather increase the efficiency of animals by decreasing the cost of input (Groen et al., 1997). In general the focus of selection is shifting from traits that increase returns to the traits that decrease the costs (Eaglen et al., 2012).

Functional traits used in the total merit index breeding value estimation of Austrian Fleckvieh include longevity, persistency, fertility, calving ease, stillbirth, somatic cell score and milk ability (Miesenberger and Fuerst, 2006) and the weight given to functional traits is 45.7% of total merit index (Bayern-Genetik GmbH). The selection models are based on the additive infinitesimal model and require the genetic parameters: heritability and genetic correlations (Bulmer, 1980).

Genetic correlations are used to describe the relationship between traits and help to estimate how related traits will change under selection (Sölkner and Fürst-Waltl, 1996). By construction, correlations measure linear relationships. So, the genetic parameters are estimated assuming linear relationship between the traits. Genetic associations of traits are often curvilinear (Mulder et al., 2015; Sölkner and Fürst-Waltl, 1996), more than at the phenotypic scale. The conclusions drawn from approaches assuming linear relationships differ markedly from those derived from the non-linear association approach (Fuerst-Waltl et al., 1998). Inclusion of traits that are nonlinearly related in a linear selection index will produce suboptimal heritability (Sölkner and Fürst-Waltl, 1996) and affect the multivariate selection strategies used in breeding programs (Fuerst-Waltl et al., 1998). In the nonlinear genetic association, selection should target an optimum (Hansen et al., 2004), rather than the extremes.

Various methods have been used to search for non-linear relationship between traits. Offspring-parent polynomial regression method is one of the earliest (Fuerst-Waltl et al., 1997). Non linearity in the genetic relationship between milk yield and type traits have already been described in Simmental (Sölkner and Fürst-Waltl, 1996) and Holstein Friesian (Fuerst-Waltl et al., 1998) cattle by using this method. Similarly weak non-linear relationship between milk yield and protein percent (Fürst-Waltl et al., 1996) has also been described by

the same method. Further, Mulder et al. (2015) have shown that heritable environment variances cause non-linear relationships between traits in pigs. Hence in this study, we are trying to find the non-linear relationships of gestation length, rate of still births, early reproductive disorders and milk production in Fleckvieh cattle by offspring-parent polynomial regression method of Fürst-Waltl et al. (1996).

2. Literature Review

Austrian Fleckvieh has the average production of 7214 Kg milk with 4.13% fat, 3.41% protein and 544 kg fat + protein. The average of first lactation is 6519 kg milk with 4.11% fat, 3.38% protein and 488 kg fat + protein (Fleckvieh Austria, 2014). Calving is one of the important events in a dairy farm and it is directly linked to the financial success of the farm. Various factors may affect the calving, but the reproductive traits calving ease and stillbirth are the key ones and are used in the genetic evaluations. Genetic evaluation for gestation length is currently considered to be included in routine genetic evaluation.

2.1. Gestation length

Average gestation length in Fleckvieh has been reported to be 288.23 days with standard deviation of 5.54 (Sattlecker, 2014). Gestation length is dependent on many environmental factors like calving age, age of dam, parity, sex of calf, birth weight and disease conditions (Andersen and Plum, 1965; King et al., 1985; Norman et al., 2011; Hansen et al., 2004; Johanson and Berger, 2003).

Sattlecker (2014) found gestation length in Fleckvieh to increase with the lactation number, being 287.12 days in first lactation, 288.08 days in second and 288.57 days in third lactation.

Andersen and Plum (1965) reported in a review based on many studies that older cows were found to carry calves longer (≤ 1 day) than the younger cows. King et al. (1985) cited more than five studies with gestation length to be shorter for heifers than in cows. In first parturition gestation length has been shown to be related to dystocia and stillbirth (Johanson and Berger, 2003; Hansen et al., 2004).

Cows having male calves were found to have 1 to 2 days longer gestation length than those having female calves (Norman et al., 2011). In Ngaoundere Gudali cattle, the sex of the calf was found to affect the gestation length significantly ($P < 0.05$), with males being carried in utero approximately 3 days longer than their female counterparts (294.1 ± 1.2 versus 291.1 ± 1.2 days) (Messine et al., 2007). In Danish Holsteins, gestation length was 1.1 days longer with male calves than with female calves (Hansen et al., 2004).

2.2. Stillbirth

Stillbirth not only includes a calf born dead but also includes a calf dead within 48 hours of birth (Philipsson et al., 1979) and is a huge economic loss to the farmers (Meyer et al., 2000). Sattlecker (2014) reported 4.35% of stillborn calves in Fleckvieh cattle.

Genetic, environmental and management factors, which are diverse in nature, have varying degrees of influence on stillbirth (Philipsson et al., 1979). Dystocia has been considered one of the chief reasons of stillbirth (Meyer et al., 2000) but 50% of the stillborn calves are from unassisted births (Philipsson, 1996). Parity, sex of calf, gestation length and their interactions has been primarily suspected for the main reasons of stillbirth (Meyer et al., 2000).

Stillbirth in primiparous and multiparous cows should be considered as the separate traits (Berger et al., 1992; Meyer et al., 2000). In Holsteins, Meyer et al. (2000) reported higher rate of stillbirth in primiparous cows (11%) rather than multiparous (5.7%). Heins et al. (2006) reported fewer stillbirths in Holstein heifers from use of Scandinavian Red bulls (7.7%) compared with use of Holstein bulls (15.1%). In multiparous dams non-Holstein breeds of sire were found to induce significantly fewer stillbirths than Holstein sires (Heins et al., 2006).

At phenotypic level, nonlinear relationship between gestation length and still birth, and gestation length and calving ease has been reported (de Maturana et al., 2009). Hansen et al. (2004) and Johanson and Berger (2003) reported of more stillbirths in phenotypic extremes, i.e. short and long gestation length. In his master thesis on the genetic relationships of gestation length with stillbirth, calving ease and early fertility disorders, Sattlecker (2014) reported the phenotypic association of gestation length and stillbirth to be markedly curvilinear in Fleckvieh, with high risk of stillbirth for very short and very long durations of pregnancy. Intermediate gestation length is optimal because of the curvi linear relationship between gestation length and still birth and it would be highly beneficial to avoid the use of bulls transmitting extreme gestation length (Norman et al., 2011).

2.3. Calving ease

Calving is a stressful event for the cow. Calving difficulty has been associated with reduced survival of both cow and calf, as well as lower production, fertility, and longevity for the cow. Calving difficulty can lead to increased rates of neonatal calf mortality, lower milk production, and overall reduced health of cow (Heins et al., 2006). Calving difficulty increases veterinary and labour costs, culling risk, and mortality in cows and calves. Further, it decreases milk production in the next lactation, and leads to lower female fertility in the next reproductive cycle (de Maturana et al., 2009)

Calving ease has been primarily associated with parity, birth weight, age and sex of calf. Calving difficulty in primiparous cows significantly differs from that in cows in all other parities (Fiedlerová et al., 2008). These problems are mainly due to foeto-pelvic

incompatibility (discrepancy between the size of the calf and the pelvic dimensions of the dam). Unlike matured cows, primiparous cows have not achieved their full development (including the development of pelvic inlet area) and have not reached their mature body size yet (Fiedlerová et al., 2008).

Phenotypic significant non-linear relationship between calving difficulty and gestation length was found in Czech Holstein population. More difficulties are associated with short or long gestation periods (Johanson and Berger, 2003). For shorter gestation, the authors found a higher incidence of perinatal mortality that could cause an earlier onset of parturition and more difficulties during calving. Long gestation represented a higher incidence of difficult calvings that are probably associated with higher birth weight and size of the calf (Strapák et al., 2000). An essential relationship between birth weight and the calving process was found (Fiedlerová et al., 2008)

3. Methodology

3.1. Description of data

The data were available from ZuchtData in four parts:

3.1.1. Health data

Health data contained the information about early fertility disorders. It includes the health conditions such as early infertility, cysts, mastitis and milk fever. It was available in the following format.

ISO life number of cow

Lactation * calving age

Calving year * calving month

Registration type * calving year

Farm id * calving year

Early fertility disorders: 1 = healthy, 2 = sick

3.1.2. Calving data

The dataset contained all the calvings of Fleckvieh from Styria since 2007. It contained 1 record per parturition. The format of data available is given below.

Table 1: Format of calving data

Columns	Format	Description
1-2	i2	Province at the time of parturition
3-13	I11	Farm id
14-28	I15	ISO life number of cow
29-36	YYYYMMDD	Date of birth of the cow
37-38	i2	Breed of cow
39-53	I15	Father (ISO life number)
54-61	YYYYMMDD	Date of the birth of the calf
62-64	i3	Gestation period
65-66	i2	Lactation number
67	I1	Sex of the calf (1=male, 2=Female)

68	I1	Use of the calf ^(1.2.1)
69	I1	Calving ease ^(1.2.2)
70	A1	Birth type ^{1.2.3}
71-85	I15	ISO life number of calf
86-100	I15	ISO life number of the father of the calf
101	A1	Indicator for calving codes (Austria scale)
102	A1	ET flag (from ET = J, else = N)

3.1.2.1. Use of the calf

The codes used to show the use of the calf is presented below

Table 2: Coding for use of calf

Codes	Description
0	Not recorded
1	Calf stays in the farm
2	Sold
3	Slaughtered
4	Stillborn
5	Died within 48 hours
6	Died from day 3 onwards
9	Not known

3.1.2.2. Calving ease

Austrian scale was used to code the calving ease. The scale ranged from 1 to 5.

