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Prochymosin Variants in the Abomasa of Calves and Milk Protein Variants of Their Mothers

Warianty prochymozyny w żołądkach cieląt i warianty mleka białkowego u ich matek

In natural conditions milk secreted by mother serves to nourish the newborn. Its stomach is not only a reservoir but it secretes large amounts of proteases which participate in digestion of proteins. Prochymosin (previously called prorennin), the precursor of chymosin (EC 3.4.23.4), is the predominant aspartic protease in the abomasa of newborn calves. Previous investigations of individual calf stomach showed three prochymosin variants (3, 19), and recently four prochymosins which differed in electrophoretical mobility (20). These prochymosins occurred singly and in pairs being controlled by four codominant alleles.

Principal constituents of milk are caseins and whey proteins. The caseins are: αS_1-casein, αS_2-casein, β-casein and κ-casein while α-lactalbumin, β-lactoglobulin, blood serum proteins and a small amount of immunoglobulins constitute major proteins of whey (10,13). Caseins and β-lactoglobulins in all cattle breeds occur in multiple molecular forms or genetic variants (9, 12). The alleles of the given locus of milk proteins are codominants.

Calf chymosin is largely known as milk-clotting enzyme which is utilized for cheese production and today for this purpose chymosin is produced by biotechnological techniques (11, 17). There is a contrast between the very rich literature dealing with clotting of milk for cheese production and our ignorance of the physiological importance of chymosin and its nutritional significance for the newborn (7). It is not known if any relationship exists between milk protein and chymosin variants. Possibly, polymorphisms of milk proteins and prochymosins has the same genetic background. Because of lack of information on this subject we found it useful to screen the prochymosin kinds in the abomasa of calves and the genetic milk protein variants of their mothers as a principal and natural nutrient.

MATERIALS AND METHODS

Specimens of abomasal mucosa (3 to 5 g) from 110 suckling 3- to 6-week-old calves of both sexes of Black-and-White cattle were randomly collected on slaughtering. Extract preparation and determination of the presence of active enzymes in crude extracts to exclude the possibility of errors in

sample identifications were described previously (19). All crude extracts showed, from 2% to 10% of potential clotting activity which had no effect on the identification of prochymosin variants.

Prochymosin variants were determined in individual calves using agarose gel electrophoresis followed by detection of proteolytical activity. Electrophoresis was carried out in 1% agarose gel (Serva, Heidelberg, Germany) containing 1 mg of casein (Sigma, Chemical Co., St. Louis, USA) in 1 ml of gel (18) in "mixed" buffer at pH 8.3 (15). Samples of crude extracts and those acidified to pH 4.7 (then both adjusted to pH 6.2) underwent electrophoresis two times. Crude extracts of the abomasum containing prochymosin AB and BC designated according to Foltman (7) and previously identified electrophoretically (19) were used as reference enzymes. From mother cows of calves of which prochymosin variants were examined milk samples were collected and prepared as follows: to 0.9 ml of freshly taken milk 0.1 ml of 0.2% sodium azide was immediately added as preservative. Milk samples were freeze-dried in ampulles and sent to the Institute of Husbandry and Animal Genetics in Giessen (Germany) for milk protein phenotyping by the isoelectric focusing method (15).

Statistics. Frequencies of prochymosins and the major milk protein variants were examined by χ^2 test for agreement with Hardy-Weinberg law. Relationships between prochymosins in calves and milk protein variants were analysed by proper $\chi 2$ test (22).

RESULTS AND DISCUSSION

Four prochymosin (Pch) variants which differed in elelctrophoretical mobilities were present in the examined calves. These variants were designated as Pch A, D, B and C described earlier (20), with regard to their decreasing relative mobility. The relative mobilities of these variants in agarose gel electrophoresis are shown in Fig. 1. Mutual occurrence of Pch variants was observed in eight phenotypes. The variants which occurred singly were considered as homozygotes and those which occurred in pairs with equal proteolytic activity of both components – as heterozygotes.

The distribution of the observed and expected number of Pch phenotypes in the examined calves is presented in Tab. 1. The results show that their distribution is in accordance with the Hardy-Weinberg law. This is in agreement with our earlier results (20). Low frequency of Pch C in this and earlier studies (19, 20) may suggest that the variant has a deleterious effect on calf survival, or that it is linked to an undesirable locus and is eliminated during selection.

The analysed milk protein variants from the mother-cows of the examined calves are seen in Tab. 1. No significant differences between the observed and expected numbers of milk protein variants showed that these phenotype frequencies were in genetic equilibrium. The genetic structure of analysed milk proteins was similar to that described previously for Black-and-White cattle (14, 17), except for some rare $\varkappa$-casein E present as heterozygotes AE and BE. Casein α-S_1 C was present in seven BC heterozygotes: three of them were associated with β-cn A_1A_3 and the remaining three with A_1A_2 and one with A_2. However, α-S_1BC heterozygotes were in part associated, with and this was discordant with the result described (1), α-S_1-cn C and β-cn A_3.

