Genotype plus Environment
Integration for a more sustainable dairy production system

Biomarkers del latte per la valutazione dello stato sanitario della mammella in vacche ad elevata produzione

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KBBE.2013.1.1-01: Development and exploitation of genomic data and tools, phenotyping approaches and breeding concepts to sustainable animal production systems

Participant organization name	Country
Schools of Veterinary Medicine and agricolture and food science. University College of Dublin (UCD) (COORDINATOR)	IE
Royal Veterinary College (RVC)	UK
Agri-Food and Biosciences Institute (AFBI)	UK
Faculty of Veterinary Medicine, University of Gent (UGent)	BE
Faculty of Veterinary Medicine, University Utrecht (UU)	NL
Aarhus University (AU)	DK
Consiglio per la Ricerca in agricoltura e l'analisi in Economia Agraria (CREA)	IT
Irish Cattle Breeding Federation Society Ltd (ICBF)	IE
Huazhong Agricultural University (HZAU)	CN
Service EAAP srl (S-EAAP)	IT
Unifarm	BE
Videncentret for Landbrug (KCA)	DK
University of Missouri, Animal Science Research Center (MU)	USA
University of Liège, Gembloux Agr-Bio-Tech (ULg-Gx) University of Liège, Faculty of Veterinary Medicine (ULg-FVM)	BE
Walloon Agricultural Research Centre (CRA-W)	BE
Leibniz Institute for Farm Animal Biology - FBN Dummerstorf	DE
SimHerd, Niels Pedersens Allé 2, 8830 Tjele (SimHerd)	DK

Genotype and **E**nvironment contributing to the sustainability of dairy cow production systems through the optimal integration of genomic selection and novel management protocols based on the development and exploitation of genomic data and supporting novel phenotyping approaches.

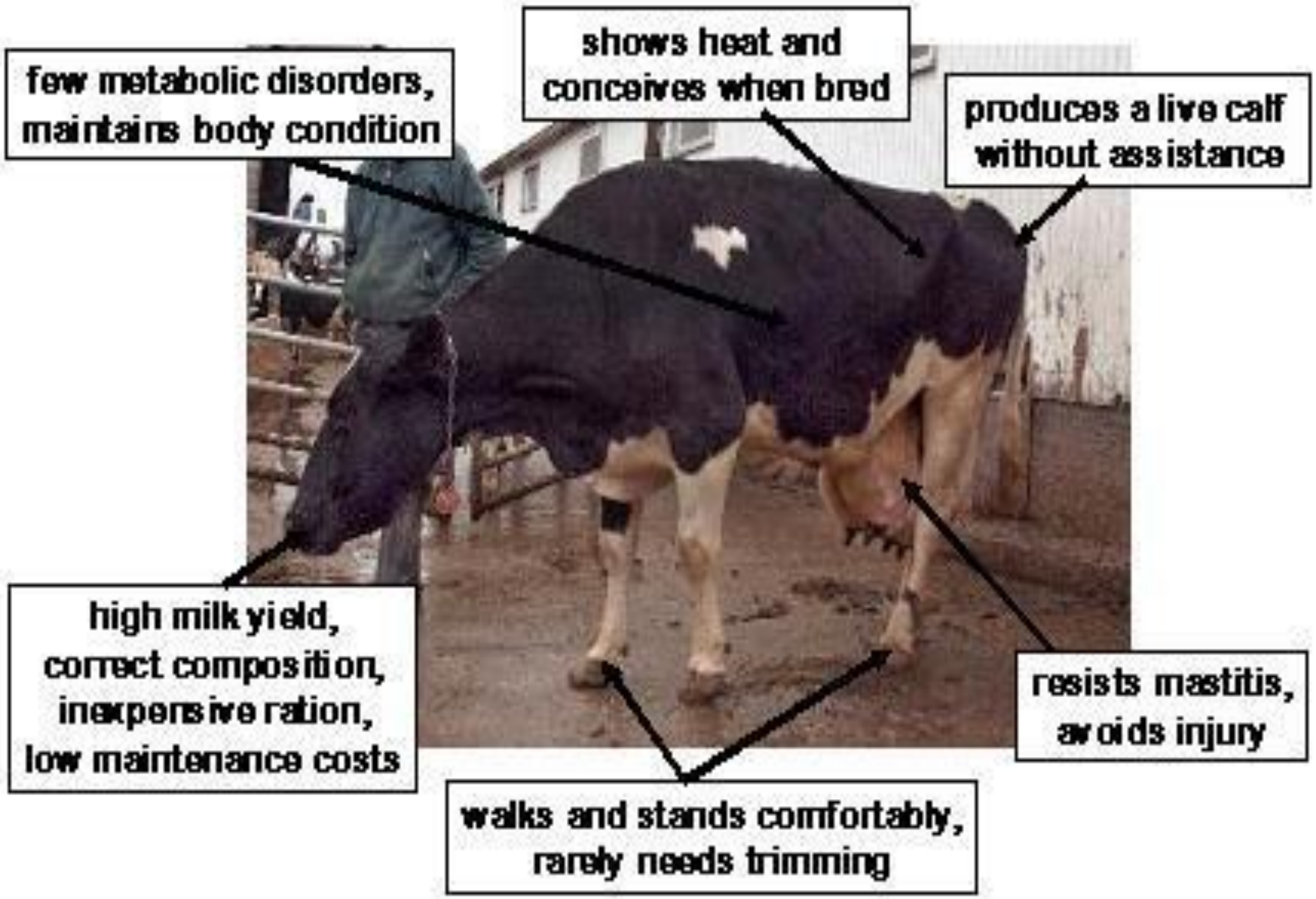
Duration: 60 months

Total estimated eligible cost: 11,607,551.40 euro

Total requested EU contribution: 8,997,235.00 euro

GplusE aims to identify those genotypes that control biological variation of the important phenotypes of dairy cows, to appreciate how these are influenced by environmental and management factors, and thus to allow more informed and accurate use of Genomic Selection (GS).