Table 3: Coding for calving age

Codes	Calving ease
0	Unspecified
1	Easy (no obstetrics required)
2	Normal (birth assistance by a person required)
3	Difficult (birth assistance by more than one person or mechanical obstetrician required)
4	Caesarean section
5	Embryotomy

3.1.2.3. Birth type

The codes used to represent the number of calves born in a single parity are shown in below table

Table 4: Coding for birth type

Codes	Number of calves (Single and multiple births)
E	1 (Single birth)
Z	2 (Twins)
D	3 (Triplets)
V	4 (Quadruplets)
F	5 (Quintuplets)
S	6 (Sextuplets)

3.1.3. Pedigree

Pedigrees of the animals were available with the maternal and paternal information. It was available in following format

ISO number of animal

ISO number of father

ISO number of mother

Date of birth

Gender

Breed

Type of use (K = milk cow, F = meat, U = mother cow, V = Sire, A = breeding, M = mast)

Information about dead (T) or alive (L)

3.1.4. Milk production data

The data set contained the production performance of the animals and was available in following format

ISO number of animal

Lactation number

Date of calving

Milk yield (Kg)

Fat percentage

Fat (Kg)

Protein percentage

Protein (Kg)

Lactation length (days)

3.2. Cleaning of data

Several editing steps were conducted in the raw available data prior to analysis in R software (R Core Team, 2014).

3.2.1 Calving data

The original raw data had 327,478 observations. All the data were of Fleckvieh and were from the Austrian province Styria. The data had the observations with lactation numbers 1 to 17. All observations (76,482) with lactation number greater than 3 were deleted. Records about the cows with unspecified calving ease (2,666 records) and embryotomy (28 records) were removed. Records with gestation length had to be within 269 to 302 days (1,827 records deleted) and the records with multiple births (18,434 records) were omitted. One of the most important editing rules was that the calving age was restricted for the cows as per Sattlecker, 2014 (Table 6). Furthermore, records of calves having sire from other breeds (18,490 records) were not included. Records of ET calves, unknown sex of the calf and missing observation for stillborn were also cleaned. These editing steps resulted in 157,732 observations (Table 5)

Table 5: Delete criterion and data remaining in calving data

	Deleted records	Remaining records
Original data		327,478
Lactation number > 10	1,380	326,098
Calving ease: 0 and 9	2,666	323,432
GL: <269 and >302	1,827	321,605
Removing multiple births	18,434	303,171
removing embryotomy	28	303,143
restrictions for the calving age	51,589	251,554
father of calf other breeds	18,490	233,064
unknown sex of calf	88	232,976
ET calves	140	232,836
Lactation number >3	75,102	157,734
Missing observation for rate of stillborn	2	157,732

Table 6: Restriction of calving age in months

Calving	Less than	More than
1	24	39
2	36	53
3	48	65

3.2.2. Milk production data

Milk production data had 252,363 observations from 230 to 305 day milk yield. Only 305 day milk yield and corresponding fat and protein yield were used for the analysis (86,014 observations were deleted) (Table 7).

Table 7: Delete criterion and data remaining in milk production data

	Deleted records	Remaining records
Original milk production data		252,363
milk production: <305 days>	86,014	166,349

3.3. Merging data

Data were merged to connect different datasets. Calving data and health data were merged to see the relationship between gestation length and early fertility disorders. Similarly, calving data and milk production data were merged to analyse gestation length and milk production parameters. While merging data for the individuals, care was taken to merge the records of individual animals with the same lactation number. For example: Animal 1 on second lactation present in calving data was merged with the animal 1 on second lactation of health data.

Table 8: Records available after merging datasets

Merging of data	Records available for analysis
Calving and milk production data	87,132
Calving and health data	82,452

3.4. Calculating residuals

Systematic environmental effects were adjusted by calculating the residuals for different traits using linear model in different and/or merged data. Residuals for different traits were calculated by treating lactation number (1-3), sex of calf (1-2), herd-year-season and calving age in months (both linear and quadratic) as the fixed effects. The obtained residuals were standardised to the mean of 0 and standard deviation of 1.

3.5. Making dam-daughter pairs

The data with standardised residuals were combined into dam daughter pairs, i.e. pairing of the records of dam in lactation 1 and daughter of the same dam also in lactation 1. The number of records available after dam-daughter pairing is given on Table 9.

Table 9: Number of records available after dam daughter pairing

Data	Dam-daughter pairs	Observations	Used for analysis between
Calving data	All 3 lactations	127,590	Gestation length, calving ease and stillbirth
	Lactation 1	19,186	
	Lactation 2	13,101	
	Lactation 3	8,464	
Calving and health data	All 3 lactations	50,721	Gestation length and early fertility disorders
	Lactation 1	7,290	
	Lactation 2	5,058	
	Lactation 3	3,182	
Calving and milk production data	All 3 lactations	43,706	Milk production traits with gestation length and calving ease
	Lactation 1	6,812	
	Lactation 2	4,505	
	Lactation 3	2,823	

3.6. Calculation of phenotypic and genetic regression coefficients

The quadratic regression analysis was performed on the dam-daughter pairs to compute the estimates of phenotypic regression coefficients (b_1 and b_2). Genetic regression coefficients were calculated only if the quadratic phenotypic regression coefficient was found to be significant to avoid misinterpretations as stated by Fuerst-Waltl et al. (1998). Estimates of genetic regression coefficients (a_1 and a_2) was computed as per Sölkner and Fürst-Waltl (1996) as:

$$a_1 = \frac{b_1}{0.5h^2(x)} \quad \text{and} \quad a_2 = \frac{b_2}{0.25h^4(x)}$$

where,

$h^2(x)$ denotes the heritability of x

$h^4(x)$ denotes squared value of heritability of x [under normality, it was assumed that $E\{h^2(x^2)\} = h^4(x)$]

x = independent trait during regression

After, phenotypic and genetic regression curves were plotted only for those traits having significant quadratic regression coefficient in R.

4. Results and Discussions

4.1. Descriptive statistics:

The mean gestation length and age at first calving for Fleckvieh was 288.02 ± 0.01 days and 30.74 ± 0.01 months respectively (Table 10). This is similar as reported by Sattlecker (2014). Boxplots (Figure 1) showed higher mean gestation length for cows giving birth to male calves (Figure 1-(i)). As expected, mean gestation length was higher with the increase in lactation number (Figure 1-(v)) and calving age (Figure 1-(vi)), as gestation length is shorter in heifers than the cows (Nogalski and Piwczyński, 2012). Figure 1-(iv) shows difficulty in calving increases with the increase in gestation length. As expected, the lower the age at first calving, the higher is the difficulty of calving (Figure 1-(xiv)).

Table 10: Basic statistics of calving age, gestation length and age at first calving

	Gestation length	Age at first calving (months)
Min	270	24
Max	302	39
Range	32	15
Median	288	30
Mean	288.02	30.74
SE.mean	0.01	0.01
CI.mean.0.95	0.03	0.02
Var	26.26	10.71
std.dev	5.12	3.27
coef.var	0.02	0.11

All the calves that were born dead or were dead within 48 hours after calving were categorised as stillborn (Philipsson et al., 1979). Those stillborns were coded as 1 and the others as 0. The incidence of still birth was found 2.90% in the first three parities and was higher in first parity (Table 11), which is consistent to other studies suggesting higher stillbirth rate in primiparous cows compared to multiparous (Bicalho et al., 2008).

Table 11: Still birth in different parities

Parity	stillbirth	Total birth	Percent
1	2861	67931	4.21
2	973	51248	1.90
3	743	38553	1.93
Overall	4577	157732	2.90

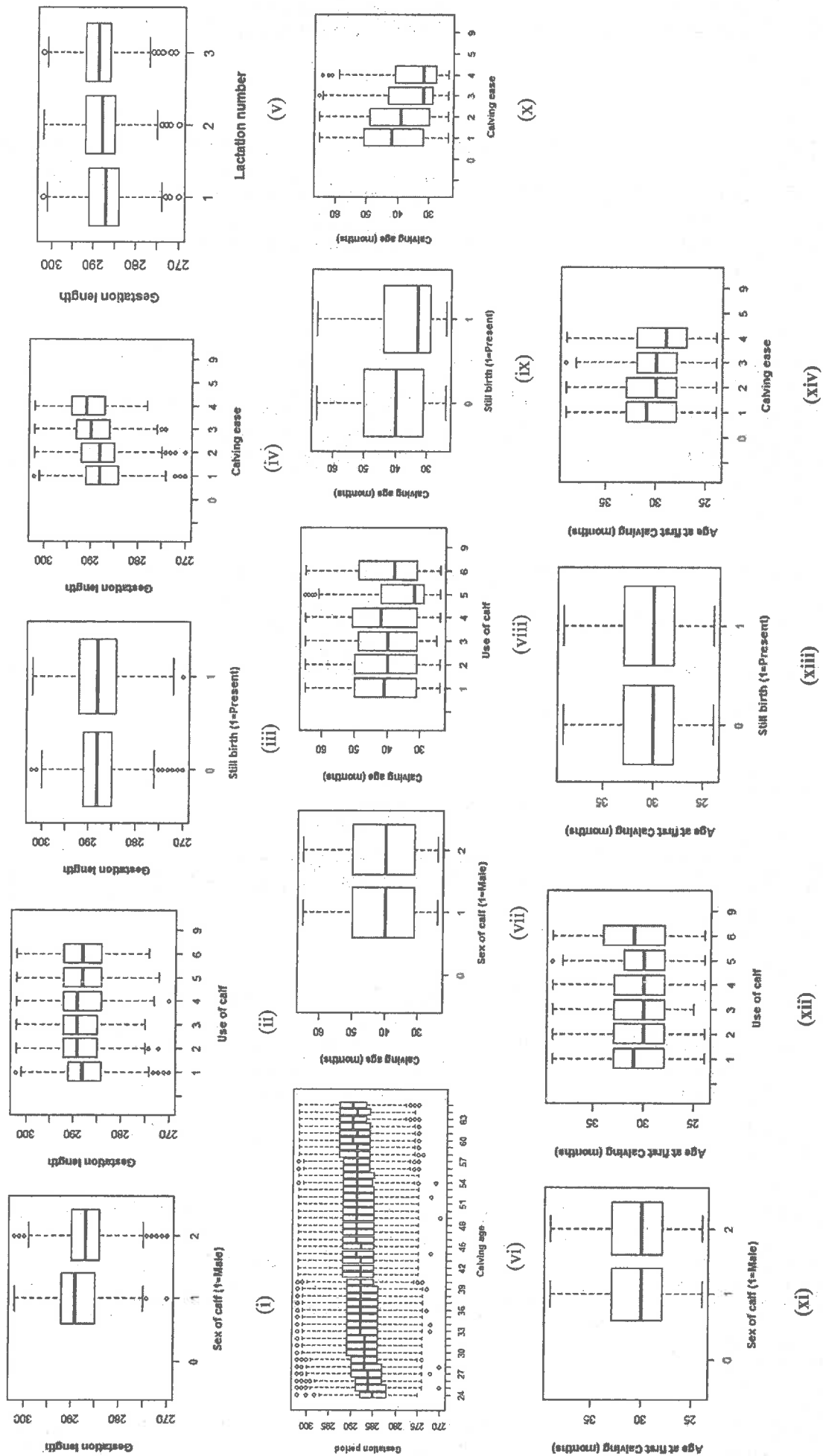


Figure 1: Boxplots for gestation length, average calving age and age at first calving