Tab. 1. Observed and expected frequencies of prochymosin phenotyopes in calves and milk protein variants of their mothers

			Phenotypes													
prochymosin	number obs.	exp.	kappa-casein					beta-casein						beta-lactoglobulin		
			A	AB	B	AE	BE	A_1	A_2	A_1A_2	A_1A_3	A_1B	A_2B	A	AB	B
AA	13	10.8	5	7	0	1	0	5	1	5	0	1	1	1	10	2
AD	10	8.0	6	2	0	1	1	5	1	3	0	0	1	1	2	7
DD	3	–	2	1	0	0	0	0	1	2	0	0	0	0	1	2
AB	33	34.0	15	14	3	0	1	11	5	11	2	3	1	6	12	15
BD	10	12.7	3	6	0	0	1	5	1	2	0	2	0	2	7	1
CD	5	–	3	0	1	0	1	2	0	2	0	1	0	2	2	1
BB	33	27.0	18	11	1	3	0	14	4	11	1	2	1	4	18	11
BC	3	–	0	2	0	1	0	2	1	0	0	0	0	0	2	1
observed			52	43	5	6	4	44	14	36	3	9	4	16	54	40
expected			53.5	40.0		7.0		42.0	10.5	42.0		8.0		17.0	52.0	40.3
$Chi_0^2 = 3.01$ $Chi_{0.05}^2 = 11.07$ d.f. = 5 ($p < 0.05$)			$Chi_0^2 = 2.49$ $Chi_{0.05}^2 = 9.40$ d.f. = 4 ($p < 0.05$)					$Chi_0^2 = 3.01$ $Chi_{0.05}^2 = 11.07$ d.f. = 5 ($p < 0.05$)						$Chi_0^2 = 0.125$ $Chi_{0.05}^2 = 6.00$ d.f. = 2 ($p < 0.05$)		

Analysis was made for number of phenotypes equal and higher than 6.

By appropriate χ^2 analysis the relationship between Pch variants in calves and κ-, β-cns and β-lactoglobulins in milk of cows has been shown (Tab. 2). Such state is not unusual because half of the codominant alleles of these proteins in the calves were the same as in their mother-cows.

The results in Table 1 show that when 33 calves had Pch AB in the milk of their 15 mothers κ-cn A, in 11 βcn A_1 and in 15 β-lg B occurred. Pch BB having a similar frequency (33 cases) coincided in their mothers mostly with κ-cn A in 18 cases, β-cn A_1 in 14 cases and β-lg AB in 18 cases. The frequencies of Pch AA and BD were three times smaller than those mentioned above. These phenotypes were mostly associated with κ-cn AB, β-cn A_1 and β-lg AB. Another association between Pch AD, both caseins and β lg was observed. In this case Pch AD occurred in those calves whose mothers' milk contained κ-cn A, β-cn A_1 and β-lg B.

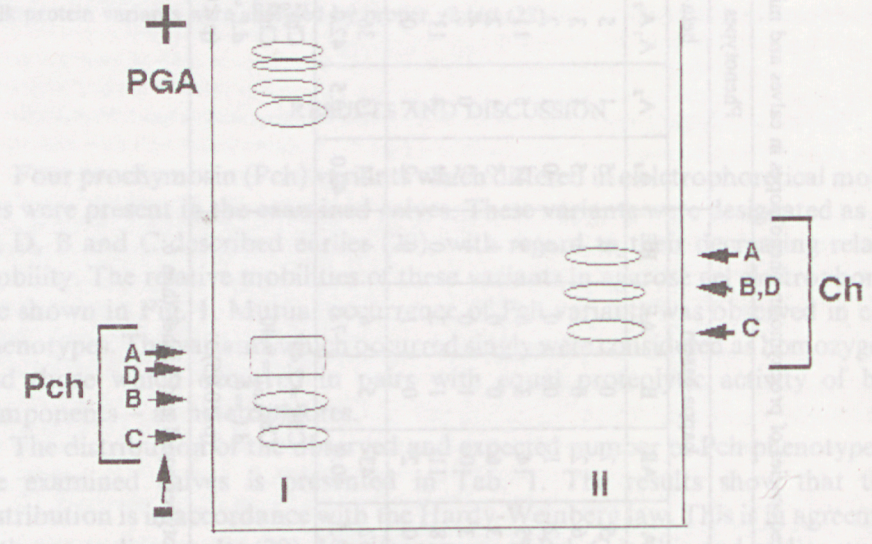

Fig. 1. Scheme of proteolytic fractions following agarose gel electrophoresis at pH 8.3 showing relative mobilities of prochymosin (I) and corresponding chymosin variants (II) which occurred in calf abomasa (PGA – pepsinogen A isozymes: Pch – prochymosin, Ch – chymosin)

