Effects of heat stress on semen characteristics of Holstein bulls estimated on a continuous phenotypic and genetic scale

A. Al-Kanaan [a,b], S. König [a], K. Brügemann [a,*]

[a] Department of Animal Breeding, University of Kassel, Witzenhausen 37213, Germany
[b] Department of Animal Production, University of Basrah, Basrah, Iraq

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ABSTRACT

Semen volume (SV), semen output (SO), semen motility (SM), number of semen doses per ejaculate (NSD) and semen concentration (SC) were analyzed on a phenotypic and quantitative genetic scale in dependency of the continuous environmental descriptor THI (=an index describing combinations of temperature with humidity). Longitudinal semen data included 10,341 observations from 562 Holstein sires kept on an AI station in the northwestern part of Germany. Statistical modeling was based on fixed and random regression methodology (RRM). In this context, the phenotypic and genetic trajectory of traits in dependency of THI was modeled by orthogonal polynomials, i.e. Legendre polynomials of order 3. A general heat stress threshold of THI=60 was identified, and indicating detrimental effects of heat stress beyond this threshold, especially on SV, SO, NSD, and SC. Least square means for semen productivity were generally higher for adult bulls, but compared to young bulls, adult bulls responded with stronger semen production losses for extremely high THI. The optimal environment for semen production was a THI in the range from 50 to 60. Detrimental impact of heat stress was reduced for bulls with longer intervals between consecutive semen collection dates. Heritabilities for semen traits were in a low to moderate range with the following maximal values: 0.18 for SV at THI 30, 0.29 for SM at THI 70, 0.26 for SO at THI 30, 0.26 for NSD at THI 58, and 0.27 for SC at THI 53. Genetic values of sires and additive genetic variances changed with alterations of THI, with highest genetic variances at the extreme ends of the continuous environmental descriptor. Genetic correlations in same traits from THI levels in great distance were lower than 0.80, and indicated genotype by environment interactions. Application of RRM allow to select sires according to THI specific breeding values, which implies an optimization of cow sire and bull sire selection for harsh environments.

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1. Introduction

Artificial insemination (AI) is one of the most important reproduction biotechnologies in dairy cattle. Artificial insemination enabled a rapid dissemination of superior genetic material across country borders, and boosted selection response in production and, more recently, also in functional traits. Maximizing genetic gain for the overall breeding goal implies widespread use of outstanding sires from national and international genetic evaluations (König et al., 2007). Reasons for sub-optimal utilization of outstanding sires include deficiencies of semen production, or even male infertility due to environmental or genetic

* Corresponding author at: Department of Animal Breeding, University of Kassel, Nordbahnhofstraße 1a, D-37213 Witzenhausen, Germany.
Tel.: +49 55 42/98 1678.
E-mail address: Kerstin.bruegemann@uni-kassel.de (K. Brügemann).

impact. The physiological and genetics background for male infertility was described in detail in humans (Carell, 2007), and infertility was also reported for influential bulls kept on AI stations.

Artificial insemination with fresh and frozen semen plays a role of increasing importance in countries with tropical or subtropical climate located in Asia, Africa, or South America (Thibier and Wagner, 2002). The detrimental impact of heat stress in terms of high temperatures and humidities, or a combination of both (temperature- × humidity index (THI)), on cattle physiology was summarized by Hoffmann (2010). Physiological alterations included male and female fertility traits, and also the risk of mortality. For female fertility traits, Brügemann et al. (2012) focused on temperate zones in middle Europe and identified environmental sensitivity especially for dairy cows with highest production levels and highest genetic values for test-day milk yield. Consequences of adaptation to harsh environments (=high THI), and addressing male and female pathways of selection, were evaluated by stochastic simulations on the basis of a longitudinal data structure (Yin et al., 2014).

From a phenotypic perspective, most studies designed specific experiments to assess the impact of heat stress on male fertility traits. Detrimental effects included reduced sperm counts and the phenomenon of "summer sterility", i.e. the depression of sexual activity due to heat stress (Setchell, 1998); semen deterioration based on in-vitro experiments for bulls (Rahman et al., 2013a) and rams (Malama et al., 2013); decreased fertilization and embryo development (Rahman et al., 2013a, 2013b); high percentages of abnormal sperms (Al-Makhzoomi et al., 2008; Silva et al., 2009); as well as high saturated fatty acids, low polyunsaturated fatty acids and low cholesterol concentrations in semen (Argov-Argaman et al., 2013). Direct climatic heat stress revealed undesired effects on semen ejaculate volume and on semen concentration (Snoj et al., 2013; Teixeira et al., 2011).

From a genetics perspective, heritabilities for semen quantity and semen quality traits were estimated in different countries, but without specifically focusing on climatic descriptors. Across country and across breed comparisons reviewed in Table 1 illustrate a broad range of heritability estimates for the following semen traits: volume, motility, output, number of doses per ejaculate and concentration.

The aim of the present study was to extend heat stress analyses by considering longitudinal data for semen quantity and quality traits via random regression methodology (RRM). Applications of RRM allow to infer genetic parameters and to study the phenotypic trajectory of male fertility traits as a function of continuous environmental descriptors. Brügemann et al. (2013) used THI as continuous environmental descriptor and applied RRM to longitudinal female fertility data. Following their results in cows, we hypothesize environmental sensitivity for semen traits of AI sires, i.e. the identification of heat stress thresholds, alterations of additive-genetic variances and heritabilities with increasing THI, and indications for genotype by environment interactions.