As expected, higher calving assistance was required most frequently in first parity (Table 12). This supports the general consensus that the complications in calving occur more frequently in heifers than the cows (Meijering, 1984; Eaglen et al., 2012). Foeto-pelvic incompatibilities due to lack of full development and mature body size cause calving complications in primiparous and young cows (Fiedlerová et al., 2008).

Table 12: Calving ease in different parities

Calving ease	Parity 1	Parity 2	Parity 3	Overall
Easy	46.45	57.55	57.22	52.69
Normal	46.95	39.67	40.38	42.98
Difficult	6.28	2.66	2.30	4.13
Caesarean section	0.32	0.12	0.11	0.20

The average milk, protein and fat yield per lactation were 6968.18 ± 4.15 , 291.69 ± 0.18 and 241.86 ± 0.15 Kg respectively. Average fat and protein percent in the milk were 4.20 and 3.46 % respectively (Table 13). The average milk yield is somewhat lower than reported by Fleckvieh Austria (2014) (7214 Kg), whereas other milk parameters are similar. This is consistent with the records completed over a range of years in this data set.

Table 13: Basic statistics of milk production

	Milk yield	Fat %	Fat(kg)	Protein (Kg)	Protein%
min	1413.00	2.18	55.00	46.00	2.44
max	20890.00	6.75	888.00	716.00	4.90
median	6787.00	4.16	285.00	235.00	3.46
mean	6968.18	4.20	291.69	241.86	3.46
SE.mean	4.15	0.00	0.18	0.15	0.00
CI.mean.0.95	8.14	0.00	0.35	0.30	0.00
var	2869345.57	0.20	5439.11	3910.67	0.06
std.dev	1693.91	0.44	73.75	62.54	0.25
coef.var	0.24	0.11	0.25	0.26	0.07

Boxplots in Figure 2 and 3 show increase in average 305 day milk yield, fat yield and protein yield with the increase in lactation number. Based on the model used to calculate the residuals (Table 14), sex of the calf born had the significant effect ($P < 0.001$) on the gestation length and milk production parameters. Cows giving birth to male calves had longer gestation length, which was evident in all the parities {Figure 4(i)}. Highest difference was observed in second parity (1.682 days), followed by third parity (1.600 days) and first parity (1.495 days).