The Perfect Cow

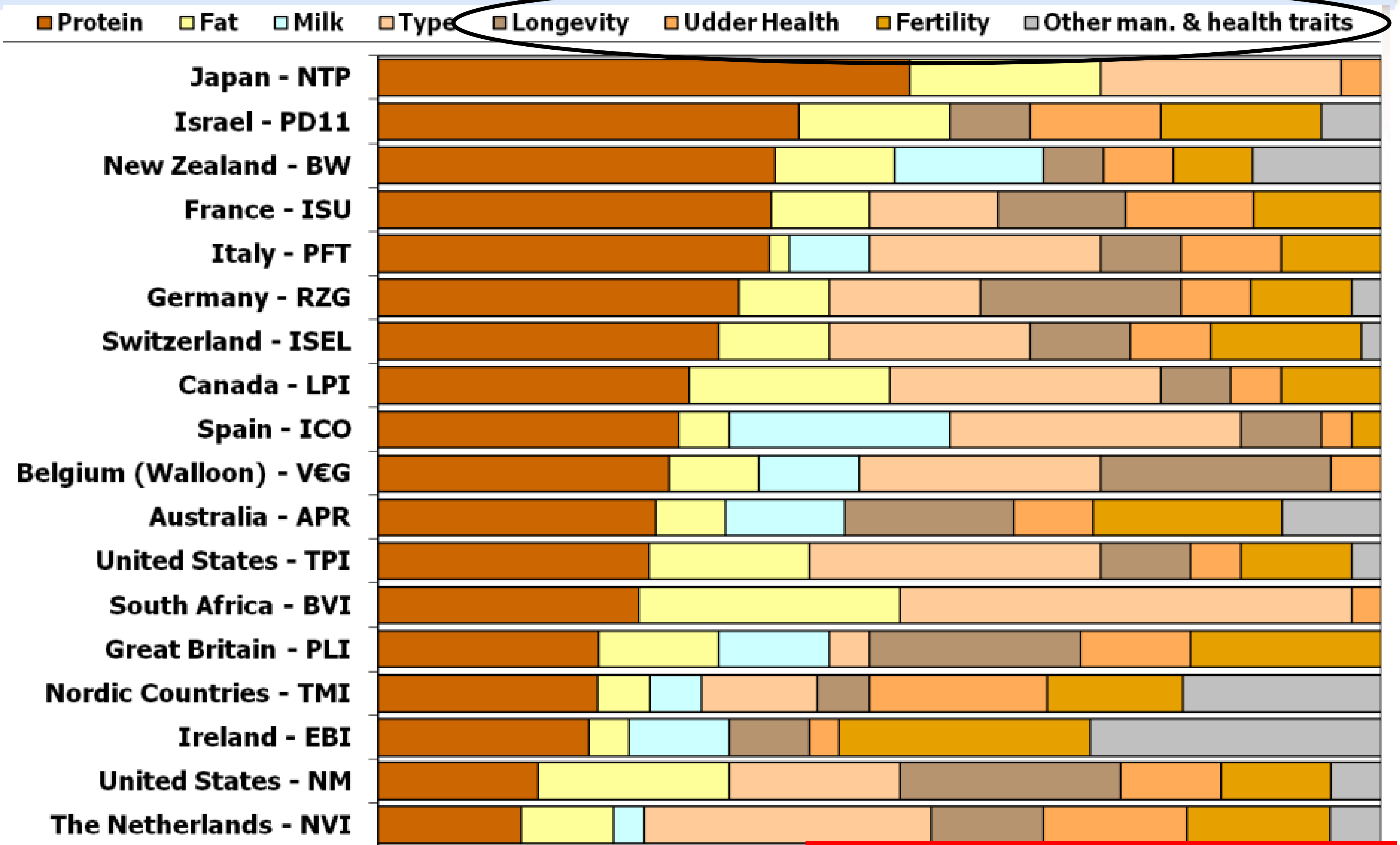


What are functional traits?

- The ICAR* Functional Traits Working Group currently is working on:
  - General health traits
  - Female fertility
  - Feet and legs problems
  - Udder health
  - Workability

*International Commitee for Animal Recording

Functional traits are being used



Source: Miglior et al., 2012

GplusE vs functional traits

- Milk biomarkers include metabolites, mastitis parameters, hormones, glycan profile and infrared spectra obtained using the MIR technology.

- The biomarkers measured in milk will be compared with the production, health and welfare traits observed in the same animals.

- A potential biomarker will be identified as a valid when an observed trait can be predicted with good precision from that particular biomarker measured in milk.

1.Materials and methods

Country	N° COW	N° MILK SAMPLES
AFBI	21	79
AU	14	56
CRAITA	45	173
CRAW	31	121
FBN	13	43
UCD	38	151
TOTAL COW	162	623

PARITY	Country						TOTAL cows
	AFBI	AU	CRAITA	CRAW	FBN	UCD	
FIRST	6	2	7	13	1	3	32
SECOND	2	9	15	9	3	11	49
≥THIRD	13	3	23	9	9	24	81
TOTAL cows	21	14	45	31	13	38	162

PARITY	Country						N° MILK SAMPLES
	AFBI	AU	CRAITA	CRAW	FBN	UCD	
FIRST	22	8	31	52	4	12	129
SECOND	8	36	58	34	9	44	189
≥THIRD	49	12	84	35	30	95	305
N° MILK SAMPLES	79	56	173	121	43	151	623

1.Materials and methods

DAY POST PARTUM	Country						N° MILK SAMPLES
	AFBI	AU	CRAITA	CRAW	FBN	UCD	
7±3	20	14	42	29	9	37	151
14±3	21	14	43	30	11	38	157
21±3	20	14	44	31	12	38	159
35±3	18	14	44	31	11	38	156
N° MILK SAMPLES	**79**	**56**	**173**	**121**	**43**	**151**	**623**

SEASON	Country						N° MILK SAMPLES
	AFBI	AU	CRAITA	CRAW	FBN	UCD	
SPRING	0	0	65	33	15	80	193
SUMMER	1	0	50	59	12	0	122
AUTUMN	78	54	5	5	7	0	149
WINTER	0	2	53	24	9	71	159
N° MILK SAMPLES	**79**	**56**	**173**	**121**	**43**	**151**	**623**

2.Materials and methods

Milk recording
milk
protein
fat
lactose
SCC

Protocols of milk standard laboratory

Milk metabolite
Glu6P
GluFree
BOHB
IsoC
Urea
NAGase
Uric acid
Progesteron

Protocols of Larsen, 2005; Larsen and Nielsen, 2005; Chagunda et al, 2006; Larsen and Moyes, 2010; Larsen 2014.

MIR DATA	
C4:0	SAT
C6:0	MONO
C8:0	POLY
C10:0	INSAT
C12:0	SCFA: C4 to C10
C14:0	MCFA : C12 to C16
C14:1 cis	LCFA : C17 and more
C16:0	iso + anteiso
C16:1 cis	omega 3
C17:0	omega 6
C18:0	ODD
Tot18_1trans	Total_Trans
C18:1 cis9	Total_C18_1
Tot18_1cis	BHB
Tot18_2	Acetone
C18_2c9c12	Citrates
C18_3c9c12c15	Na
C18_2c9t11	Ca
SAT	P
MONO	Mg
POLY	K
INSAT	Lactoferrin

Fourier transform mid-infrared (FT-MIR) spectroscopy according to Soyeurt et al, 2009, 2011, 2012; Grelet et al , 2016

3.Materials and methods
Statistical analysis

SCC class	Country						N° MILK SAMPLES
	AFBI	AU	CRAITA	CRAW	FBN	UCD	
low (≤100,000)	74	43	81	69	24	122	413
medium (101,000≤X≤400,000)	4	11	56	30	6	16	123
high (≥401,000)	1	2	36	22	13	13	87
N° MILK SAMPLES	79	56	173	121	43	151	623

Shapiro-Wilks test for normality

Correlation

Canonical discriminat analysis

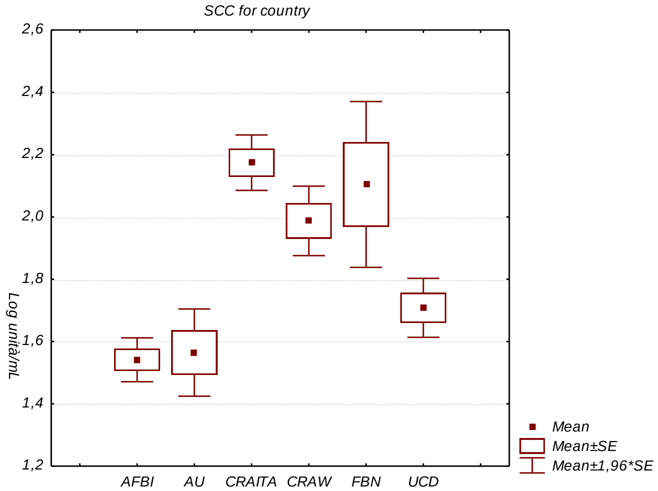
MIXED GLM model

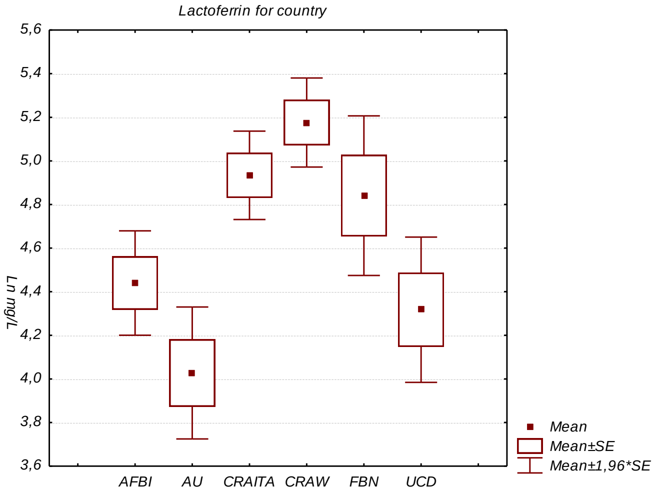
$$Y_{ijklmn} = \mu + P_i + S_j + C_k + H_l + \alpha_m + \beta(MY)_{ijklmn} + e_{ijklmn}$$