2. Materials and methods

2.1. Data

Semen quality and quantity traits in the present study were: semen volume (SV, in ml), semen output (SO, in no. of sperm), semen motility (SM, in %), number of semen doses per ejaculate (NSD) and semen concentration (SC, in no. of sperm per ml ejaculate). Data included 10,341 observations for all traits from 562 Holstein Friesian sires kept on one AI station in the federal state of Lower Saxony in the north-western part of Germany. Traits were recorded in the years 2009, 2010, and 2011 from 10 to 136 months old bulls. Semen volume is the quantity of a bull's ejaculation as measured in a scaled tube. Semen concentration was determined in the laboratory of the AI center using a NucleoCounter®. Semen output is the product of SV with SC and reflects the no. of sperm per ejaculation. Semen motility (percentage of motile sperm in relation to all sperm) was visually analyzed with a microscope by trained technicians. Number of semen doses strongly depends on SO, i.e. a single semen doses requires 15 millions of motile sperm. Descriptive statistics of semen traits is summarized in Table 2.

Meteorological data were daily average measurements for ambient temperature (in °C) and relative humidity (in %) from two weather stations at a distance of 24 and 39 km from the AI station. Daily average THI was calculated by applying the formula of the National Research Council (1971):

$$THI = (1.8 \times T \ (°C) + 32) - [(0.55 - 0.0055 \times RH\%) \times (1.8 \times T \ (°C) - 26)]$$

where T (°C) = temperature of air measured by a thermometer and RH% = relative humidity. Maximal daily THI (69.64) and minimal daily THI (30.29) were realized in June 2010 and in December 2010, respectively. Highest monthly THI indicating periods of heat stress in dairy cattle (THI > 60) were observed in June 2010 and 2011, and in July and August for all three years (Fig. 1).

2.2. Statistical analysis

2.2.1. Analyses on the phenotypic scale
A fixed regression model was used to study alterations of semen traits by THI on the phenotypic scale. For this purpose, a linear mixed model as implemented in the SAS 9.2 procedure PROC MIXED (SAS Institute, Cary, NC) was applied. The statistical model 1 in matrix notation used for analysis of variance (test of significance of fixed effects based on sum of squares (SS), type 3) was defined as follows:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Zu} + \mathbf{e} \qquad (1)$$

where $\mathbf{y}$ = vector of observations for semen traits, $\mathbf{\beta}$ = vector of fixed effects including age of sire (age groups: < 12, 12–18, 18–48 and > 48 months), year-season of semen collection (years: 2009, 2010 and 2011; seasons: spring = March–May, summer = June–August, autumn = September–November, and winter = December–February), the intervals between consecutive semen collection dates (intervals: < 3 days and ≥ 3

Table 1
Literature overview of heritabilities for the following semen traits of AI sires: Semen volume (SV), semen motility (SM), semen output (SO), number of semen doses per ejaculate (NSD), and semen concentration (SC).

Author	Number of records/ bulls	Region	Breed	Heritability				
				SV	SM	SO	NSD	SC
Knights et al. (1984)	717 bulls	Anita, Iowa, USA	Angus		0.13	0.24		0.13
Taylor et al. (1985)	149,339 records	Ohio,USA	Holstein	0.16– 0.18		0.03– 0.05		0.10– 0.16
Stålhammar et al. (1989)	215 bulls	Sweden	Swedish R&W+Swedish Friesian	0.2	0.18		0.17	
Ducrocq and Humblot (1995)	2387 records	L'Aigle, France	Normande	0.65			0.23	0.37
Diarra et al. (1997)	294 bulls	Québec, Canada	Holstein	0.53	0.51			0.37
Mathevon et al. (1998a)		Guelph, Canada	Holstein	0.24– 0.44	0.01– 0.31	0.38– 0.54		0.36– 0.52
Mathevon et al. (1998b)	16,242 records	Ceyzeriat, France	Montbéliard	0.08– 0.49		0.12– 0.15		0.08– 0.32
Gredler et al. (2007)	12,746 records	Austria	Simmental	0.18	0.04	0.22		0.14
Kealey et al. (2006)	841 bulls	Montana, USA	Hereford	0.09	0.22			0.21
Carabaño et al. (2007)	8773 records	Madrid, Spain	Holstein	0.23– 0.36				
Druet et al. (2009)	2131 records	Jouy-en-Josas, France	Holstein	0.22	0.43	0.09		0.19
Karoui et al. (2011)	42,348 records	Aberekin, Spain	Holstein	0.22	0.16	0.18		0.19
Silveira et al. (2012)	5903 bulls	São Paulo, Brazil	Nellore		0.11			
Siqueira et al. (2012)	21,186 bulls	São Paulo and MatoGrosso do Sul, Brazil	Nellore		0.08– 0.18			

Table 2
Descriptve statistics for semen traits of AI sires as used in the present study.

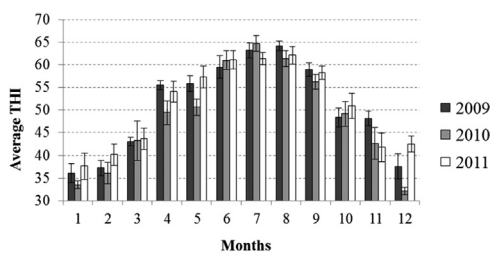| Semen trait	Unit	Mean	SD	Min	Max
Semen volume (SV)	[ml]	5.97	2.18	0.5	17
Semen motility (SM)	[%]	69.45	2.71	65	75
Semen output (SO)[a]	[no. of sperm]	7.89	4.02	0.33	30.80
No. of semen doses (NSD)	[count variable]	449.53	276.24	3	2008
Semen concentration (SC)[a]	[no. of sperm per ml ejaculate]	1.33	0.46	0.09	3.00

[a] $\times 10^9$.