On the basis of the results obtained in this study it is not possible to say exactly which kind of relationship exists between Pch and milk protein variants. For this reason further studies are needed which should be aimed at determining both protein polymorphisms in the same animals obtained by their special crossing. Family data concerning Pch variants were not known and it was impossible to confirm their mode of inheritance. Also the milk protein variants in the examined calves were not estimated, therefore it was not possible to calculate the correlation coefficient and regression equation between Pchs and milk proteins.

From the results obtained we know what milk protein variants have been digested by Pch types which were present in the abomasa of the calves.

Considerable attention has been focused on milk protein polymorphism and its effect on milk yield and the composition of milk proteins and cheese making properties. The significance of the prochymosin variants in digestion of milk proteins is still unknown. This is probably due to lack of a suitable method for differentiation of Pch types.

Tab. 2. Relation between prochymosin types in calves and milk proteins variants in their cow-mothers (n = 110)

Types of prochymosin	AA, AD, AB, BD, BB	AA, AD, AB, BD, BB	AA, AD, AB, BD, BB
Statistic value	χ_0^2	$\chi_{0.02}^2$	$\chi_{0.98}^2$
β-caseins	4.66	24.05	4.178
ϰ-caseins	4.63	11.67	0.429
β-lactoglobulins	14.61	18.17	2.03

($p < 0.05$).

The Pch variants may be related to inheritance of milk production traits and its composition. We can suppose that a relationship exists between Pch types and their effectiveness. The specific milk-clotting activity of chymosin A is higher than that of chymosin B and C (7). The activation of Pch C at pH 5 is completed in a shorter time than that of other components (2). The clotting mechanism includes splitting of the Phe-Met bond in all ϰ-casein variants. According to F o x (9) the sequence around this bond rather than the bond itself contains the important determinants of hydrolysis. Milk proteins not only provide amino--acids for the young, but the products of protein digestion are a source of biologically active peptides (6, 21). Further studies are required to use the molecular biological techniques by means of DNA analysis (13) which is currently used for determination of milk protein gentypes at any age of animal. These methods should be adapted to Pch locus analysis which permits determination of prochymosin genotypes. Attempts, using restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR) methods, were made but without detecting Pch variants (11).

CONCLUSIONS

1. Eight prochymosin phenotypes were observed in 110 examined calves: the most frequent among them were Pch AB and BB in each occurring in 33 calves.

2. The most frequent protein phenotypes, in the dams milk were: ϰ-cn A and AB in 52 and 43 cows respectively; β-cn A_1 and A_1A_2 in 44 and 36 cows; β-lg AB and B in 54 and 40 cows respectively.

3. The distribution of prochymosins in calves and analysed milk protein variants from the mothers occurred in genetical equilibrium.

4. The relationship between prochymosin variants in calves and κ-cn, β-cn and β-lg variants in their dams milk has been demonstrated.

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STRESZCZENIE

Badano związek między rodzajami prochymozyny występującymi w trawieńcu cieląt i genetycznymi wariantami głównych białek w mleku ich matek. Skrawki błony śluzowej trawieńca od 110 cieląt obojga płci i wieku 3-6 tygodni rasy ncb pobierano podczas uboju. Fenotypy prochymozyny (Pch) określano przy pomocy elektroforezy w żelu agarozowym. Białka mleka matek określano metodą ogniskowania izoelektrycznego. U badanych cieląt stwierdzono 4 warianty Pch: A, D, B i C, nazwane zgodnie z ich malejącą migracją elektroforetyczną. Występowały one w 8 fenotypach. Ich dystrybucja w populacji badanych cieląt zgodna była z prawem Hardy-Weinberga. Prochymozynie AB występującej u 33 cieląt odpowiadały: u 15 matek κ-cn A, u 11 β-cn A_1 i u 15 matek β-lg B. Obecna również u 33 cieląt Pch BB związana była z występowaniem w mleku 18 matek κ-cn A, 14 matek β-cn A_1 i u 18 β-lg AB. Częstość Pch AA i BD była trzykrotnie mniejsza w porównaniu z typami AB i BB. Warianty te związane były najczęściej z występowaniem w mleku matek κ-cn AB, β-cn A_1 i β-lg AB. W przypadku pojawienia się Pch AD w mleku matek stwierdzono zwykle κ-cn A, β-cn A_1 i β-lg B. Otrzymane wyniki świadczą o istnieniu związku między rodzajami Pch w trawieńcu cieląt i wariantami genetycznymi białek mleka u matek.