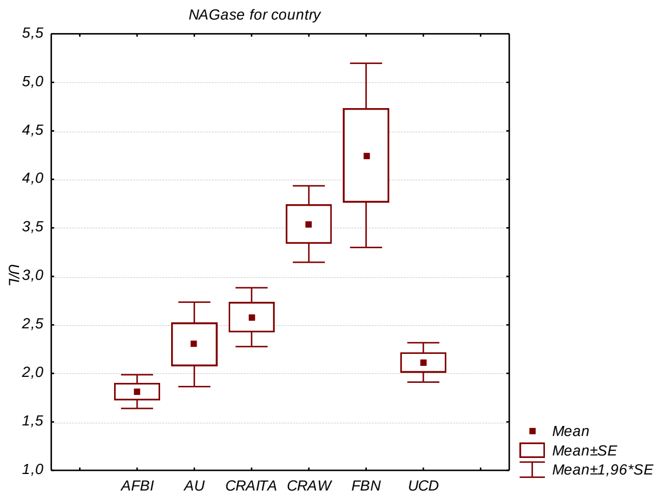
Y_{iklmn}= observed value of milk parameters
μ=overall mean
P_i= fixed effect of parity
S_j=fixed effect of season of calving
C_k= fixed effect of the days in milk
H_l= fixed effect of the SCC
α=random effect
$\beta(MY)_{ijklmn}$=regression effect of milk yield with the regression coefficient
e_{ijklmn}=random error

