**Heterogeneity in Whey Proteins of Mare's Milk**

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**ABSTRACT**

The possible existence of multiple forms in the whey proteins of mare's milk was investigated. When individual milk samples from over 300 animals of various breeds were examined, four forms of an undescribed whey protein could be observed. Based on chemical properties and electrophoretic behavior, this protein has been identified tentatively as Whey1 (Wh1). A single case of heterogeneity in mare's α-lactalbumin also was observed. Previously described variation in β-lactoglobulin could not be confirmed. The results of this study provide further evidence of the widespread, perhaps universal, occurrence of polymorphism in milk proteins.

**INTRODUCTION**

Heterogeneity in whey proteins has been observed in the milk of numerous species. Genetically controlled variation has been described in β-lactoglobulin of cattle (11), sheep (9), pigs (6, 7), and humans (4). α-Lactalbumin heterogeneity has been reported in cattle (3), sheep (14), goats (14), and pigs (1, 14). As a result of the widespread interest in polymorphic proteins and the absence of such studies in the horse, this investigation was to determine the variation of whey protein in mare's milk. While this work was in progress, Máchová and Nováčková (10) reported a two allele system controlling variation in mare's β-lactoglobulin.

**MATERIALS AND METHODS**

Samples of skim milk which had been collected at various stages of lactation and stored at −20 °C were separated by electrophoresis in a modified version of the discontinuous polyacrylamide gel system described by Kiddy et al. (8). To identify positively all of the proteins, the amount of electrode buffer used in each sample was reduced to .5 ml and the sample application increased to 50 μl in 5.0 mm slots. This modification resulted in a high concentration of certain proteins which can be seen in the photographs. To insure that all the common milk protein types would be found, samples were collected from 353 animals representing 22 locally available breeds.

**RESULTS AND DISCUSSION**

Electrophoretic resolution of the whey fraction of mare's milk showed three distinct proteins, two of which varied in individual animals. Typical results of the procedure are in Fig. 1. A fast moving protein, which shows individual variation and is in a low concentration, cannot be identified precisely at this time. It tentatively has been called Whey1 (Wh1) on its chemical properties and electrophoretic mobility. All of the other proteins have been identified by reports (2, 12). In anticipation of possible genetic control over this variable protein, letter designations for some of the patterns are in Fig. 1.

Published photographs of mare's milk separated by electrophoretic methods show Wh1. Antila et al. (2) showed the protein but did not identify it. Similarly, the photograph presented by Jenness (5) showed a fast moving pair of bands. These were not identified but appeared similar to what has been designated Wh1 AB in this study. Minieri and Intrieri (12) considered this fast moving protein was serum albumin. This seems unlikely, as extensive studies of equine serum albumin have demonstrated only two common types (15) and a third much rarer type (13). If four relatively common serum types existed in the horse, it is unlikely that they would have been missed by previous investigators. When blood serum and milk obtained from the same animal were separated using the procedure described here, serum albumin had a mobility slower than the protein designated as α-lactalbumin. This tech-
patterns in individual samples of mare's milk and three genetically determined patterns of cow $\beta$-lactoglobulin separated under similar conditions is shown in Fig. 2. The similar electrophoretic mobility of $\beta$-lactoglobulin in the two species is evident. In slot 5 of Fig. 2 is the pattern produced by a sample of mare's milk.

A comparison of the four single banded Wh$_1$ pattern proved unsuitable for separating genetic variants of serum albumin of horse blood.

A comparison of the four single banded Wh$_1$ patterns produced by acid precipitated casein (1) and skim milk (2) from the same animal, providing confirmation of the identification of associated casein and the whey fraction.
colostrum. This sample shows the high concentration of whey proteins expected in colostrum. The variation in mare's β-lactoglobulin recently reported by Mácha and Nováčková (10) could not be confirmed. Their variation was in an acid system (pH 2) while our buffer had a pH of 8.3.

Confirmation of the identification of the associated casein is shown in Fig. 3. The banding pattern of a skim milk sample of Wh1 BD type is compared with the pattern produced by a sample of acid precipitated casein recovered from milk of the same animal. Casein was recovered by precipitating twice at pH 4.3. A slight separation is in the precipitated casein which is not in the skim milk.

Milk from a single animal of the Appaloosa breed showed heterogeneity in the α-lactalbumin fraction. The two banded pattern in this sample is in Fig. 4. No other abnormalities in either the appearance of the animal or its milk sample were noted.

Identification of the protein designated α-lactalbumin in this study has been questioned by Jenness (personal communication). He is of the opinion that the protein identified here as α-lactalbumin is actually serum albumin. He also believes that α-lactalbumin, which he has demonstrated in mare's milk, actually migrates in the area of the associated casein fraction. While this interpretation remains a possibility, we believe that the greatest proportion of available evidence supports the identification in this report.

The variation of mare's milk indicates the possible existence of at least two genetic systems not described in this species. It also provides further evidence that milk protein systems vary in nearly all species adequately studied. This widespread, and perhaps universal, genetic diversity is of concern to population geneticists and may affect future selection of domestic animals.

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REFERENCES