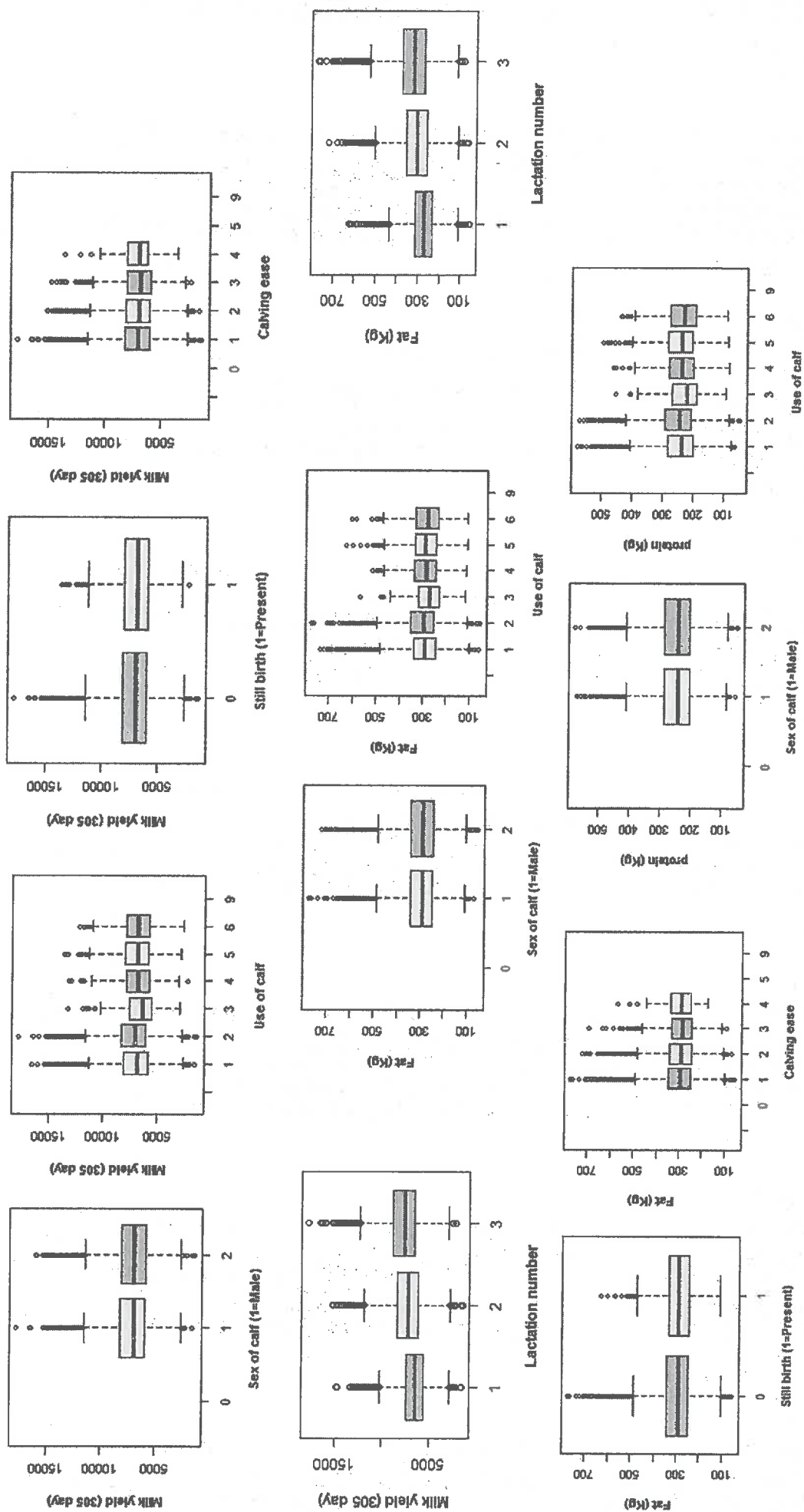


Figure 2: Boxplot of some milk production parameters

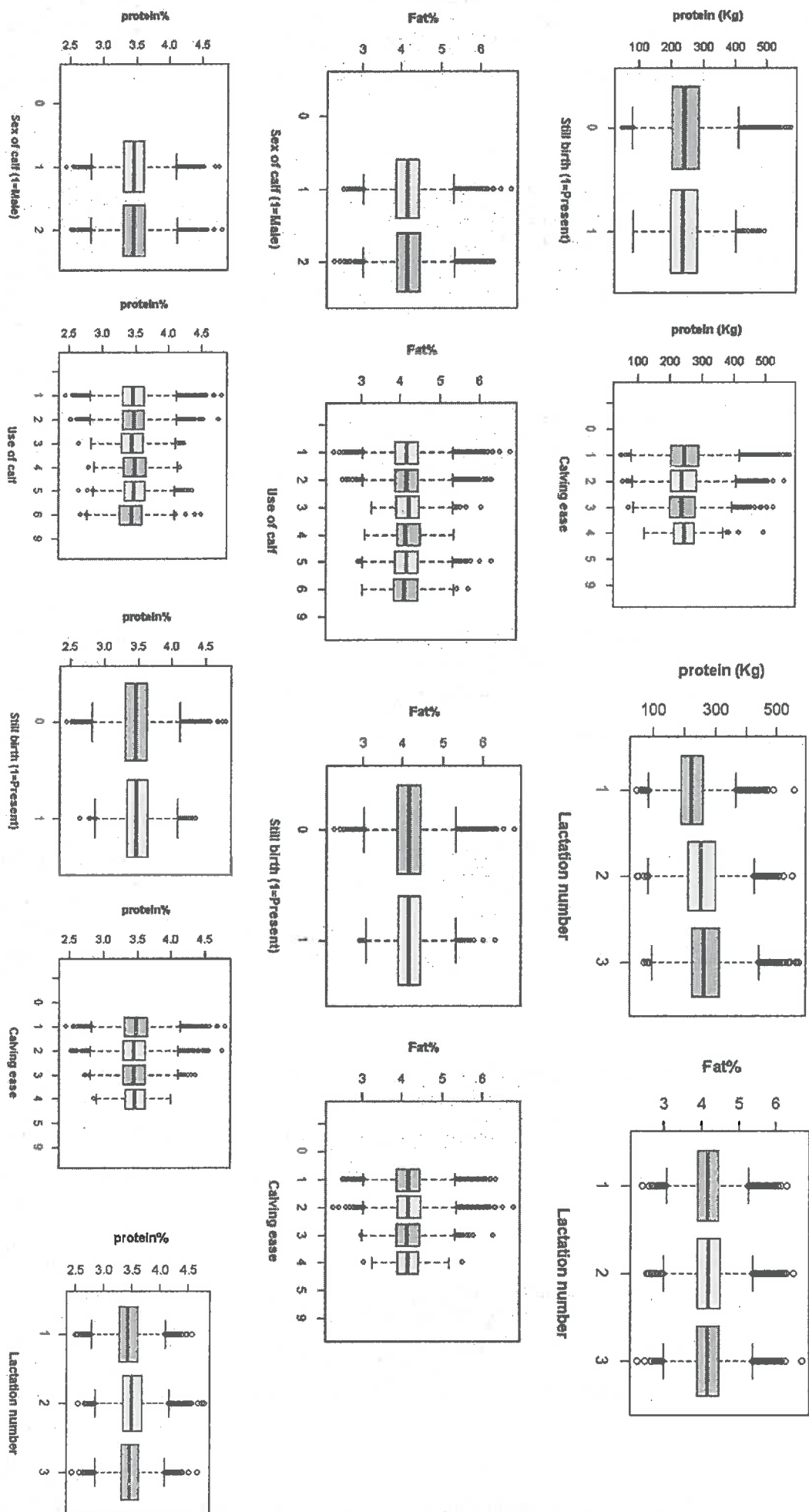
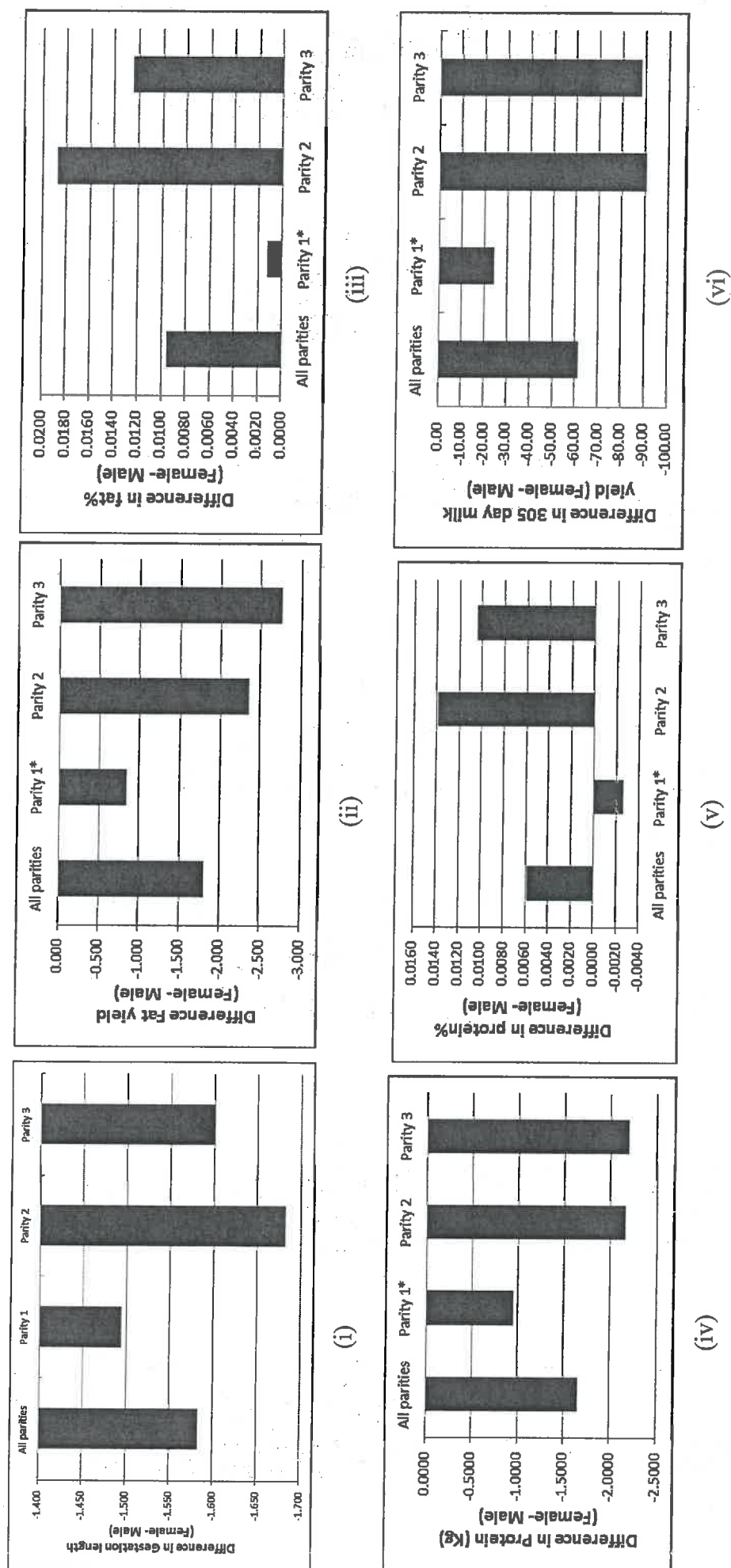


Figure 3: Boxplot of some milk production parameters



* Effect of sex on parity 1 is non-significant

Figure 4: Effect of sex of calf on gestation length and milk production parameters

These all findings match with the conclusion of Messine et al. (2007) (citing 10 studies) that male calves are carried 1 to 2 days longer than females. This has also been reported both in *Taurus* (Hansen et al., 2004) and *Indicus* (Messine et al., 2007) breeds.

For milk production parameters, there was no significant effect of sex in the first parity (Figure 4). But, cows giving birth to male calves were found to produce significantly more 305 day milk ($P<0.001$), fat (Kg) ($P<0.01$) and protein (Kg) ($P<0.001$) yield in second and third parities. Yet, cows calving females had significantly higher fat% ($P<0.05$) and protein% ($P<0.001$) in the milk during second and third parities. This agrees with the findings reported by Hinde et al. (2014) and Beavers and Doormaal (2014) in Holsteins. Our study is based the model used to calculate the residuals, further study may be required including genetic effects of sires in the model.

Table 14: Predicted means and p-values of fixed effects

Trait	Mean	Fitted fixed effects				
		Calving age (months)	Calving age ²	Lactation number	Sex of the calf	HYS
Gestation length	282.60	***	***	***	***	***
Still birth	0.16	***	***	***	***	>0.1
Calving ease	4.42	***	***	***	***	***
Early fertility disorders	0.06	**	*	>0.1	***	>0.1
Merging calving data with milk production data						
Gestation length	282.4	***	***	***	***	***
Milk yield	6208	**	***	***	***	***
Fat %	4.088	*	***	***	**	>0.1
Fat (Kg)	249.7	>0.1	***	***	***	***
Protein %	3.303	***	***	***	***	***
Protein (Kg)	202.4	>0.1	***	***	***	***
Protein+fat (kg)	452.1	>0.1	***	***	***	***
Protein+fat (pc)	7.391	***	***	***	***	*
Stillbirth	0.1463	***	***	*	***	>0.1
Calving ease	4.428	***	***	***	***	*

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

4.2. Gestation length and stillbirth

Non-linear relationship on the phenotypic scale could be clearly seen between gestation length and stillbirth. Stillbirth rate is high for lower and higher gestation length (Figure 4). Stillbirth percentage was 33.33% (1 stillbirth in 3 records) and 50% (1 out of 2 records) when the gestation length was 270 and 273 days respectively. 271 and 272 days of gestation period had 0% of stillbirth (have only 1 and 5 records respectively). Stillbirth rate was 3.17% when the gestation length was 274 days. Then it decreased gradually until 287 days, where it reached at 0.97%. Then after it started to rise steadily and the stillbirth was 2.37% in 302 days of gestation period. The data is presented in Annex 1.

Calves born from short gestation length are small and weak (Norman et al., 2011), increasing the chances of stillbirth. Calves in the uterus for longer period may grow too big causing difficulty in calving and increasing the chances of stillbirth.

Significant ($P < 0.001$) phenotypic regression coefficient for the quadratic term was found for the regression of gestation length of offspring and stillbirth of dam when all the lactations were considered (Table 15). Genetic regression coefficients were calculated as described before assuming the heritability of stillbirth to be 0.05.