Fig. 1. Average monthly temperature-humidity indices (THI) for the years 2009, 2010, and 2011. (Stripes in the bars indicate daily minima and maxima for THI.)

days), and regressions for the covariate THI from the ejaculation date (regressions were modelled using Legendre polynomials) nested within age classes of bulls, $\mathbf{u}$=vector of random bull effects, and $\mathbf{X}$ and $\mathbf{Z}$ are the associated incidence matrices, respectively. Model evaluation for regression specification was based on AIC-values and BIC-values for models with either Legendre polynomials of order 3 and of order 4 in consecutive runs. Based on previous experiences with longitudinal THI simulation studies (Yin et al., 2014), and based on

smaller AIC- and BIC values, we chose Legendre polynomials of order 3 for the final modeling of THI regressions.

For the analyses of the physiological background of semen traits in detail, not only THI measured at the ejaculation dates (=semen collection dates) were considered. In different runs, we analyzed the impact of THI from dates previous to semen collection date on semen traits. Fuerst-Waltl et al. (2006) identified that the period of epididymal maturation (1–11 days before semen collection) and the period of spermatogenesis (days 12–65 before semen collection) are important intervals when analyzing semen traits. Hence, average THI from the following intervals was used as continuous environmental descriptor: Interval I=1–11 days, interval II=12–65 days, interval III=22–28 days, and interval IV=29–35 days before the ejaculation date.

2.2.2. Analyses on the genetic scale

For genetic analyses, a linear RRM as used by Brügemann et al. (2012) for heat stress studies of female fertility traits, and considering the full relationship matrix among animals, was specified. Inclusion of random additive-genetic effects of bulls via the genetic relationship matrix allow to separate random

bull effects into genetic and permanent environmental effects. In matrix notation, the RRM (model 2) was:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Za} + \mathbf{Wpe} + \mathbf{e} \qquad (2)$$

where $\mathbf{y}$=vector of observations for semen traits, $\mathbf{\beta}$=vector of fixed effects and regressions as specified for model 1, $\mathbf{a}$=vector of additive genetic effects for random regression coefficients on THI using third-order Legendre polynomials, $\mathbf{pe}$=vector of random permanent environmental effects using third-order Legendre polynomials, $\mathbf{e}$=vector of random residual effects, and $\mathbf{X}$, $\mathbf{Z}$ and $\mathbf{W}$ are the associated incidence matrices, respectively.

The (co)variance structure of random effects was defined as:

$$\mathrm{Var}\begin{bmatrix} \mathbf{a} \\ \mathbf{pe} \\ \mathbf{e} \end{bmatrix} = \begin{bmatrix} \mathbf{G} \otimes \mathbf{A} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{P} \otimes \mathbf{I_{pe}} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{R} \otimes \mathbf{I_n} \end{bmatrix}$$

where $\mathbf{G}$=additive genetic (co)variance matrix of random regression coefficients, $\mathbf{A}$=additive genetic relationship matrix considering genetic relationships traced back to base animals born in 1924, $\mathbf{P}$=permanent environmental variance matrix of random regression coefficients, $\mathbf{I_{pe}}$= identity matrix for 562 bulls, $\mathbf{R}$=residual variance matrix, $\mathbf{I_n}$=identity matrix for n observations, and $\otimes$=Kronecker product.

Genetic statistical analyses were done in a Bayesian framework using the THRGIBBS1F90 software-package (Tsuruta and Misztal, 2006). In total, 100,000 Gibbs samples were generated, whereof 10,000 samples were used for the "burn-in period", and 90,000 samples were used to calculate posterior statistics. Assessment of the length of the burn-in sample and of the sampling period was based on visual inspections of (co)variances for genetic effects, and is illustrated for the additive genetic variances of SC at THI 50 (Fig. 2).

3. Results and discussion

Results from the fixed effects model (1) were used to stretch the topic of environmental sensitivity for bulls kept on AI stations (Section 3.1: Environmental impact on semen traits). This topic of environmental sensitivity includes comparisons of least square means for semen traits within classes of effects for ejaculation seasons and
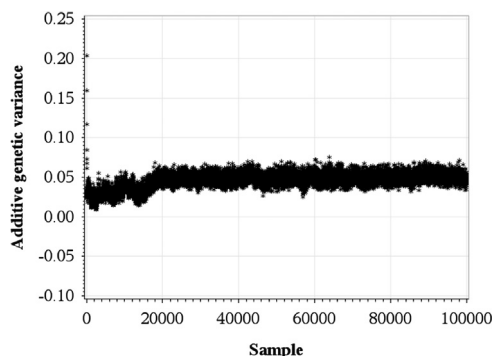

Fig. 2. Genetic variances for semen concentration at THI 50 for the different rounds of iterations (samples).

ejaculation years. Simultaneously, the effects of THI (continuous environmental descriptor) from the ejaculation date on semen traits was studied, and allowing the identification of so called heat stress thresholds. Heat stress thresholds indicate a THI value associated with an obvious increase or decrease of semen traits. The THI threshold in the present study was identified at THI 60 for all semen traits, and indicating detrimental effects on SV, SO, NSD, and SC. Only SM slightly increased with increasing THI. Significant effects ($P < 0.05$) of years and of seasons reflect the impact of feeding and of husbandry, and of daylight changes on semen traits. Furthermore, environmental impact includes the management of semen collection as practiced on AI stations. A close interval between consecutive ejaculation dates (< 3 days) was associated with impaired male fertility, and might be seen as an additional stress component for AI bulls.

Model (1) was used in ongoing runs to study the THI effect from dates previous to the ejaculation date on semen traits. Significant THI effects were identified for THI from 1 to 11 days previous to the ejaculation date, and from 29 to 35 days before the ejaculation date. These results underline that heat stress during the periods of epididymal maturation (1–11 days before semen collection) and of spermatogenesis (days 12–65 before semen collection) negatively effects semen quantity and quality.