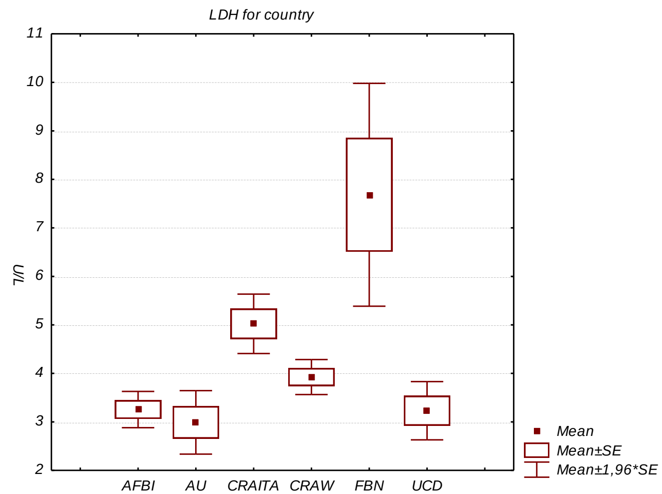
Results 1

Differences between country



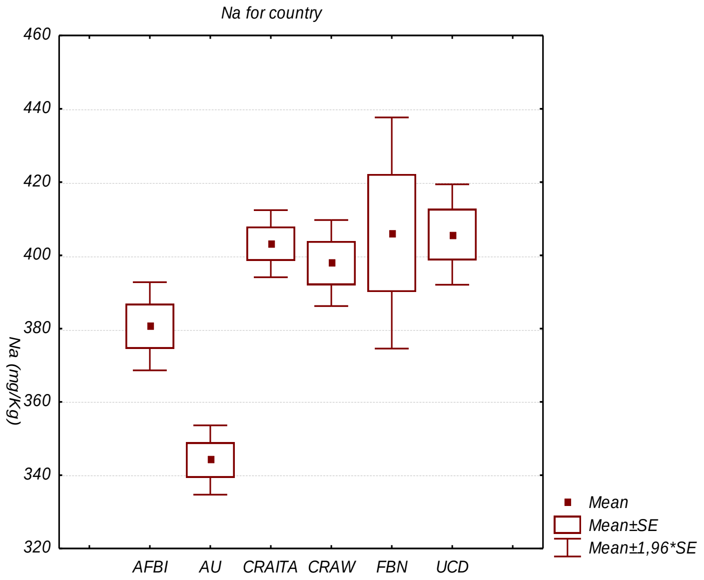


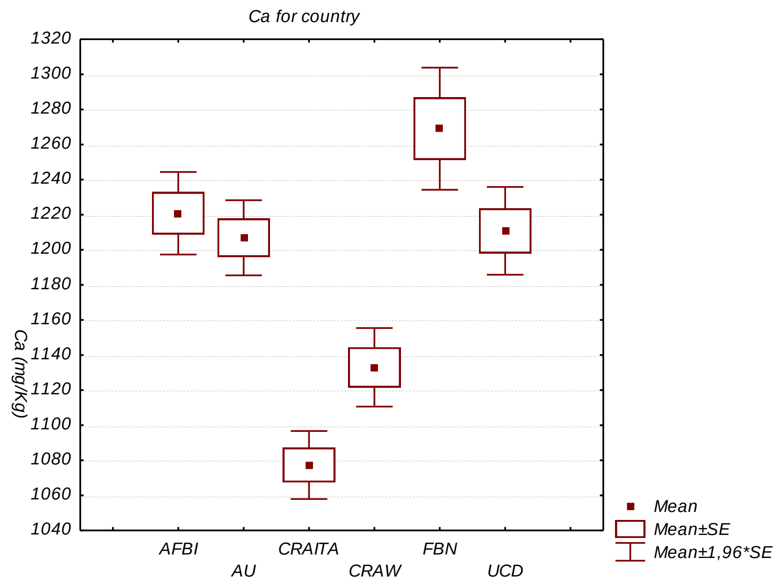


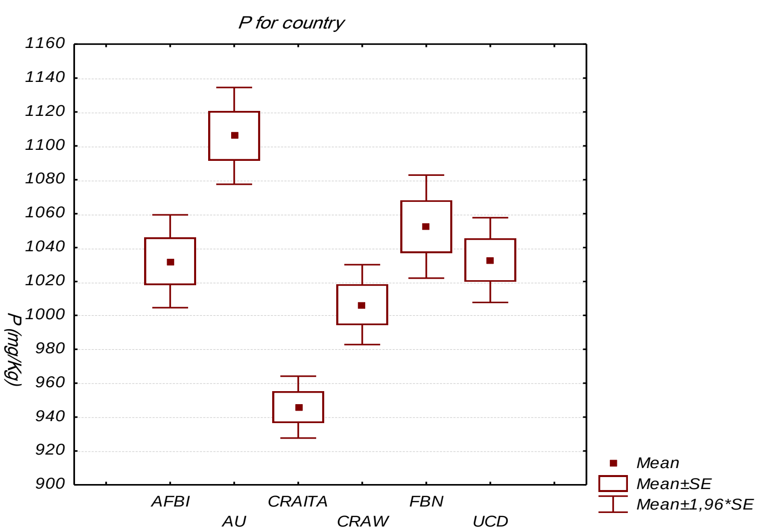


Results 1

Differences between country

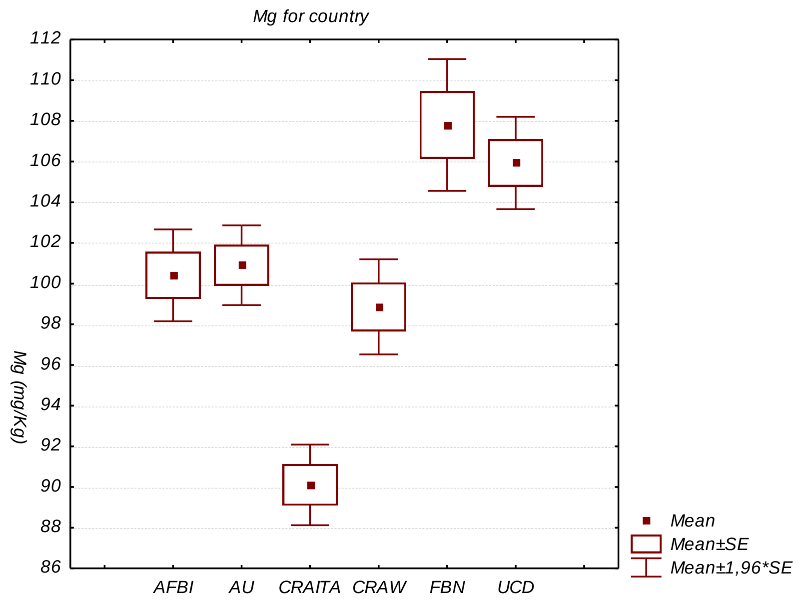


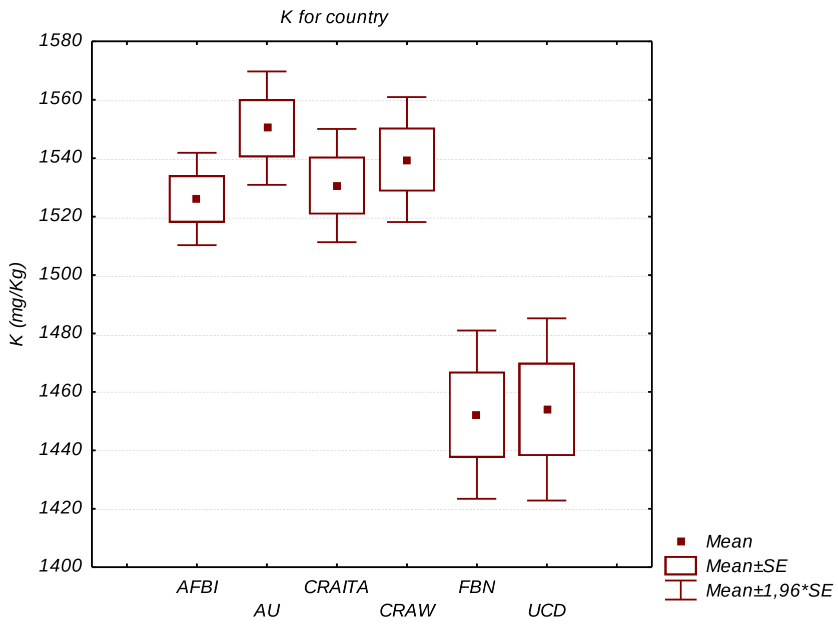




Results 1

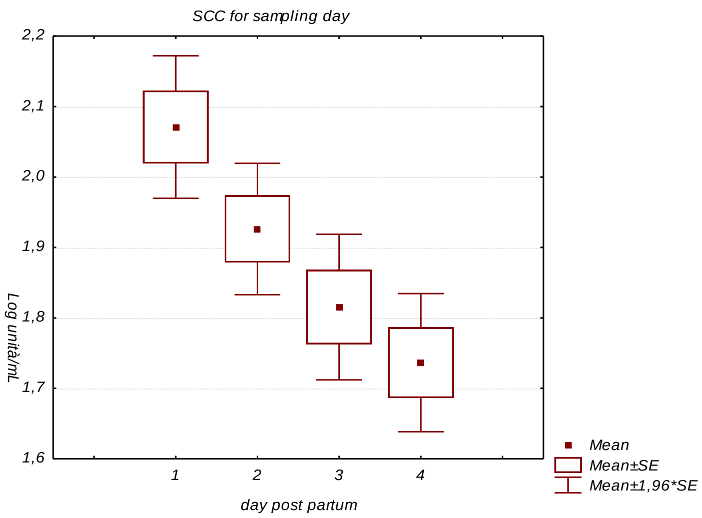
Differences between country

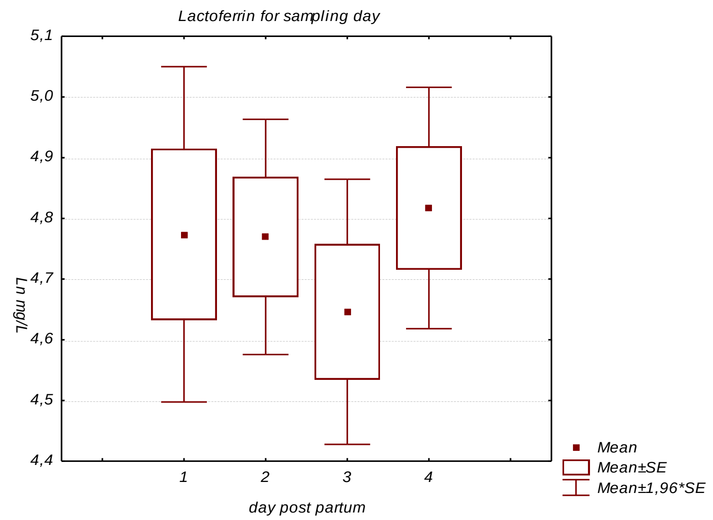