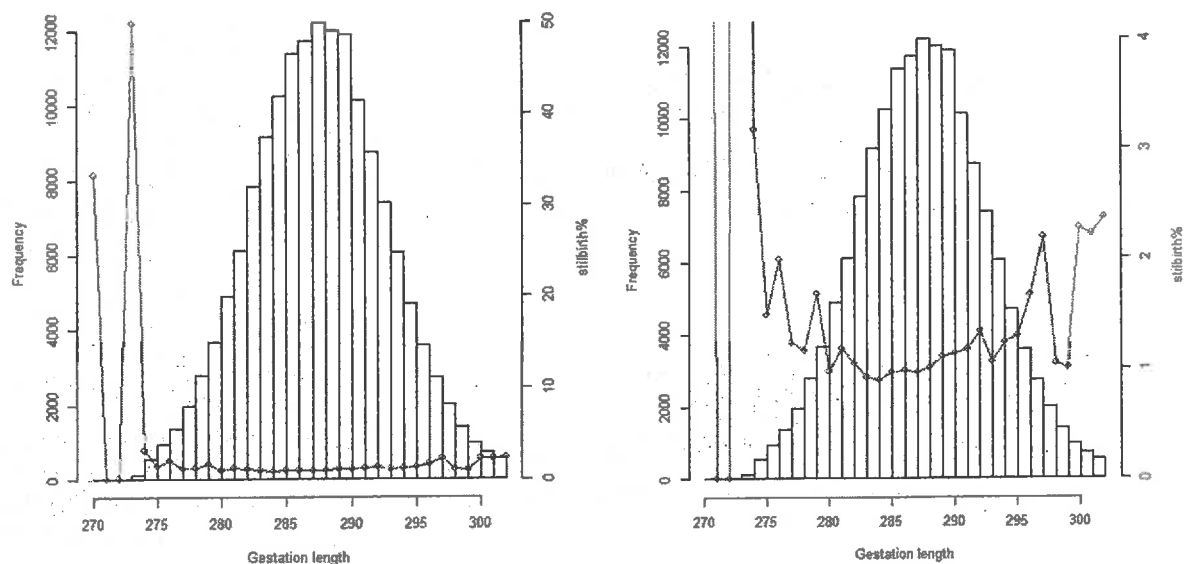


Figure 5: Relationship between gestation length and stillbirth

{Second figure shows only the stillbirth up to 5% (zoomed figure of first). Line graph represents stillbirth percentage and bar plot represents frequencies of gestation length}

Table 15: Estimates of phenotypic and genetic regression coefficients obtained by offspring parent regression between gestation length and stillbirth

Offspring trait	Parent trait	Lactation number	b ₁	P	b ₂	p	a ₁	a ₂
Model: still birth _{Daughter} = μ + Gestation length _{Dam} + Gestation length _{Dam} ² + e								
Stillbirth	Gestation length	1	0.0131	>0.1	0.0025	>0.1		
		2	-0.0009	>0.1	-0.0073	>0.1		
		3	0.0150	>0.1	-0.0076	>0.1		
		All	0.0103	***	-0.0033	>0.1		
Model: Gestation length _{Daughter} = μ + Still birth _{Dam} + Still birth _{Dam} ² + e								
Gestation length	Stillbirth	1	0.0805	>0.1	-0.0141	>0.1		
		2	0.2723	>0.1	-0.0441	>0.1		
		3	-0.1162	>0.1	0.0216	>0.1		
		All	0.1527	***	-0.0251	***	6.1062	-40.095

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1

Phenotypic curve (Figure 6- Zoomed) shows higher gestation length of daughter born from the cows having stillbirth. As expected from formula, extreme curvilinearity was observed at genetic level than in the phenotypic level (Figure 5).

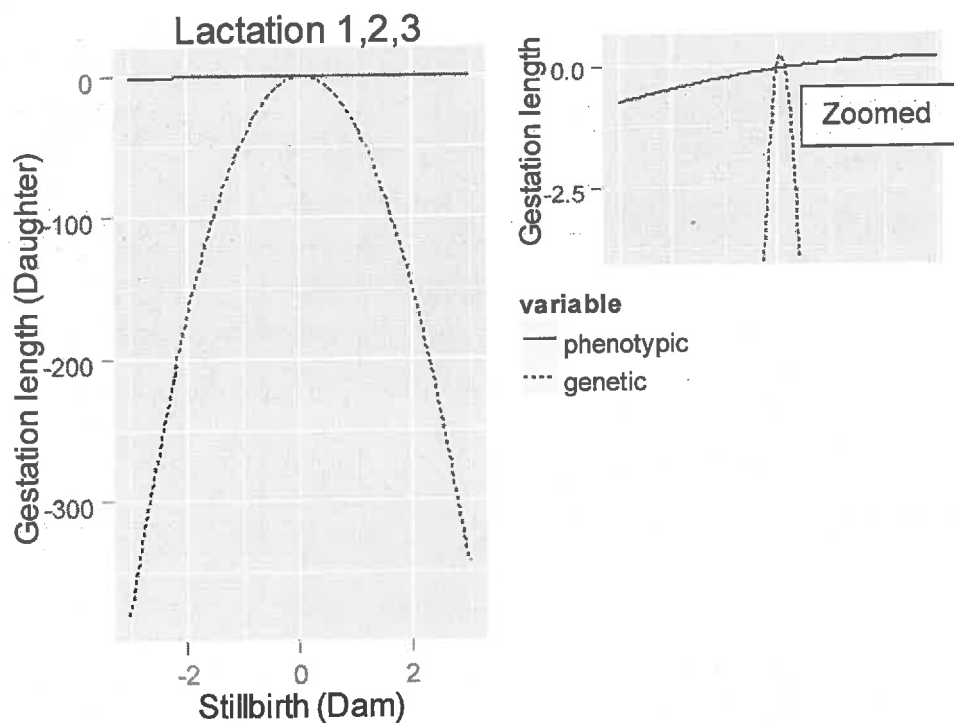


Figure 6: Phenotypic and genetic regression curves of gestation length and stillbirth
(The figure on the right is the zoomed portion showing quadratic phenotypic curve)

4.3. Gestation length and calving ease

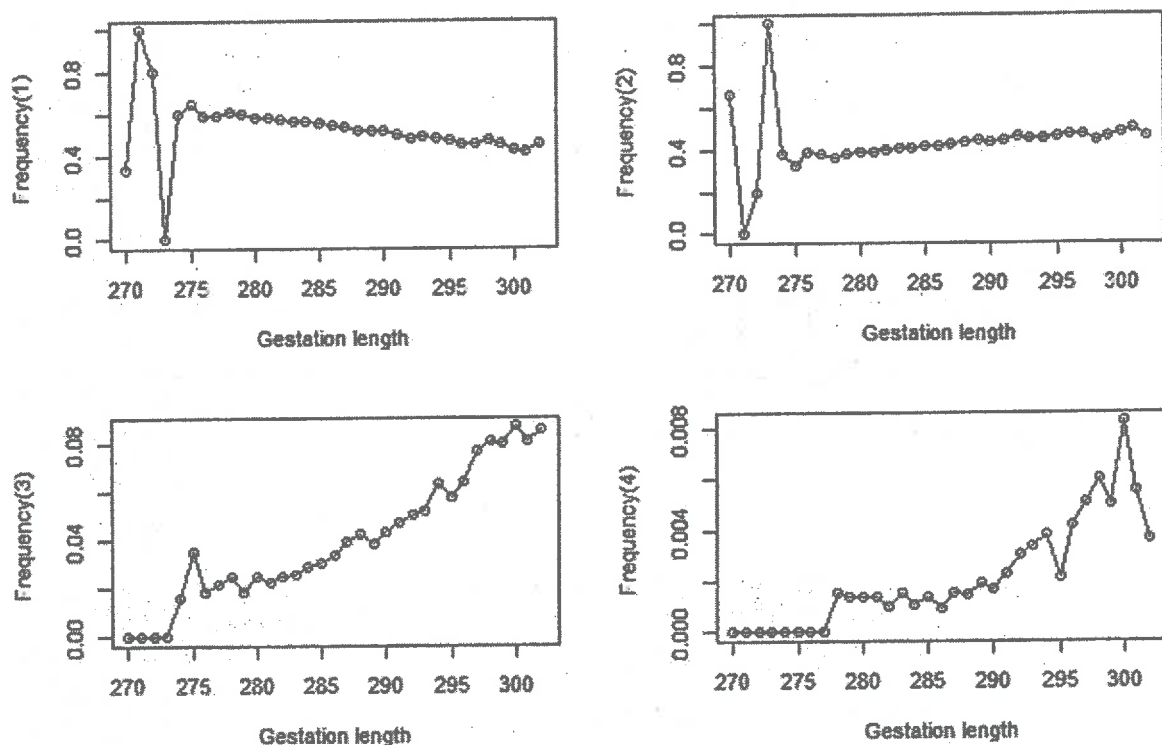


Figure 7: Phenotypic relationship between gestation length and calving ease
(calving ease: 1=easy, 2=Normal, 3=Difficult, 4=Caesarean section)

Until 273 days of gestation length, there were only few observations(<5). After that the frequency of easy calving decreased gradually (Figure 7). Whereas the frequency of difficult calving and caesarian section increased gradually with the increase in gestation length; this may be probably associated with higher birthweight and size of the calf (Strapák et al., 2000).

Phenotypic quadratic regression coefficients were not found significant for gestation length and calving ease by parent offspring regression (Table 16). This suggests that lower gestation length (for the range of 270 to 302 days) is good for Fleckvieh in terms of calving ease. This is in contrast to Norman et al. (2011) suggesting for intermediate gestation length for optimal calving ease.

Table 16: Estimates of phenotypic and genetic regression coefficients obtained by offspring parent regression between gestation length and calving ease

Offspring trait	Parent trait	Lactation number	b ₁	P	b ₂	p	a ₁	a ₂
Model: Calving ease _{Daughter} = μ + Gestation length _{Dam} + Gestation length _{Dam} ² + e								
Calving ease	Gestation length	1	0.0471	***	0.0008	>0.1		
		2	0.0060	>0.1	0.0061	>0.1		
		3	0.0051	>0.1	0.0023	>0.1		
		All	0.0051	***	0.0023	>0.1		
Model: Gestation length _{Daughter} = μ + Calving ease _{Dam} + Calving ease _{Dam} ² + e								
Gestation length	Calving ease	1	0.0172	*	0.0079	>0.1		
		2	0.0159	>0.1	-0.0071	>0.1		
		3	0.0311	*	-0.0059	>0.1		
		All	0.0214	***	-0.0036	>0.1		

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1

4.4. Calving ease and stillbirth

There have been studies reporting more than half of all stillborn to be born from normal and easy calving (Steinbock et al., 2003; Philipsson and Steinbock, 2003; Steinbock et al., 2006). Similarly, prolonged parturitions which are observed during difficult calving cause extended hypoxia and significant acidosis (House, 2002; Breazile et al., 1988; de Maturana et al., 2009) resulting in stillbirth. These facts suggest the possibility of non-linear relationship between them.