Model (2) additionally includes the additive-genetic component via the full relationship matrix among animals, and allowing the estimation of heritabilities for semen traits for specific THI (Section 3.2: Genetic impact on semen traits). We identified alterations of heritabilities for semen traits with alterations of THI. Model (2) also allows to prove the existence of genotype by environment interactions. A genetic correlation lower than 0.80 in same traits measured at different THI indicate genotype by environment interactions, and was identified for THI in great distance (e.g. SV measured at THI 30=trait 1 with SV measured at day 60=trait 2).

3.1. Environmental impact on semen traits

3.1.1. Temperature–humidity index (THI)

Analyses of variance (Type 3 test of fixed effects) revealed highly significant impact ($P < 0.001$) of THI from the ejaculation date modeled with Legendre polynomials of order 3 on SV, SO, and NSD. Significant impact of THI at the ejaculation date ($P < 0.05$) was identified for SM and SC. Least square means from model (1) as a function of THI, and stratified by age classes of bulls, are depicted on the phenotypic scale for SV (Fig. 3), SM (Fig. 4), SO (Fig. 5), NSD (Fig. 6), and SC (Fig. 7). Semen traits where quite constant or only showed minor fluctuations for THI < 60. Such environmental comfort zones for dairy cattle were described by e.g. Aceves et al. (1987). Detrimental impact of heat stress on semen productivity and semen quality was identified for THI > 60, and indicating decreasing SV, SO, NSD and SC beyond this upper critical "THI threshold". The identified upper THI threshold for semen quality and semen quantity traits from Holstein bulls is in agreement with results from Holstein cows located in a temperate climatic zone in the northern part of Germany for test-day
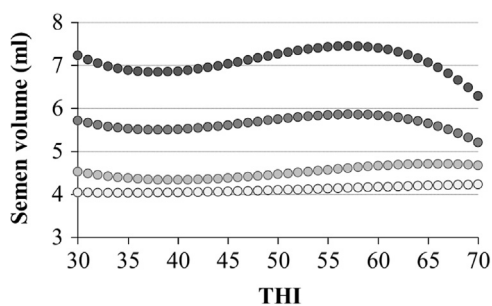
Fig. 3. Least squares means for semen volume (SV) of AI sires by THI measured at the ejaculation date for different age classes of bulls. (Age classes of bulls: from light to dark grey: < 12 months, 12–18 months, 18–48 months and > 48 months.)
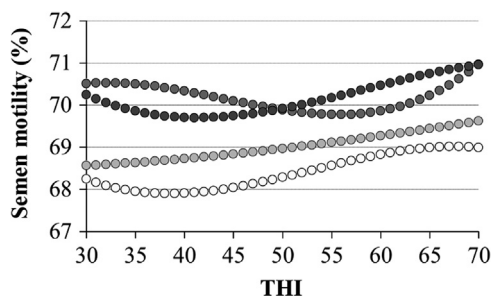

Fig. 4. Least squares means for semen motility (SM) of AI sires by THI measured at the ejaculation date for different age classes of bulls. (Age classes of bulls: from light to dark grey: < 12 months, 12–18 months, 18–48 months and > 48 months.)
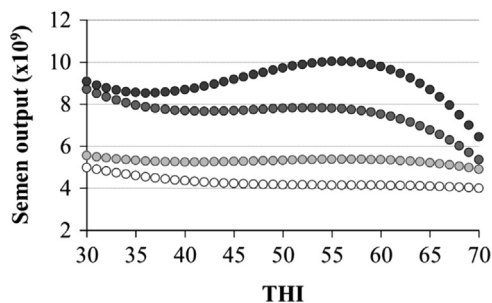

Fig. 5. Least squares means for semen output (SO) of AI sires by THI measured at the ejaculation date for different age classes of bulls. (Age classes of bulls: from light to dark grey: < 12 months, 12–18 months, 18–48 months and > 48 months.)
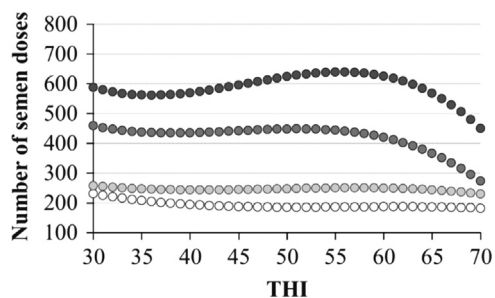

Fig. 6. Least squares means for number of semen doses per ejaculate (NSD) of AI sires by THI measured at the ejaculation date for different age classes of bulls. (Age classes of bulls: from light to dark gray: < 12 months, 12–18 months, 18–48 months and > 48 months.)
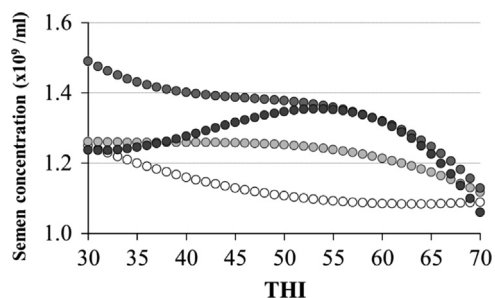

Fig. 7. Least squares means for semen concentration (SC) of AI sires by THI measured at the ejaculation date for different age classes of bulls. (Age classes of bulls: from light to dark grey: < 12 months, 12–18 months, 18–48 months and > 48 months.)

protein yield (Brügemann et al., 2011), and for female fertility traits (Brügemann et al., 2012). Only SM steadily increased for THI > 60. However, apart from 18 to 48 month old bulls, increase was small (on average lower than 1% between THI 60 and THI 70), and within the range of standard errors (SE) for SM at the extreme end of the THI scale. Due to the reduced dataset for extremely low and extremely high THI, SE of least square means were larger at the extreme ends of the environmental scale. In the case of SM, SE was 0.11% at THI 30, decreased to 0.03% at THI 50, and again increased to 0.08% at THI 70. Gredler et al. (2007) found a slightly negative phenotypic correlation between SM and SV indicating an antagonistic association between both traits for the whole dataset of dual-

purpose Simmental bulls on the phenotypic scale. Following the results from the present study (comparison of Fig. 3 with Fig. 4), a decrease of SV and an associated increase of SM was most obvious under heat stress conditions for adult bulls. This finding was partly verified when using THI measurements in greater distance from the ejaculation date (Suppl. Fig. S1).