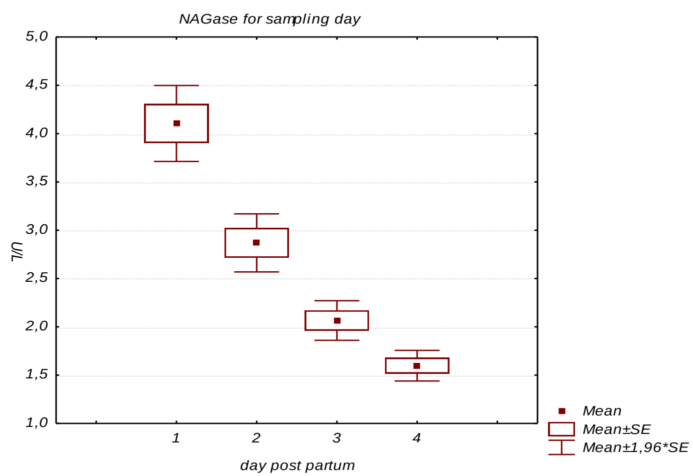


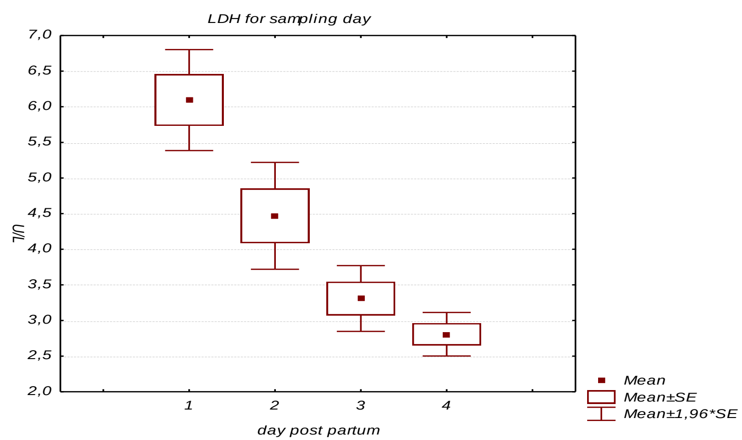
Results 1

Differences between sampling day



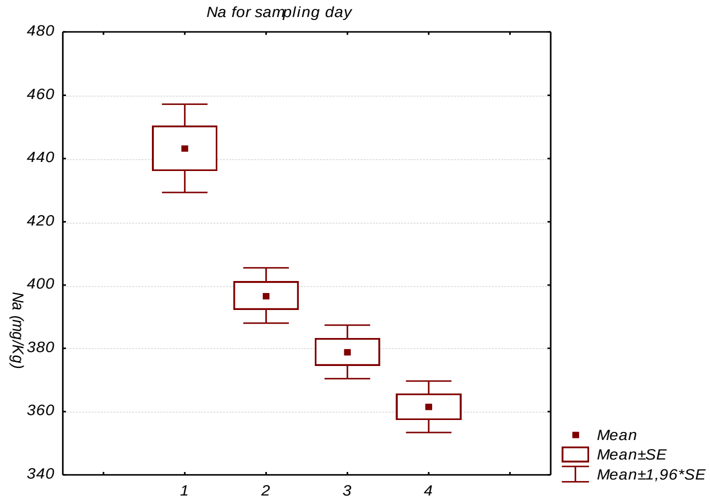


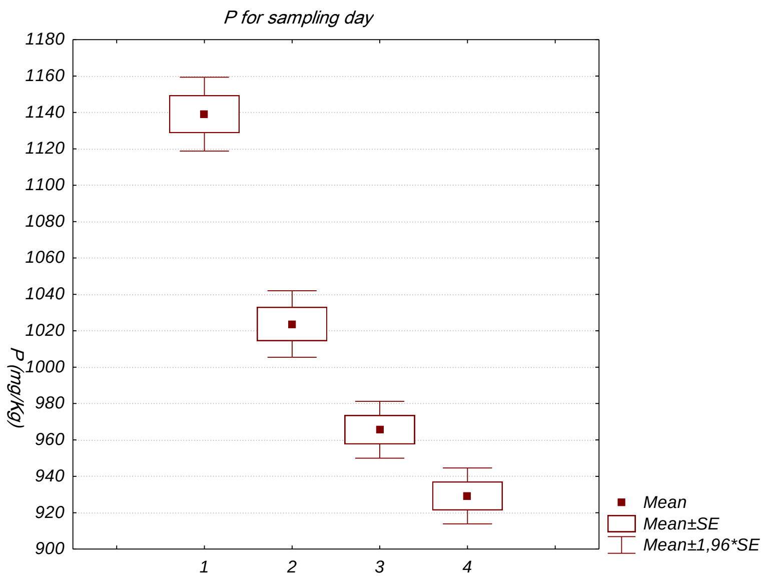


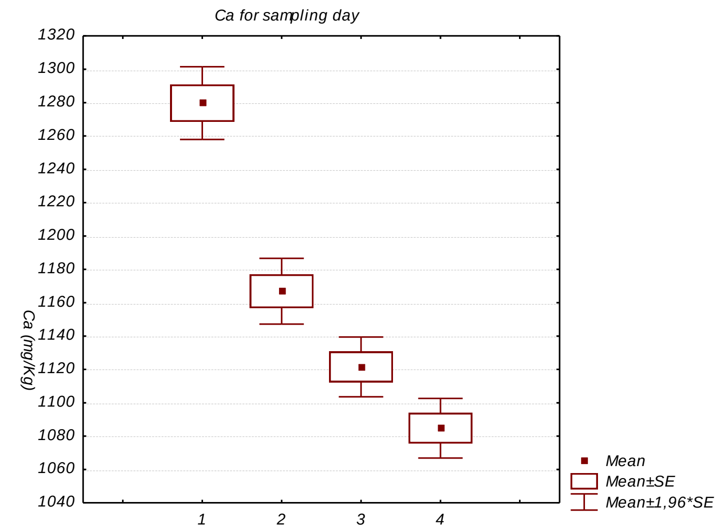


1= 7±3
2= 14±3
3= 21±3
4= 35±3

Results 1 — Differences between sampling day

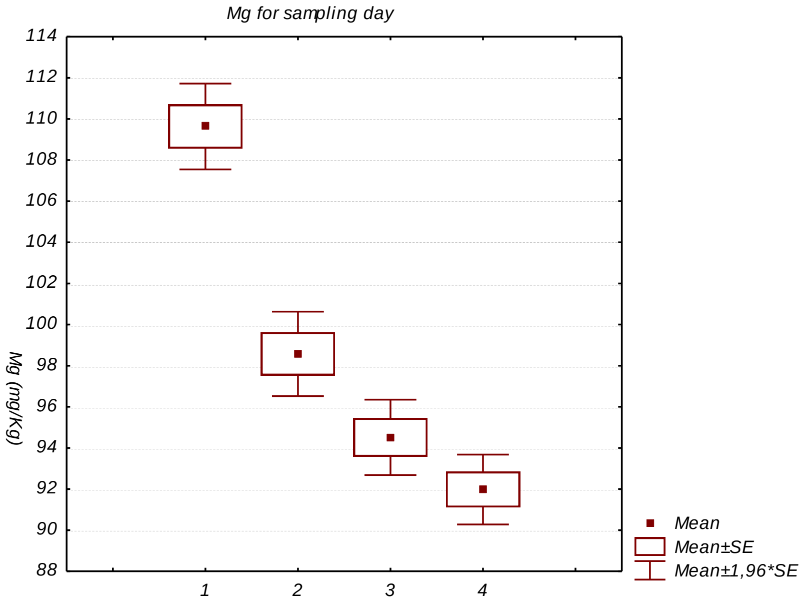


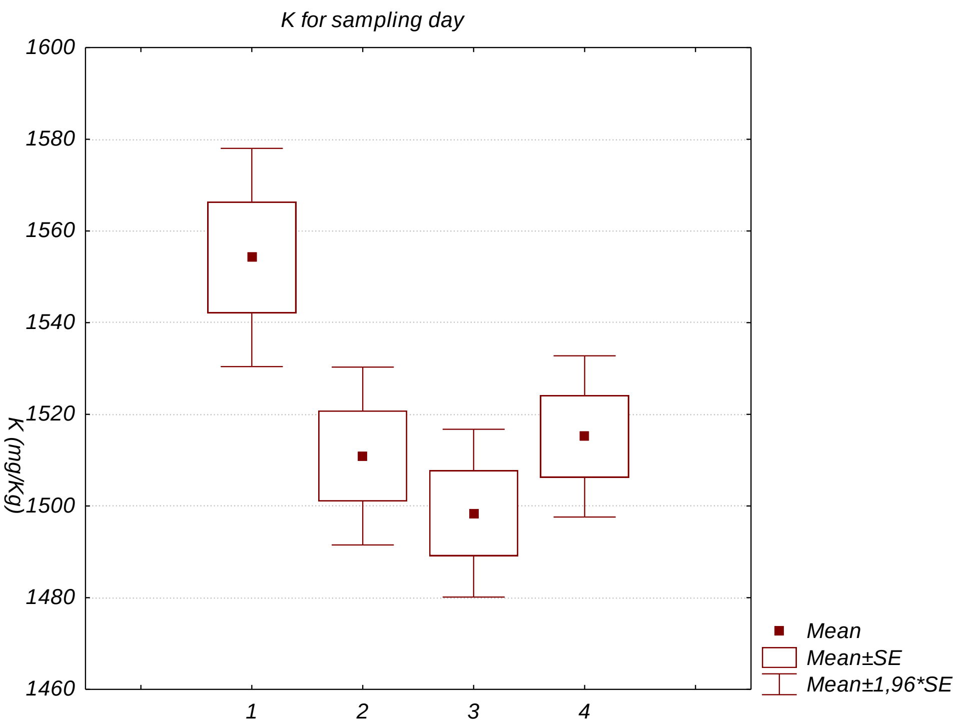




1= 7±3
2= 14±3