In the offspring (calving ease) – parent (stillbirth) regression, significant phenotypic regression coefficient for the quadratic term were found in the first parity ($P < 0.001$) and in the combined parity ($P < 0.05$) as shown in Table 17. The phenotypic linear regression coefficient is negative when the records of first parity are used. But when the records of all three parities are analysed, the coefficient becomes positive. This means that calving traits act as separate traits according to the parity, which has been suggested in various reports (Wiggans et al., 2008, 2006) and hence calving traits by parity should be accommodated in the net merit indices rather than calving trait as a whole.

In the primiparous cows, phenotypic graph shows us that more stillbirths were born from easy calving (Figure 8- Zoomed). Whereas, when the records of all parities are analysed, the phenotypic graph shows less stillbirths from easy calving.

Similarly in offspring (stillbirth) – parent (calving ease) regression significant ($P < 0.05$) phenotypic regression coefficient for the quadratic term was found in the first parity only (Table 17). Further genetic regression coefficients were calculated assuming heritability for calving ease as 0.09. This suggests that genetically too easy (without any assistance) and too difficult calving ease has more probability for stillbirth (Figure 8). Hence, animals having normal calving ease should be selected in regards to stillbirth. Further calving ease and stillbirth should be used as different traits, rather than using calving ease as the predictor of stillbirth.

Table 17: Estimates of phenotypic and genetic regression coefficients obtained by offspring parent regression between stillbirth and calving ease

Offspring trait	Parent trait	Lactation number	b ₁	P	b ₂	p	a ₁	a ₂
Model: Calving ease _{Daughter} = μ + Stillbirth _{Dam} + Stillbirth _{Dam} ² + e								
Calving ease	Stillbirth	1	-0.6470	***	0.1187	***	-25.881	189.949
		2	-0.3367	.	0.0582	>0.1		
		3	0.2215	>0.1	-0.0385	>0.1		
		All	0.0978	*	-0.0171	*	3.911	-27.362
Model: Stillbirth _{Daughter} = μ + Calving ease _{Dam} + Calving ease _{Dam} ² + e								
Stillbirth	Calving ease	1	0.0098	>0.1	0.0170	*	0.2182	8.4187
		2	-0.0060	>0.1	-0.0045	>0.1		
		3	-0.0189	>0.1	0.0140	>0.1		
		All	0.0023	>0.1	0.0037	>0.1		

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1

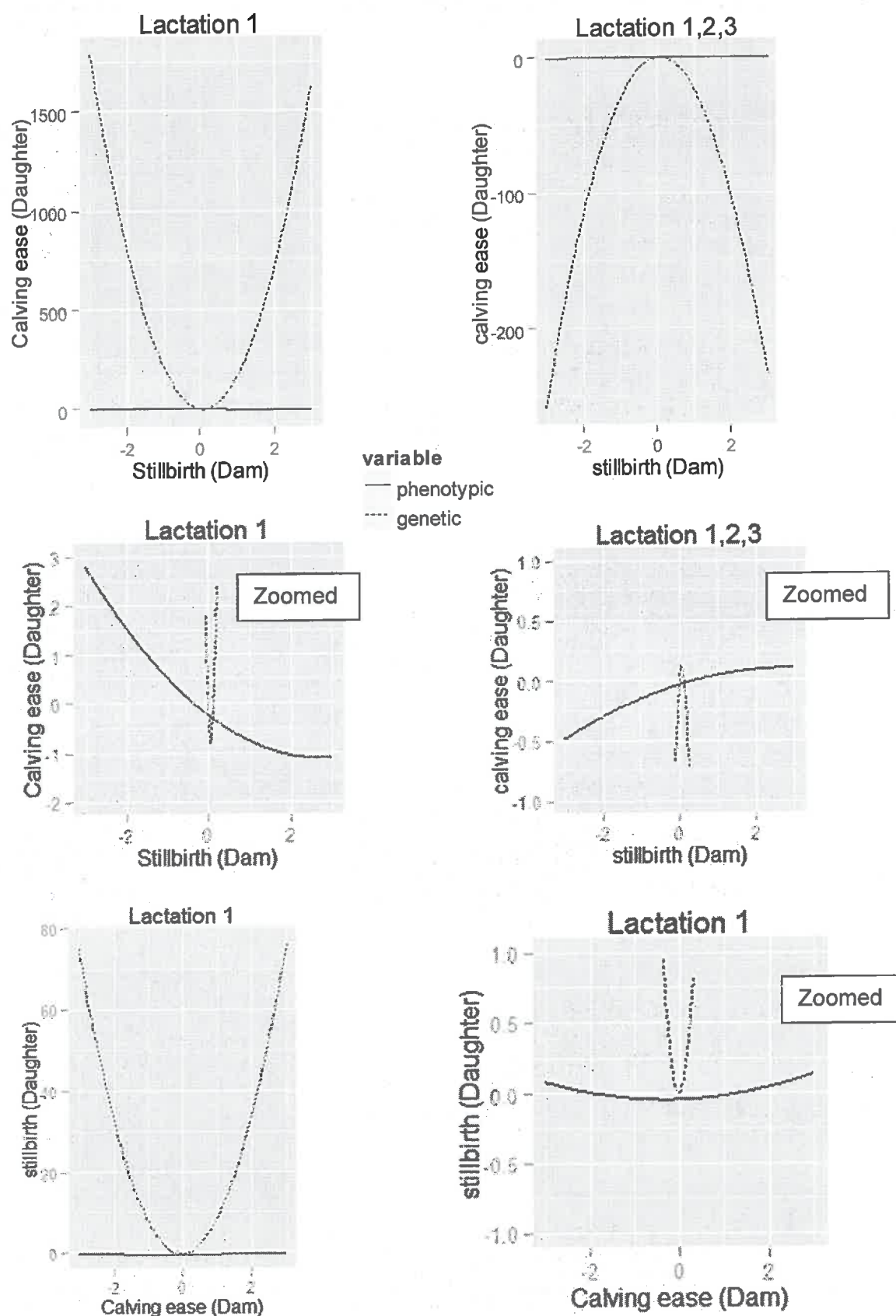


Figure 8: Phenotypic and genetic regression curves of calving ease and stillbirth

(The figure marked 'Zoomed' are the zoomed portion showing quadratic phenotypic curve)

4.5. Gestation length and early fertility disorders

The early fertility disorders (EFDs) were found in 33.33% (1 EFD in total 3 births) of total births when the gestation period was 270 days. After that EFDs were found in 10.7% (26 out of 243 total births) of total births when the gestation period was 275 days. Then it declined gradually and remained below 3.1% until 300 days. Then-after it gradually began to increase as shown in figure 9.

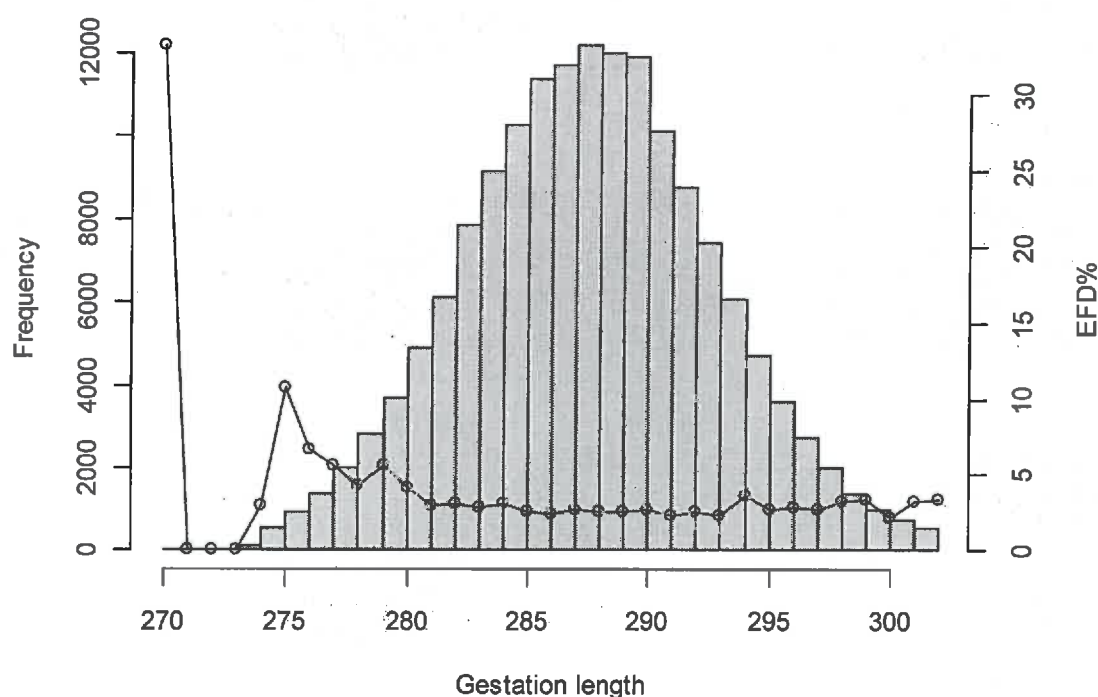


Figure 9: Phenotypic relationship between gestation length and early fertility disorders.

{Line graph represents early fertility disorder (EFD) percentage and bar plot represents frequencies of gestation length}

Significant ($P < 0.05$) phenotypic regression coefficient for the quadratic term was found when we regressed gestation length (offspring) and early fertility disorders (dam) of second parity (Table 18). Further genetic regression coefficients were calculated by assuming heritability of EFD to be 0.023 (Koeck et al., 2010).

At phenotypic level, animals having early fertility disorders were found to have longer gestations (Figure 10). Using early fertility disorder as the independent traits gave a highly curvilinear genetic curve and suggests that selection should focus on optimal gestation length with regard to early fertility disorders.