For SV, SO, and NSD, we identified an optimal THI range for semen production, i.e. THI 50 to THI 60. This range of optimal THI is in agreement with results for Simmental bulls kept in moderate climates of Austria (Fuerst-Waltl et al., 2006). These authors suggested an optimal ambient temperature for semen production ranging between 5 and 15 °C. The optimal ambient temperature for semen production of AI bulls kept in hot environments of the US was higher (15–21 °C, Parkinson 1987; Taylor et al., 1985), and indicating adaption to the production environment. An impressive example for adaptation is given by Farooq et al. (2013). They found that physical and biochemical semen parameters in Pakistani Zebu bulls adapted to hot environments were in a desired range only during hot seasons. In contrast, detrimental effects of heat stress on semen traits in dairy and beef cattle bulls from commercial populations were most obvious under heat stress scenarios. Such findings were described by Boujenane and Boussaq (2013) for semen production of Holstein bulls housed in Morocco during the hot season, or by Meyerhoeffer et al. (1985) for decreasing percentages of motile and normal spermatozoa of Angus bulls. Biological explanations were given by Setchell (1998), e.g. physiological

limits for the testicular temperature because of increasing oxygen demand.

Critical heat stress periods also included intervals I (interval I=THI calculated from 1 to 11 days before the semen collection date) and IV (interval IV=THI calculated from 29 to 35 days before the semen collection date). Especially for adult bulls, pattern of solutions for semen traits in dependency of THI in the period previous to the ejaculation date reflect result from THI measured at the ejaculation date (Suppl. Fig. S1). Undesired impact of heat stress measured before the ejaculation date on semen production is in agreement with result by Fuerst-Waltl et al. (2006) for dual-purpose cattle. These authors found undesired effects of high ambient temperature during epididymal maturation (=1 to 11 days before the ejaculation date), and during spermatogenesis (=12 to 65 days before the ejaculation date). These authors referred to Dorst (1991), who reported that spermatogenesis and epididymal maturation include a time period of 65 days. Time lagged detrimental heat stress impact of ~2 weeks on semen quality was identified by Meyerhoeffer et al. (1985) for AI sires in the US. In the study by Vogler et al. (1993), spermatogenic processes were more sensitive to heat stress compared to sperm maturation in the epididymis.

3.1.2. Year-season of semen production

Statistical modeling (model 1) simultaneously considered effects of year-season and of THI. Seasonal effects are strongly confounded with temperature and humidity, but additional seasonal characteristics include animal associated components (e.g. changing sexual activity of mammals during the year (Swanson and Herman, 1944)), as well as further environmental descriptors (e.g. length of daylight, fodder components). Despite consideration of THI, the effect of year-season was highly significant ($P < 0.0001$) for all semen traits. When comparing solutions from different seasons for the years 2009, 2010, and 2011, SV and SM were throughout highest in 2010 (Table 3). Such a strong effect of the year might be due the quality of the feeding ratio, which especially depends on the quality of the first cut of grass silage. Grass silage is the major component of the feeding ration of bulls which are kept indoors on AI station throughout the whole year.

Solutions for seasonal effects in our study revealed inconsistencies in different years. This might be due to our statistical modeling by including both environmental effects season and THI, whereas most of the previous "heat stress studies" solely analyzed seasonal impact on semen traits. Provided that bull management and feeding rations are quite constant throughout the year, seasonal impact is considerably explained by variations of temperature and humidity. Further seasonal impact on semen traits uncoupled from THI include changes of daylight. In our study, "Autumn-2011" was identified as a year-season combination with highest values for SO and NSD. Lowest values for SO and NSD in "Autumn-2009" indicate a pronounced effect of the year. During summer and spring seasons 2009 and 2010, SO and NSD were higher compared to semen production levels as achieved in autumn

and winter. Despite the detrimental effects of temperature and humidity on semen characteristics, a multiplicity of studies (van Os et al., 1997; Nichi et al., 2006; Teixeira et al., 2011; Chacur et al., 2013) also reported highest ejaculate volumes of bulls during the summer months. Based on their 31-year retrospective study conducted under moderate climatic conditions in Slovenia, and including four Bos Taurus breeds, Snoj et al. (2013) reported highest ejaculate volumes and highest total sperm output in summer, and lowest values in winter. In our study, SC was not significantly different in different year-season combinations (significantly higher values ($P > 0.05$) only in "autumn-2011"). In analogy, Snoj et al. (2013) also denied relationships between seasons and levels of SC. In 2010 and 2011, SM was lowest in the summer season (Table 3). Argov-Argaman et al. (2013) found almost identical semen production levels (SV and no. of sperms) across seasons, but in their study, SM was higher in winter than in summer. Detrimental effects of summer seasons on semen quality were shown by Teixeira et al. (2011), who investigated sperm morphology and acrosome integrity.

3.1.3. Age of bulls

For all levels of THI, semen production characteristics SV (Fig. 3), SO (Fig. 5), and NSD (Fig. 6) were throughout highest for oldest bulls (>48 month). For all traits in our study (also including SM (Fig. 4) and SC (Fig. 7)), youngest bulls (<12 month) achieved lowest values. Solutions (least square means) for fixed effects of defined "age of sire classes" (Table 3) generally support superiority of older bulls, and reflect results from previous studies (e.g. Boujenane and Boussaq, 2013; Snoj et al., 2013). In the study by Brito et al. (2002), ejaculate volume was strongly related to age classes of bulls, because increasing body mass was correlated with testicular growth. In causality, increase in semen quantity was positively correlated with the number of mature spermatozoa, and with pronounced progressive semen motility (Majić Balić et al., 2012).