3= 21±3
4= 35±3

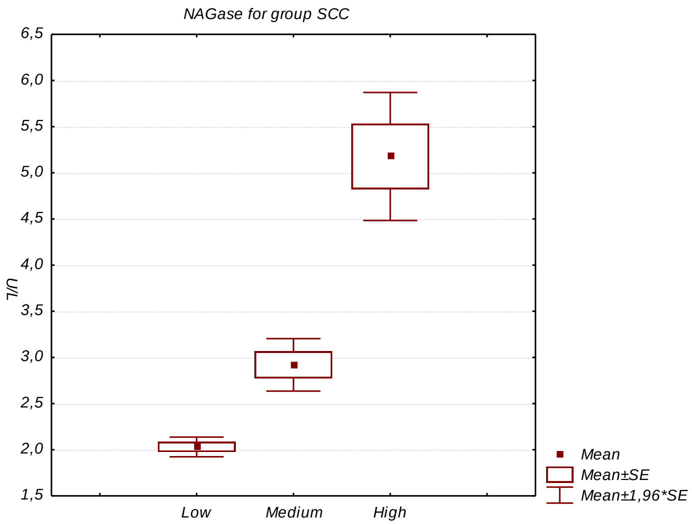
Results 1 Differences between sampling day

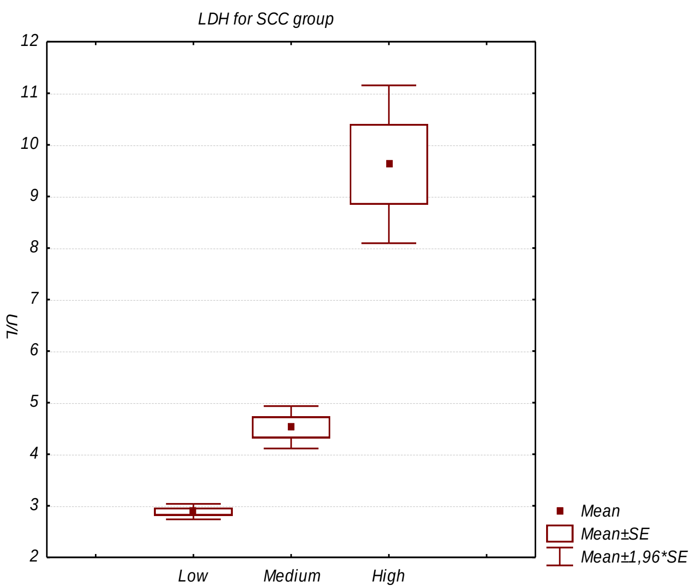


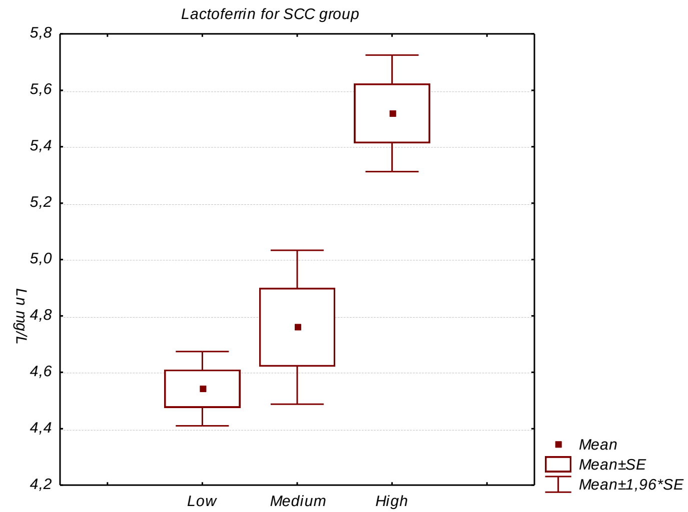


1= 7±3
2= 14±3
3= 21±3
4= 35±3

Results 1 Differences between SCC group





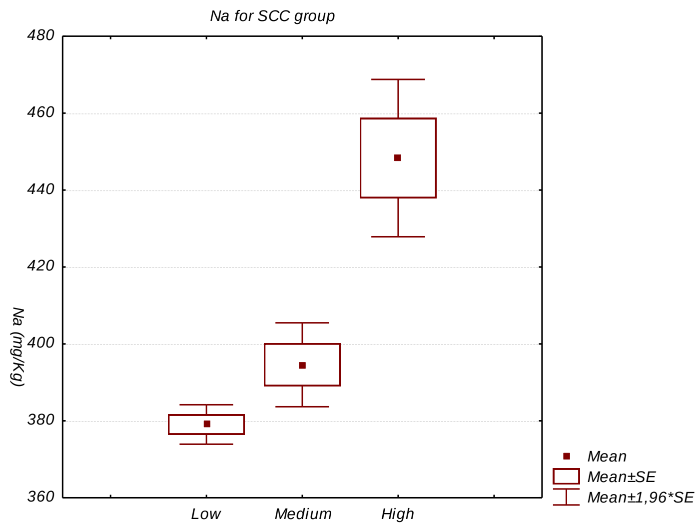


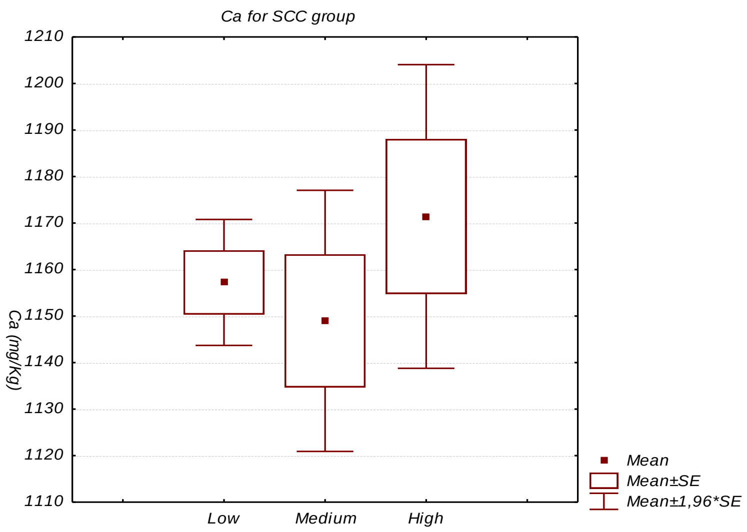
low (≤100,000)

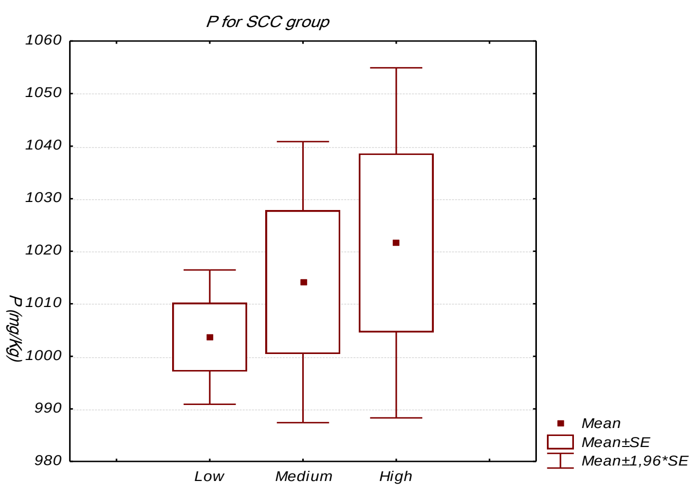
medium (101,000≤X≤400,000)

high (≥401,000)

