Chymosin Variants In Different Cattle Breeds And Their Influence On Growth Traits In Consideration Of κ-casein Variants

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Introduction

Chymosin (EC 3.4.23.4) is one of the major enzymes in the fourth stomach of the unweaned calf (Foltmann, 1970), where it accounts for approx. 80% of the total proteolytic activity. Synthesis of chymosin in the stomach mucosal tissue occurs only during the neonatal period, which is followed by synthesis of pepsin as the predominant aspartyl protease in the gastric juice of adult cattle (Foltmann *et al.,* 1979). Kappa casein (κ-CN) is the unique casein fraction affected by chymosin during the primary phase of the milk clotting process. At the beginning of the enzyme reaction a Phe-Met linkage (Phe^{105}-Met^{106}) is specifically split into the insoluble para- κ-CN (amino acid 1-105) and the soluble caseinomacropeptide (amino acid 106-171). This is an essential process for the nutrition of the suckling calf but also for the production of cheese (Mercier *et al.,* 1973).

It is well known that 14 kappa casein (*CSN3*) variants exist within the κ-CN in the *Bos* genus (Caroli *et al.,* 2009). These genetic variants of κ-CN, caused by amino acid exchanges are associated with micelle size, micelle stability, milk casein and κ-CN concentrations, renneting time, quality of cheese curds and therefore with cheesemaking properties. For example milk with the κ-CN B coagulates significantly faster than milk with κ-CN A (Losi *et al.* 1973). Less common *CSN3* alleles such as *CSN3*E* within Italian Friesian or *CSN3*G* within Pinzgauer are also associated with more unfavorable coagulation properties than *CSN3*A* (Caroli *et al*., 2000, Erhardt *et al.,* 1997). Jakob (1994) reported a difference in the chymosin hydrolysis rate of the κ-CN C variant as opposed to the A and B variants. This was confirmed by Plowman *et al*., (1997) demonstrating that the interaction between Arg 97 (replaced by His in κ-CN C) and Asp 249 stabilize the enzyme–substrate complex and account for the higher rate of hydrolysis of κ-CN A over C.

An aminoacid exchange (Gly-Asp 286) within the prochymosin gene (Harris *et al.,* 1982) results in the two variants A and B of chymosin. Data about the occurrence and frequency as well as differences in the activity of the two chymosin variants are rare.

The aim of this study is to estimate the chymosin allele frequencies in different cattle breeds and to analyze the influence of chymosin variants in suckling calves on growth traits depending on the different κ-CN variants of their dam.

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Material and methods

Genotyping.
Genomic DNA from different cattle breeds (table 1) was used for DNA analyses. Forward and reverse primer within the flanking regions of Exon 7 (Genbank No. NM_180994.1) were designed using Primer 3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) to verify the SNP causing the A and B variant of chymosin. The analyzed SNP in this study is located in exon 7 of the prochymosin gene. This SNP was chosen because exon 7 is not affected by the splicing process described by Zinovieva *et al.* (2002) that causes multiple isoforms. A PCR was performed with the primers CHYM_exVII_F = 5´-GAGGCTAGAAGGGGTCCAAG-3´ and CHYM_exVII_R = 5´-GGTTCCCTCTCCAGA GGTTC-3´ to amplify a 270 bp product containing the SNP. The volume of each PCR reaction was 25 µl which included 50 to 10 ng genomic DNA, 2.5 µl of 10x Taq Bufffer advanced (5 prime, Hamburg, Germany), 200 µM of each dNTP, 0.8 µM of each primer and 1 U of Taq DNA polymerase (5 prime, Hamburg, Germany). Cycling was performed in a thermocycler (iCycler, BioRad, Munich, Germany) with the following cycles: 94°C for 90 sec, followed by 30 cycles of 94°C for 30 sec, annealing at 64°C for 30 sec, and elongation at 72°C for 45 sec and a final elongation at 72°C for 7 min. The PCR product was digested with the restriction endonuclease *Taa*I (Fermentas, St. Leon-Roth, Germany) resulting in fragment lengths of 215bp and 55bp for allele B while allele A was not cut. The fragments were separated on a 3.5% agarose gel.
Kappa-casein phenotyping was done using milk samples according to Erhardt (1989).

Animals.
We selected dams with three to five progenies within the years 1998 to 2002 from the two beef cattle breeds German Angus (n=41) and German Simmental (n=29). From these dams 183 German Angus and 125 German Simmental calves were recorded at the Experimental Farm of the Institute of Animal Breeding and Genetics of the Justus Liebig University Gießen in Rudlos, Germany.

Growth traits.
Birth weight (BWT) and weaning weight (WWT) was recorded for all calves within 24 h after birth and at weaning. The daily gain from birth to weaning was calculated as (WWT-BWT)/(age at weaning).