Adult bulls are characterized by higher semen productivity than younger bulls, but they reacted with a stronger decline in production levels on increasing THI beyond the identified THI threshold (THI >60). Higher environmental sensitivity for older bulls might be due to the increasing distance between the testicular artery and venous blood with increasing age. Hence, a strong decline in semen quality with increasing THI is explained by reduced heat transfer efficiency between arterial and venous blood, and impaired testicular thermoregulatory ability (Brito et al., 2012). Based on negative correlations between THI and SM, Majić Balić et al. (2012) reported environmental sensitivity to increasing ambient temperatures for young Simmental bulls. These authors identified an intensification of pro-oxidative processes in semen plasma and in spermatozoa from young bulls as a major reason for decreasing sperm progressive motility, along with semen quality deterioration. König et al. (2005a) studied environmental sensitivity of health disorders by parity classes of cows. They identified trait specific reactions: For some

Table 3

Least square means with corresponding standard errors within classes of fixed effects for the following semen traits of AI sires: Semen volume (SV), semen motility (SM), semen output (SO), number of semen doses per ejaculate (NSD), semen concentration (SC).

Effects	Groups	SV ± SE	SM ± SE	SO ± SE	NSD ± SE	SC ± SE
Year-season	Spring-2009	$5.37^a \pm 0.09$	$69.23^a \pm 0.13$	$6.70^a \pm 0.18$	$372.90^a \pm 11.03$	$1.26^a \pm 0.02$
	Spring-2010	$5.59^b \pm 0.07$	$70.09^b \pm 0.11$	$6.80^a \pm 0.15$	$382.22^a \pm 9.28$	$1.25^a \pm 0.02$
	Spring-2011	$5.35^a \pm 0.08$	$68.99^a \pm 0.12$	$6.85^a \pm 0.16$	$366.16^a \pm 9.94$	$1.27^a \pm 0.02$
	Summer-2009	$5.33^a \pm 0.10$	$69.72^a \pm 0.15$	$6.59^a \pm 0.20$	$363.23^a \pm 12.14$	$1.26^a \pm 0.03$
	Summer-2010	$5.62^b \pm 0.09$	$68.98^b \pm 0.13$	$6.78^a \pm 0.17$	$375.72^a \pm 10.74$	$1.24^a \pm 0.02$
	Summer-2011	$5.25^a \pm 0.09$	$68.83^b \pm 0.14$	$6.66^a \pm 0.19$	$364.43^a \pm 11.55$	$1.25^a \pm 0.03$
	Autumn-2009	$5.16^a \pm 0.09$	$69.85^a \pm 0.13$	$6.09^a \pm 0.18$	$335.90^a \pm 11.26$	$1.22^a \pm 0.02$
	Autumn-2010	$5.54^b \pm 0.07$	$69.31^b \pm 0.11$	$6.56^b \pm 0.15$	$367.43^b \pm 9.24$	$1.22^b \pm 0.02$
	Autumn-2011	$5.28^a \pm 0.08$	$69.30^b \pm 0.12$	$7.34^c \pm 0.16$	$414.79^c \pm 10.21$	$1.37^b \pm 0.02$
	Winter-2009	$5.29^a \pm 0.11$	$69.62^a \pm 0.16$	$6.24^a \pm 0.22$	$346.49^a \pm 13.37$	$1.20^a \pm 0.03$
	Winter-2010	$5.56^b \pm 0.12$	$69.60^a \pm 0.18$	$6.54^a \pm 0.24$	$357.83^a \pm 15.02$	$1.22^a \pm 0.03$
	Winter-2011	$5.12^a \pm 0.08$	$69.61^a \pm 0.13$	$6.17^a \pm 0.17$	$337.62^a \pm 10.32$	$1.24^a \pm 0.02$
Age of bull (in months)	≤ 12	$4.11^a \pm 0.10$	$68.44^a \pm 0.15$	$4.25^a \pm 0.19$	$190.34^a \pm 12.07$	$1.13^a \pm 0.03$
	12–17	$4.52^b \pm 0.06$	$69.03^b \pm 0.09$	$5.31^b \pm 0.12$	$246.66^b \pm 7.20$	$1.23^b \pm 0.01$
	18–47	$5.69^c \pm 0.08$	$70.08^c \pm 0.11$	$7.59^c \pm 0.15$	$424.77^c \pm 9.26$	$1.35^c \pm 0.02$
	≥ 48	$7.16^d \pm 0.05$	$70.15^c \pm 0.08$	$9.28^d \pm 0.11$	$599.81^d \pm 6.60$	$1.30^c \pm 0.01$
Interval (in days)	< 3	$4.97^a \pm 0.10$	$69.53^a \pm 0.15$	$5.61^a \pm 0.19$	$302.93^a \pm 12.03$	$1.11^a \pm 0.02$
	≥ 3	$5.77^b \pm 0.03$	$69.32^a \pm 0.04$	$7.60^b \pm 0.06$	$427.86^b \pm 3.74$	$1.32^b \pm 0.01$

Different superscripts [a,b] denote significant differences ($P < 0.05$) within blocks of effects (blocks are separated by horizontal lines).

traits, incidences decreased with increasing parity (effects of resistance, adaptation, and also of selection), but for other traits, opposite results were found.