Results 1 Differences between SCC group





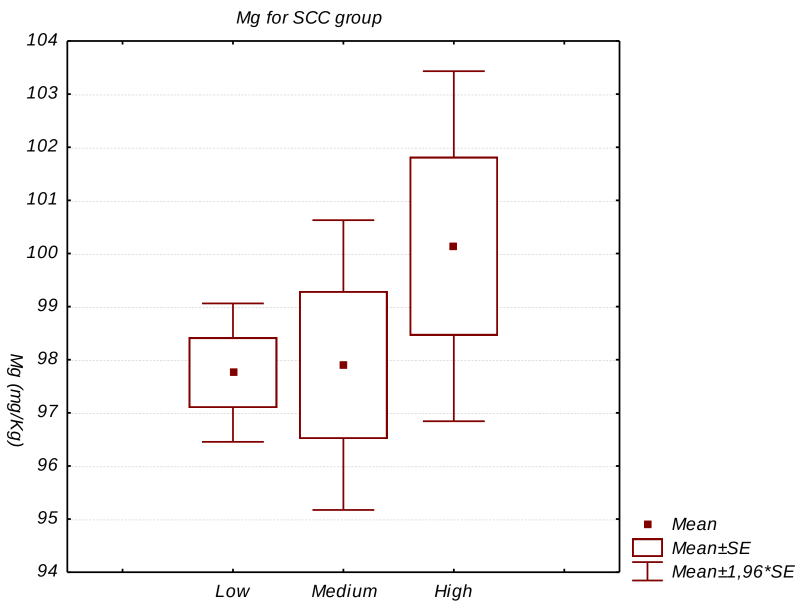


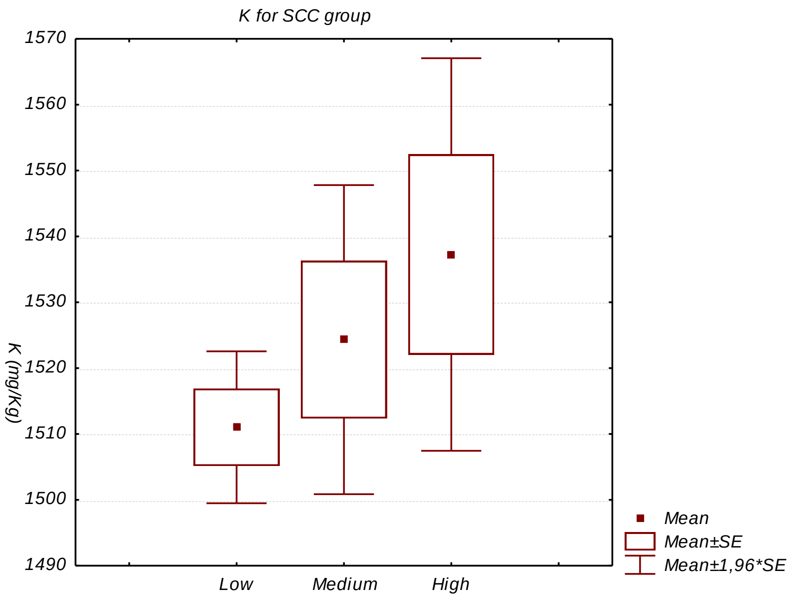
low (≤100,000)

medium (101,000≤X≤400,000)

high (≥401,000)

Results 1 Differences between SCC group





low (≤100,000)

medium (101,000≤X≤400,000)

high (≥401,000)

Results 2

MIXED GLM Model

Item	Obs	MSE	Country	Parity	Control	LevelSCC	LevelSCC		
			P value				High	Medium	Low
Milk (Kg/d)	623	35.6	***	***	***	***	**27.7^{c}**	**29.9^{b}**	**31.6^{a}**
Fat (g/100mL)	623	0.93	***	Ϯ	***	Ϯ	4.42^{AB}	4.55^{A}	4.35^{B}
Protein (g/100mL)	623	0.06	***	-	***	-	3.28	3.24	3.23
Lactose (g/100mL)	548	0.04	Ϯ	***	***	***	**4.56^{b}**	**4.71^{a}**	**4.70^{a}**
Glu6P (mM)	623	0.004	***	**	***	***	**0.245^{a}**	**0.204^{b}**	**0.182^{c}**
GluFree (mM)	623	0.005	***	***	***	***	**0.185^{bB}**	**0.201^{bA}**	**0.218^{a}**
BOBH (Ln mM)	623	0.18	***	***	-	-	4.01	4.01	3.94
IsoC (mM)	623	0.002	***	-	***	Ϯ	0.178^{B}	0.193^{A}	0.186^{AB}
Urea (mM)	619	0.95	***	*	Ϯ	-	**2.88^{b}**	**3.13^{a}**	**3.13^{a}**
NAGase (U/L)	623	1.83	***	**	***	***	**4.79^{a}**	**2.75^{b}**	**2.34^{c}**
LDH (U/L)	623	8.17	***	***	***	***	**8.89^{a}**	**4.27^{b}**	**3.27^{c}**
Uric acid (mM)	622	2429	***	-	***	-	162	165	165
Prog (U/L)	622	4.14	*	-	***	Ϯ	**4.02^{b}**	**4.18^{ab}**	**4.54^{a}**

Ϯ P < 0.10; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ **A, B**: $P < 0.10$; **a, b, c**: $P < 0.05$ - MSE: Mean Standard Error

Results 2

MIXED GLM Model

Item	Obs	MSE	Country	Parity	Control	LevelSCC	LevelSCC		
(g/100mL)			P value				High	Medium	Low
C4 : 0	458	0.11	***	*	***	-	2.89	2.87	2.94
C6 : 0	458	0.04	***	*	***	†	**1.70 ^{ab}**	**1.67 ^{b}**	**1.72 ^{a}**
C8 : 0	458	0.04	***	***	***	†	1.03 ^{AB}	1.01 ^{B}	1.05 ^{A}
C10 : 0	458	0.46	***	***	***	-	2.12	2.07	2.15
C12 : 0	458	0.68	***	***	***	-	2.46	2.40	2.48
C14 : 0	458	2.67	***	***	***	-	9.58	9.27	9.48
C14 : 1 cis	458	0.03	***	***	***	-	0.66	0.67	0.64
C16 : 0	458	14.2	***	***	***	†	28.0 ^{A}	27.3 ^{AB}	27.0 ^{B}
C16 : 1 cis	458	0.10	***	**	***	-	1.76 ^{AB}	1.81 ^{A}	1.74 ^{B}
C17 : 0	458	0.002	***	*	***	-	0.685	0.683	0.680
C18 : 0	458	1.96	***	***	***	†	**12.23 ^{b}**	**12.42 ^{ab}**	**12.63 ^{a}**
Tot C18 : 1 trans	458	0.79	***	**	**	†	3.12 ^{B}	3.09 ^{b}	3.33 ^{aA}
C18 : 1 cis-9	458	20.7	***	***	***	†	**23.3 ^{b}**	**24.8 ^{a}**	**24.1 ^{ab}**
Tot C18 : 1 cis	458	23.2	***	***	***	†	**25.0 ^{b}**	**26.6 ^{a}**	**25.8 ^{ab}**

† P < 0.10; * P < 0.05; ** P < 0.01; *** P < 0.001 **A**, **B**: P < 0.10; **a**, **b**, **c**: P < 0.05 - MSE: Mean Standard Error