Statistical analyses.
Allele frequencies have been calculated for the polymorphism determined by direct counting. A variance component analysis was done using SAS (version 9) with the following linear model for each breed separately:

$$y_{ijklm} = \mu + ZJ_i + SEX_j + CSN3_k + CHYM_l + (CSN3*CHYM)_{kl} + e_{ijkl}$$

Where y_{ijklm} is the phenotypic observation (daily gain from birth to weaning, age and weight at weaning), μ is the overall mean, ZJ_i and SEX_j are the fixed effects of the breeding year i and the sex j, respectively. $CSN3_k$ is the fixed effect of the kappa casein genotype of the dams and $CHYM_l$ is the fixed effect of the chymosin genotype l of their calves. The age and weight at weaning were included as covariate in the model for weight and age at weaning.

Results and discussion

A DNA test using the PCR-RFLP technique was developed for the SNP in exon 7 of the prochymosin gene determining variant A or B of chymosin. The estimated chymosin allele

frequencies in different European cattle breeds are shown in table 2. In each breed both alleles A and B were detected and allele B was predominant in all breeds except for Pinzgauer and Charolais. A general difference in the allele frequencies between beef cattle and dairy cattle breeds could not be verified. Remarkable is the higher frequency of allele A in German Angus (0.29) compared to Aberdeen Angus (0.01). This could be a result of the breed history of German Angus, which was established in the 1960s by crossbreeding Aberdeen Angus with German dual purpose cattle breeds like Gelbvieh and German Simmental.

Table 1: Allele frequencies of chymosin in different cattle breeds

Breed		n	Allele frequency	
			A	B
Beef cattle	Aberdeen Angus	38	0.01	0.99
	German Angus	40	0.29	0.71
	Charolais	14	0.68	0.32
Dual purpose cattle	Gelbvieh	31	0.32	0.68
	German Simmental	51	0.46	0.54
	Pinzgauer	25	0.52	0.48
Dairy cattle	Angler	43	0.32	0.68
	German Holstein	32	0.36	0.64

The chymosin allele frequency for the calves within this study of German Angus (A: 0.29; B: 0.71) and German Simmental (A: 0.50; B: 0.50) were similar to the results for these breeds in table 1. The distribution of chymosin genotypes of calves within the *CSN3* genotypes of dams for both breeds is shown in table 2. Within every occurring *CSN3* genotype of the dams every chymosin genotype was detected in their offsprings.

Table 2: Number of calves with different chymosin genotypes within the *CSN3* genotypes of the dams (number of dams) for German Angus (GA) and German Simmental (GS)

CSN3 genotype dams	Chymosin genotype calves					
	AA		AB		BB	
	GA	GS	GA	GS	GA	GS
AA (GA n= 10; GS n = 6)	2	9	21	13	19	7
AB (GA n=11; GS n= 20)	2	19	24	51	27	18
BB (GA n= 8; GS n= 3)	3	1	21	4	16	3
AE (GA n=12)	4	-	18	-	26	-

Table 3 shows means and standard deviations for the analyzed growth traits for German Angus and German Simmental calves. Both breeds are weaned at an average age of 216 to 218 days of age. As expected German Angus showed lower weaning weights and in consequence lower daily gain than German Simmental. In agreement with previous studies

(Brandt *et al.*, 2010) the year of breeding and the sex of calf showed the highly significant effects on all traits as well as the covariates age and weight at weaning (results not shown). No significant effects on daily gain from birth to weaning, weaning age and weaning weight were observed neither between *CSN3* genotypes of dams nor between chymosin genotypes of their calves. The interaction between *CSN3* genotypes of dams and the chymosin genotypes of calves was also not significant for all traits analyzed. From this we have to conclude that calves with varying chymosin genotypes within dams with different *CSN3* genotypes have no advantage in growth. The growth of calves in this study to the age of more than 200 days is probably too late to detect a possible chymosin effect because of decreasing impact of the dams' milk on growth with increasing age.

Table 3: Means ($\bar{x}$) and standard deviations (SD) for growth traits of calves

	German Angus (n=183)		German Simmental (n=125)	
	$\bar{x}$	SD	$\bar{x}$	SD
Daily gain from birth to weaning (g)	926	151.5	1108	195.4
Weaning age (day)	218	18.8	216	15.9
Weaning weight (kg)	238	31.4	283	43.7

Only three (*CSN3*A*, *B* and *E*) of the 14 described κ-CN alleles occurred within the animals analyzed in this study (table 2), the influence of chymosin variants on other variants should be the subject of ongoing studies. Furthermore it is possible that isoforms of chymosin (Zinovieva *et al.*, 2002) have an effect on the coagulation of milk with particular κ-CN variants.

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