3.1.4. Intervals between semen collection dates

Apart from SM, the interval between consecutive semen collection dates was highly significant ($P < 0.0001$) for all semen traits. Traits SV, SO, NSD and SC achieved higher values with longer intervals (more than 3 days) compared to a shortened time period (less than 3 days; Table 3). Results are in accordance with studies by Everett et al. (1978), Everett and Bean (1982), Mathevon et al. (1998a), Fuerst-Waltl et al. (2006), Karoui et al. (2011), and Boujenane and Boussaq (2013). These authors found increased ejaculate volume, sperm concentrations, and number of spermatozoa per ejaculate with an increasing time span between consecutive semen production dates.

3.2. Genetic impact on semen traits

3.2.1. Heritabilities of semen traits in dependency of THI

Genetic parameters for semen traits as listed in Table 1 are mostly the outcome from univariate or multiple trait models. An RRM with Legendre polynomials was applied by Carabaño et al. (2007), who studied the genetic trajectory of ejaculate volume by alterations of a time dependent covariate (age at semen collection). Their statistical modelling focussed on different polynomial structures, i.e. Legendre polynmomials of order 3 to order 6 for both animal genetic and permanent evnironmental effects, but heritablities from different models were almost identical at identical collection dates. In their study, heritabilities for ejaculate volume were highest at the extreme ends of the time scale. This finding is in line with results from our present study showing highest heritabilities for semen traits SV, SM, SO, NSD, and SC at the extreme ends of the coninuous THI scale (Fig. 8). However, extremely high additive-genetic variances and heritabilities at the extreme ends of the environmental
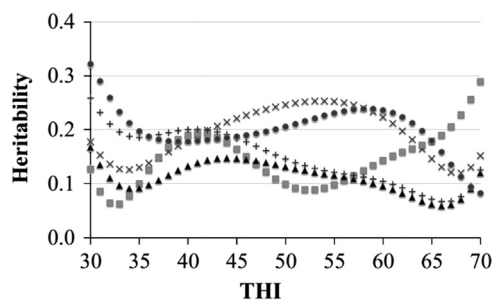

Fig. 8. Heritabilities for semen volume (▲), semen motility (■), semen output (+), number of semen doses per ejaculate (●) and semen concentration (×) by THI measured at the ejaculation date.

scale might be attributed to the artefacts of the chosen polynomial structure for random regression coefficients, and to the reduced dataset for minmal and maximal THI. Carabaño et al. (2007) emphasised a careful interpretation of genetic parameter estimates from RRM at the beginning and at the end of the time scale, being of importance especially for small datasets. Such an artefact of RRM, i.e. a deviation of genetic parameters at the extremes of the continuous scale, was described and proven in previous studies for female fertility traits (e.g. Yin et al., 2012). A second explanation for increasing genetic variances and heritabilities at the ends of the environmental parameter space is given by Schierenbeck et al. (2011). They explained those findings with a pronounced genetic differentiation of functional traits in harsh environments.

To our knowledge, the present study is a first genetic study which aims at modelling the genetic trajectory of semen traits in dependency of an environmental descriptor (here: THI). For genetic analyes, only THI from the ejaculation date was used as continuous environmental descriptor by neglecting THI measurements from time intervals in greater distance. Changes of genetic parameters for semen traits by THI indicate alterations of gene

expressions with changes of temperatue and humidty, as proven on a molecular genetic level for heat stressed male mice (Cammack et al., 2009). Heritabilities for semen quantity and semen quality traits in temperate zones (Fig. 8) reflect estimates from previous genetic analyses using univariate or multiple trait models (as summarized in Table 1). For example, heritabilities for SV in our study were quite constant (0.10 to 0.15) in the comfort zone between THI 35 and THI 60. Similar results were found for large datasets from the US (Taylor et al., 1985), from Austria (Gredler et al., 2007), or from Spain (Karoui et al., 2011). Pattern of heritability curves for SV and SO were almost identical (Fig. 8), and illustrate the close relationship between both semen quantity traits. The semen trait in our study showing major fluctuations of heritabilities during THI was SM (Fig. 8). For SM, maximal heritabilities were achieved at the extreme ends of the THI scale and at THI 43 ($h^2 = 0.20$), whereas minimal heritabilities ($h^2 < 0.10$) were identified between THI 33 and THI 54. Also across studies comparisons (Table 1) exhibit substantial variations of heritabilities for SM, e.g. from 0.01 (Mathevon et al., 1998a) to 0.51 (Diarra et al., 1997). Interestingly, both studies were conducted using data from Canadian Holstein bulls. Gredler et al. (2007) stated that variations of genetic parameter estimates in the same traits from different studies are related to effects of the breed, to effects of the age, and to the statistical modeling. Mathevon et al. (1998b) also reported impact of bull age on heritabilities, on repeatabilities, and on variances for SV and SO of French Montbéliard bulls. In their study, lowest heritabilities were found for youngest bulls. Maxima of heritability curves for SC and NSD were found in close distance to the THI threshold (THI 60, Fig. 8). The highest heritability was 0.27 for SC at THI 53, and 0.26 for NSD at THI 58. For both traits, genetic parameter estimates are in line with results from previous studies (Table 1).