Table 18: Estimates of phenotypic and genetic regression coefficients obtained by offspring parent regression between gestation length and early fertility disorders (EFD)

Offspring trait	Parent trait	Lactation number	b ₁	P	b ₂	p	a ₁	a ₂
Model: EFD _{Daughter} = μ + Gestation length _{Dam} + Gestation length _{Dam} ² + e								
EFD	Gestation length	1	0.0032	>0.1	-0.0062	>0.1		
		2	-0.0394	*	-0.0027	>0.1		
		3	-0.0334	.	0.0135	>0.1		
		All	-0.0041	>0.1	0.0009	>0.1		
Model: Gestation length _{Daughter} = μ + EFD _{Dam} + EFD _{Dam} ² + e								
Gestation length	EFD	1	0.2310	>0.1	-0.0419	>0.1		
		2	1.6216	*	-0.2808	*	141.01	-2122.88
		3	-0.1017	>0.1	0.0212	>0.1		
		All	-0.0434	>0.1	0.0093	>0.1		

Significant codes: 0 '****' 0.001 '***' 0.01 '**' 0.05 '.' 0.1

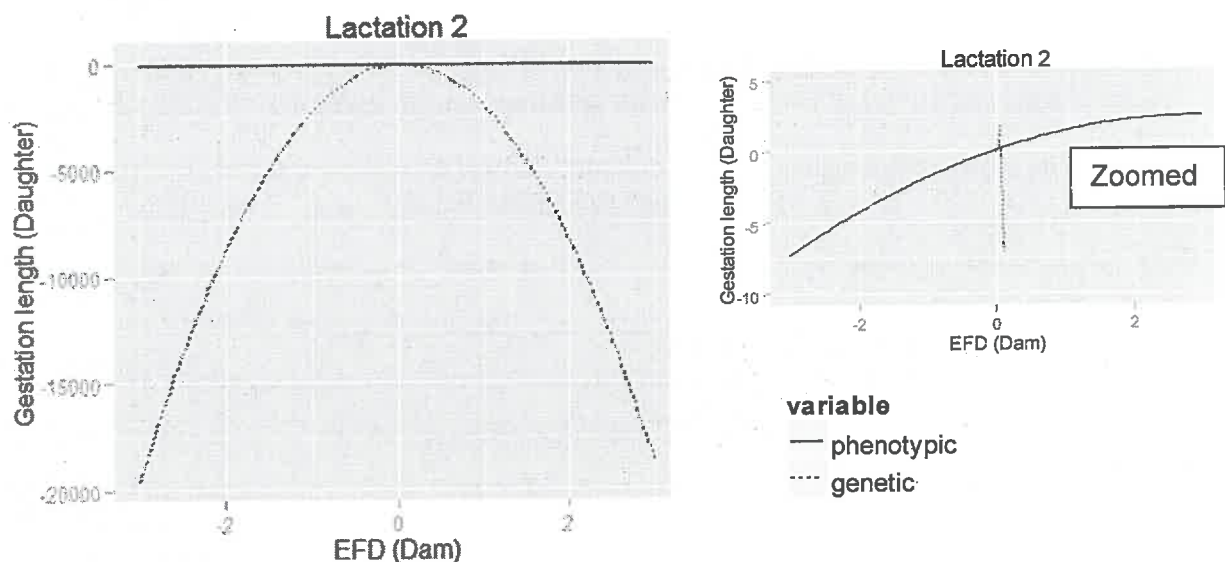


Figure 10: Phenotypic and genetic regression curves of gestation length and early fertility disorder

(The figure on the right is the zoomed portion showing quadratic phenotypic curve) (EFD codes: 1-healthy, 2-sick)

4.6. Milk yield and gestation length

A recent study indicated that length of gestation before being born is genetically related to milk production rather than the length of gestation before giving birth (Eaglen et al., 2013). Genetically, the negative signs of regression coefficient in first, second and combined parity (Table 19) suggests that the longer they stay in uterus, the lower the milk production is, which agrees to the findings of Eaglen et al. (2013). None of the quadratic phenotypic regression coefficients were found to be significant, suggesting that no non-linear relationship is present between milk yield and gestation length.

Similarly non-linear relationships were also not found for protein and fat yield (Kg) with gestation length. The data is presented in Annex 2 and 3.

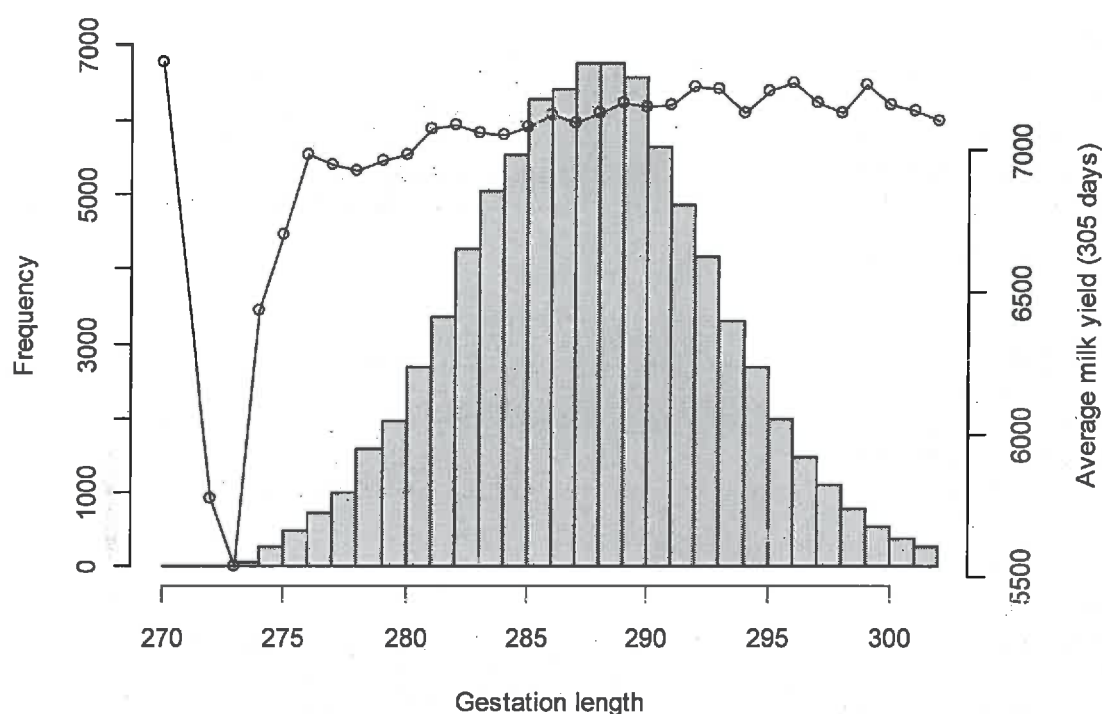


Figure 11: Phenotypic relationship between gestation length and average milk yield.

{Line graph represents average milk yield (305 days) and bar plot represents frequencies of gestation length}

Table 19: Estimates of phenotypic and genetic regression coefficients obtained by offspring parent regression between gestation length and milk yield

Offspring trait	Parent trait	Lactation number	b ₁	p	b ₂	p	a ₁	a ₂
Model: Milk yield _{Daughter} = μ + Gestation length _{Dam} + Gestation length _{Dam} ² + e								
Milk yield	Gestation length	1	-0.019085	.	-0.001018	>0.1		
		2	-0.041393	*	-0.003126	>0.1		
		3	0.00738	>0.1	0.01474	>0.1		
		All	-0.037204	***	-0.003180	>0.1		
Model: Gestation length _{Daughter} = μ + Milk yield _{Dam} + Milk yield _{Dam} ² + e								
Gestation length	Milk yield	1	0.043462	**	0.005853	>0.1		
		2	-0.035613	*	-0.003801	>0.1		
		3	-0.02030	>0.1	0.01581	>0.1		
		All	-0.002191	>0.1	0.004741	>0.1		

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1

4.7. Milk yield and calving ease

Cows born from difficult parturition have poor genetic merit for milk production. There are studies showing genetic relationship between ease of birth of a female calf and her milk production later during adult phase (Heinrichs and Heinrichs, 2011; Eaglen et al., 2011, 2013).

Parent offspring regression of milk yield and calving ease resulted significant phenotypic regression coefficient for the quadratic term in the first parity ($P < 0.05$) and combined parities ($P < 0.001$) (Table 20). Further genetic regression coefficients were calculated assuming heritability for milk yield as 0.34 (Gugger et al., 2007).

The non-linear relationship between calving ease and milk yield is somewhat surprising (Figure 12). This tells us that the cows with highest yields do not represent the 'ideal standard' on the genetic level for calving ease. This is in contrast to Eaglen et al. (2013)

suggesting easy born individuals having higher potential for milk production. Rather this study shows that average milk production is linked to optimal calving ease.