Results 2

MIXED GLM Model

Item	Obs	MSE	Country	Parity	Control	LevelSCC	LevelSCC		
(g/100mL)			P value				High	Medium	Low
C18 : 2	458	0.10	***	†	***	-	2.51	2.53	2.55
C18 : 2 cis-9,cis-12	458	0.07	***	-	***	-	1.76	1.81	1.79
C18 : 3 cis-9,cis-12,cis-15	458	0.01	***	-	**	-	0.54	0.53	0.55
C18 : 2 cis-9,trans-11	458	0.05	***	***	***	-	0.47	0.41	0.46
Saturated FA	458	25.7	***	***	***	†	64.8 A	63.2 B	63.8 AB
MUFA	458	27.1	***	***	***	-	31.0	32.3	31.8
PUFA	458	0.47	***	-	***	-	3.97	3.91	4.00
Unsaturated FA	458	31.0	***	***	***	-	34.8	36.1	35.7
Short chain	458	1.28	***	**	***	†	**7.95 ab**	**7.80 b**	**8.09 a**
Medium chain	458	30.1	***	***	***	-	44.2	43.3	43.0
Long chain	458	41.4	***	***	***	-	46.8	48.1	48.0
Isoanteiso	458	0.05	***	***	***	-	1.88	1.91	1.93
Omega3	458	0.02	***	-	***	-	0.69	0.69	0.70
Omega6	458	0.22	***	-	***	-	2.68	2.66	2.68
ODD	458	0.11	***	*	-	-	3.39	3.33	3.36
Total trans	458	1.07	***	**	***	†	3.96 AB	3.89 B	4.14 A
Tot C18 : 1	458	26.1	***	***	***	-	27.9	29.2	28.8

† P < 0.10; * *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001 **A**, **B**: *P* < 0.10; **a**, **b**, **c**: *P* < 0.05 - MSE: Mean Standard Error

Results 2

MIXED GLM Model

Item	Obs	MSE	Country	Parity	Control	LevelSCC	LevelSCC		
			P value				High	Medium	Low
Acetone (mM)	458	0.01	***	-	***	-	0.10	0.10	0.11
Citrates (mM)	458	3.16	***	-	***	-	9.13	9.65	9.43
Na (mg/Kg)	458	2069	***	***	***	***	**423 [a]**	**379 [b]**	**380 [b]**
Ca (mg/Kg)	458	6863	***	*	***	-	1189	1194	1186
P (mg/Kg)	458	6159	***	***	***	-	1048	1047	1040
Mg (mg/Kg)	458	73	***	***	***	-	103	103	101
K (mg/Kg)	458	10343	***	-	***	-	1533	1506	1511
Lactoferrin (Ln mg/L)	458	0.83	***	***	-	***	**5.14 [a]**	**4.54 [b]**	**4.40 [b]**

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ **A**, **B**: $P < 0.10$; **a**, **b**, **c**: $P < 0.05$ - MSE: Mean Standard Error

Results 3

Canonical discriminant analysis

	Eigenvalue	proportional variance	cumulative variance	P value
CAN1	1.134	0.795	0.795	<0.0001
CAN2	0,292	0.205	1.000	0.0258

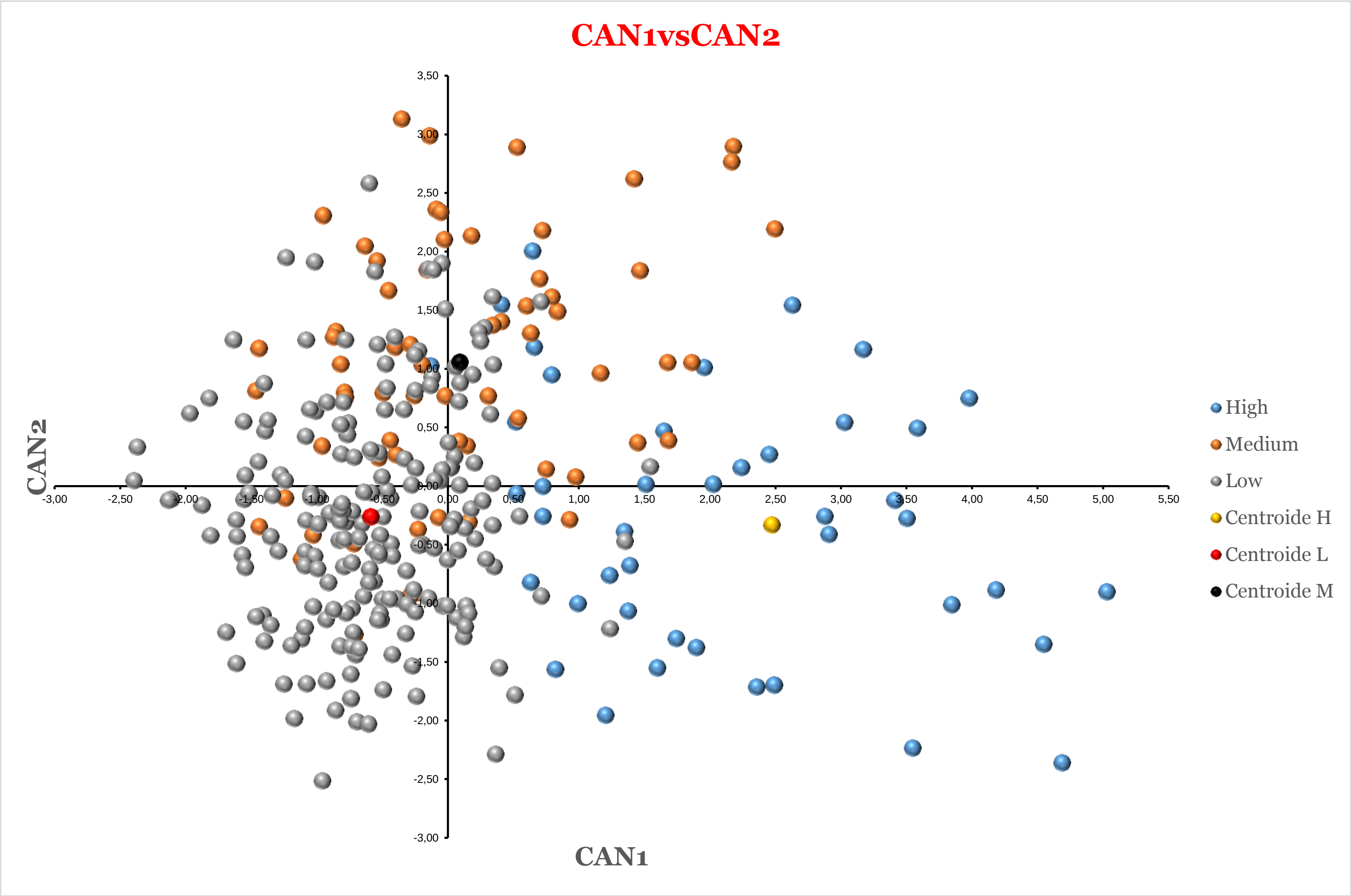
Means of canonical variables

Level of SCC	CAN1	CAN2
High	2.465	0.328
Low	-0.590	-0.261
Medium	-0.091	1.0580

Canonical value for each parametrs

Parameter	CAN1	CAN2
Lactose	**-0.561**	0.045
Glu6P	0.350	0.217
GluFree	-0.335	-0.169
NAGase	**0.772**	0.1640
LDH	**0.754**	0.119
UA	0.157	0.332
C6	-0.158	-0.323
C8	-0.259	-0.352
C10	-0.284	-0.303
C12	-0.300	-0.292
C14	-0.288	-0.332
C16_1c	0.300	0.351
C17	0.325	**0.466**
SCFA	-0.242	-0.355
Na	**0.626**	0.029
Lactoferrin	**0.457**	0.054

Results 3 Canonical discriminant analysis



Conclusion

- GLM confirmed NAGase, LDH, Lactoferrin, Na, Lactose

- Using discriminant analysis same parameters discriminate better High vs (Medium&Low), other parameters discriminate Medium vs (Low&High)

- How do these parameters interact?

- Which are the better to diagnose health status of mammary gland?

Future remarks

- Analysis of parameters obtained by milk samples of thousand cows
- GEBV
- GWAS
- The better milk parameters to detect o prevent diseases
- Use GplusE results in new management strategies and in new breeding schemes to obtain “the perfect cow”!

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