Heritabilities for semen traits larger than 0.10 justify selection strategies with the overall aim to improve bull fertility. Inclusion of semen traits into breeding goals was suggested by e.g. Fitzpatrick et al. (2002) for Santa Gertrudis bulls in Australia. Semen traits are routinely recorded in laboratories of AI stations, and the existing infrastructure can be used for large-scale phenotyping. Also pedigrees of AI bulls are well and deep documented, whereas missing or wrong identifications are mostly relevant for cows from commercial production herds (e.g. Sanders et al., 2006). Nevertheless, inclusion of semen traits into overall breeding goals imply availability of genetic covariance components with other traits of interest (especially with production and functional traits of current breeding goals). Hence, we suggest an extension of univariate RRM to multiple trait RRM in ongoing studies. Furthermore (pre-conditioning high genetic correlations), estimated breeding values for semen traits of young bulls can be used as early predictors for genetic values of male fertility traits as used in official national and international genetic evaluations (e.g. paternal non-return rates). Gredler et al. (2007) estimated correlations between estimated breeding values for semen quality traits with estimated breeding values for official male fertility traits, but correlations were in a low to moderate range (0.08–0.17).
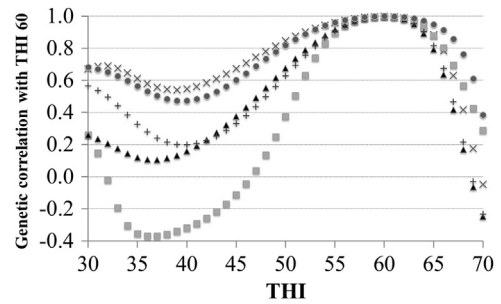

Fig. 9. Genetic correlations for semen traits at THI 60 with corresponding semen traits in dependency of THI: Semen volume (▲), semen motility (■), semen output (+), number of semen doses per ejaculate (●) and semen concentration (×).

Nevertheless, also application of univariate RRM for semen quantity and semen quality traits allow optimization of genetic selection via selection of appropriate sires adapted to specific environments. As outlined by Yin et al. (2014), economic competitiveness of German dairy cattle breeding organizations strongly depends on exports of livestock and sire semen, especially in countries located in tropical climates in Asia, Africa, and South America. Availability of "THI specific" estimated breeding values for semen traits enables selection of superior genetics for specific climate conditions of the importing country.

3.2.2. Genetic correlations in same semen traits for different THI combinations

Classically, the proof of possible genotype by environment interactions in dairy cattle is based on genetic correlation estimates from multiple trait models, where observations for a given trait are defined as different traits in different environments. Such a "research design", i.e. availability of progeny records from same sires in different environments, is given in dairy cattle through the widespread AI structure. Following Robertson (1959), genetic correlations in same traits between different environments lower than 0.80 indicate genotype by environment interactions, and suggest re-rankings of sires in different environments. König et al. (2005b) favored the application of multiple trait models for studies focusing on distinct environmental classes, e.g. different regions, countries, or production systems. In contrast, for continuous environmental descriptors (e.g. THI), applications of RRM to explain performances gradually over a range of environments, were suggested. Also form a statistical point of view (a fewer number of parameters need to be estimated), the concept of reaction norms as applied by Kolmodin et al. (2002) for production traits is a promising alternative.

RRM applications allow to infer genetic correlations in same traits between all possible combinations of environmental descriptors. From the whole grid of pairwise estimates, we focused on genetic correlations between semen traits at THI 60 with identical semen traits from the whole THI range (Fig. 9). For all semen traits, genetic correlations were $r_g \sim 0.80$ for THI levels in close distance, i.e. for THI ranging between 58 and 61. Genetic correlations continuously decreased with increasing distances between THI 60 and remaining THI. THI 60 was the identified THI threshold at the phenotypic scale. Apart from SM, genetic correlations

between THI 60 with THI > 60 decreased substantially. Opposite genetic correlations for SM support findings on the phenotypic scale, where, in contrast to remaining semen traits, a stagnation or even slight increase of SM beyond THI 60 was observed (Fig. 4). Generally, indications for genotype by environment interactions at high THI are larger for traits reflecting semen quantity when compared to sperm quality (SM). As a further particularity of SM in comparison to other traits, also additive genetic variances and heritabilities continuously increased beyond the heat stress threshold (Fig. 8). With regard to genetic effects, also Karoui et al. (2011) found particularities for SM, i.e. substantially lower heritabilities compared to semen volume, concentration, and number of spermatozoa per ejaculate. In the present study, lowest genetic correlations were found for SM from THI 60 with SM from THI < 35. Especially the negative genetic correlation ($r_g = -0.18$) between SM from THI 60 with SM from THI 33 indicate genotype by environment interactions, and substantial re-rankings of sires in different climatic conditions. Again, interpretation of results should consider artefacts of the polynomial structure, and the reduced dataset at the extreme ends of the environmental scale.

Indications for genotype by environment interactions for semen traits are in agreement with environmental sensitivity for female fertility traits (Brügemann et al., 2013). However, for similar statistical RRM, same breeds and same regions, a focus on test-day production traits revealed genetic correlations throughout higher than 0.90 for all possible THI combinations (Brügemann et al., 2011). Also for multiple trait models and production traits, limited evidence for genotype by environment interactions were found (e.g. König et al., 2005b). Hence, strong environmental sensitivity for male and female fertility traits encourage ongoing studies addressing the topic of genotype by environment interactions on the genomic scale. Routinely large scale genotyping of AI sires allows application of genomic RRM (as introduced by Yin et al. (2014) for cow traits in dependency of THI) to semen traits. In contrast to traits expressed only in female progeny (e.g. protein yield), phenotypes of semen traits can be directly assigned to genetic markers of bulls, and directly related to their environmental descriptors.

4. Conclusions

Analyses of semen quantity traits SV, SO and NSD revealed environmental sensitivity for temperature × humidity combinations which indicate heat stress in dairy cattle. A general heat stress threshold of THI 60 showed a strong decline in semen productivity. Semen motility was partly affected by increasing THI, and different reactions were observed for different age classes of bulls. A change of additive genetic variances and heritabilities with alterations of THI was found for all traits. Strong indications for genotype by environment interactions were identified for semen quantity traits. RRM applications to semen traits allow to estimate THI specific breeding values, and to select sires adapted to harsh environments.

Conflicts of interest

We confirm that we have no conflicts of interest.

Acknowledgements

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.livsci.2015.04.003.

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