Table 20: Estimates of phenotypic and genetic regression coefficients obtained by offspring parent regression between milk yield and calving ease

Offspring trait	Parent trait	Lactation number	b ₁	p	b ₂	p	a ₁	a ₂
Model: Calving ease _{Daughter} = μ + Milk yield _{Dam} + Milk yield _{Dam} ² + e								
Calving ease	Milk yield	1	0.00760	>0.1	-0.02361	*	0.04473	-0.8171300
		2	-0.0070	>0.1	-0.01226	>0.1		
		3	-0.0285	>0.1	-0.01123	>0.1		
		All	-0.0066	>0.1	-0.01230	***	-0.03887	-0.4257566

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1

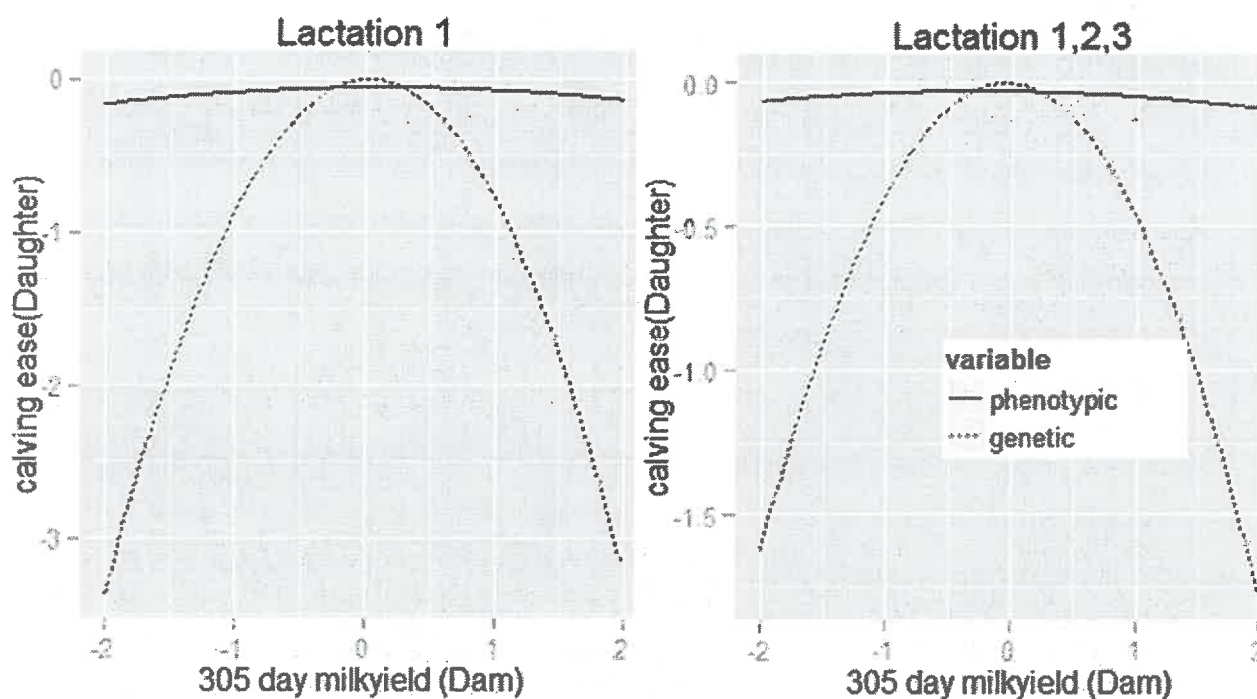


Figure 12: Phenotypic and genetic regression curves of milk yield and calving ease

4.8. Milk yield and stillbirth

Stillbirth causes reduced milk production although the biology is still unknown (Bicalho et al., 2008). Phenotypic regression coefficients were not found to be significant when the parent offspring regression was done between stillbirth and milk yield (Table 21).

Table 21: Estimates of phenotypic and genetic regression coefficients obtained by offspring parent regression between milk yield and stillbirth

Offspring trait	Parent trait	Lactation number	b ₁	p	b ₂	p	a ₁	a ₂
Model: Stillbirth _{Daughter} = μ + Milk yield _{Dam} + Milk yield _{Dam} ² + e								
Stillbirth	Milk yield	1	-0.008851	>0.1	0.006428	>0.1		
		2	0.017990	>0.1	-0.004993	>0.1		
		3	-0.001931	>0.1	-0.011445	>0.1		
		All	0.003633	>0.1	0.001202	>0.1		

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1

4.9. Suitability of the method used

While conducting a polynomial regression analysis of degree 2, traits were defined alternatively as dependent and independent. This has sometimes resulted in conflicting results, which was also observed by Fürst-Waltl et al. (1996). Hence casual flow (dependence/independence) has to be determined prior to regression analysis. Causal flow may play an important role in the prediction of changes of one variable given a second one, but not vice versa (Fürst-Waltl et al., 1996) as interpreting against the causal flow will have no meaning (Solkner and James, 1994).

Although the regression coefficients can be calculated for all the traits, the method has to be restricted. This method is extremely unsuitable for low heritable traits being used as independent trait because overestimation of genetic regression coefficients make the genetic curve incomparable to phenotypic. Fuerst-Waltl et al. (1997) has suggested that the method should be avoided for the traits having heritabilities less than 0.1, except fitness traits.

The method has also been found to be unsuitable for the binary traits like stillbirth and the categorical traits like calving ease if used as the independent variable.

5. Conclusion

The results of the study provided indications of the interplay of genetic factors involved in a set of functional traits and milk production of cows.

Calving traits behave differently in different parities. So, calving traits by parity should be accommodated in the net merit indices rather than calving trait as a whole. Furthermore, calving ease and stillbirth should be used as different traits, rather than using calving ease as the predictor of stillbirth. This is already implemented for the Fleckvieh population investigated in this study.

Cows giving birth to male calves had longer gestation length, as was evident in all the parities. For milk production parameters, there was no significant effect of sex in the first parity. Cows giving birth to male calves were found to produce significantly more 305 day milk ($P < 0.001$), fat (Kg) ($P < 0.01$) and protein (Kg) ($P < 0.001$) yield in second and third parities whereas cows calving females had significantly higher fat% ($P < 0.05$) and protein% ($P < 0.001$) in the milk during second and third parities.

Nonlinear relationships at genetic level were found between gestation length and stillbirth (combined lactation), gestation length and early fertility disorders (second lactation), calving ease and milk yield (first and combined lactation); the first trait being dependent and the second trait being independent. Choice of traits as dependent or independent was found to affect the results significantly. Calving ease and stillbirth were the only traits which had nonlinear relationship when either trait was used as independent, alternatively. When calving ease was used as dependent trait, its relationship with stillbirth was nonlinear in first lactation and in the data set combining lactations 1 to 3. Whereas using it as independent trait, the non-linearity was reduced to the first lactation only.

The non-linear relationship between calving ease and milk yield is somewhat surprising, indicating that the cows with lowest and highest milk yields are more prone to calving difficulties. The construction of the transformation from phenotypic to genetic regression coefficients involves the square of heritability of the independent trait in the denominator of the genetic regression coefficient. This yielded results that were out of the range of expectations for several cases with independent traits of low heritability. Categorical traits should also not be used as independent traits in the offspring parent regression method of polynomial degree 2 because of the missing range of values allowing nonlinearity to show.

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Annexes

Annex 1:

Table showing stillbirth in different gestation period

Gestation period of dam	No. of stillbirth	No. of total birth	% of stillbirth
270	1	3	33.33
271	0	1	0.00
272	0	5	0.00
273	1	2	50.00
274	4	126	3.17
275	8	537	1.49
276	19	952	2.00
277	17	1366	1.24
278	23	1976	1.16
279	47	2798	1.68
280	36	3664	0.98
281	58	4897	1.18
282	64	6101	1.05
283	72	7815	0.92
284	82	9144	0.90
285	99	10237	0.97
286	111	11366	0.98
287	114	11711	0.97
288	123	12202	1.01
289	133	11993	1.11
290	135	11870	1.14
291	119	10126	1.18
292	117	8735	1.34
293	79	7397	1.07
294	75	6051	1.24
295	61	4692	1.30
296	60	3597	1.67
297	60	2736	2.19
298	21	2005	1.05
299	14	1387	1.01
300	22	969	2.27
301	16	722	2.22
302	13	549	2.37

Annex 2:

Estimates of phenotypic and genetic regression coefficients obtained by offspring parent regression between gestation length and fat (Kg)

Offspring trait	Parent trait	Lactation number	b ₁	p	b ₂	p	a ₁	a ₂
Model: Fat (Kg) _{Daughter} = μ + Gestation length _{Dam} + Gestation length _{Dam} ² + e								
Fat (Kg)	Gestation length	1	-0.009230	>0.1	-0.002309	≥0.1		
		2	-0.017743	>0.1	-0.009012	>0.1		
		3	0.014873	>0.1	0.004163	>0.1		
		All	-0.026697	***	-0.003626	>0.1		
Model: Gestation length _{Daughter} = μ + Fat (Kg) _{Dam} + Fat (Kg) _{Dam} ² + e								
Gestation length	Fat (Kg)	1	0.05098	**	-0.00318	>0.1		
		2	-0.020531	>0.1	-0.004215	>0.1		
		3	-0.01103	>0.1	0.01972			
		All	0.006248	>0.1	0.003027	>0.1		

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1

Annex 3:

Estimates of phenotypic and genetic regression coefficients obtained by offspring parent regression between gestation length and protein (Kg)

Offspring trait	Parent trait	Lactation number	b ₁	p	b ₂	p	a ₁	a ₂
Model: Protein (Kg) _{Daughter} = μ + Gestation length _{Dam} + Gestation length _{Dam} ² + e								
Protein (Kg)	Gestation length	1	-0.0120 097	>0.1	-0.00026 79	>0.1		
		2	-0.0416 17	*	-0.00559 9	>0.1		
		3	0.00371 1	>0.1	0.008397	>0.1		
		All	-0.0384 49	***	-0.00410 4	>0.1		
Model: Gestation length _{Daughter} = μ + Protein (Kg) _{Dam} + Protein (Kg) _{Dam} ² + e								
Gestation length	Protein (Kg)	1	-0.0107 64	>0.1	-0.00140 4	>0.1		
		2	-0.0294 17	.	-0.00762 5	>0.1		
		3	0.00998 0	>0.1	0.006259	>0.1		
		All	-0.0328 82	***	-0.00393 9	>0.1		

Significant codes: 0 '****' 0.001 '**' 0.01 '*' 0.05 '.' 0.1

Annex 4:

Relationship between gestation length with average fat (Kg), average fat (%), average protein (Kg) and average protein (%) in the 305 day milk yield

