Protein Composition of Whey from Subclinical Mastitis and Effect of Treatment with Levamisole

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ABSTRACT
Subclinical mastitis caused a rise in the noncasein protein concentration of milk. This mainly was caused by an increase in the concentration of serum albumin and immunoglobulin derived from blood. In most cases, the concentration of the major whey proteins β-lactoglobulin and α-lactalbumin decreased. This decrease can be attributed to both inflammatory damage of mammary secretory tissues and destruction of blood-milk permeability barriers. When used orally, levamisole reduced leukocyte count and intramammary pathogens. Although changes of concentration were least for relative amounts of β-lactoglobulin, serum albumin, and immunoglobulin, we observed enhanced α-lactalbumin production and simultaneous reduction of the total whey protein as mastitis score decreased. In normal quarter milk, the relative amount of immunoglobulin increased with levamisole. This suggests there was increased transport of immunoglobulin to aid udder health.

INTRODUCTION
In dairy countries it appears that up to 50% of cows may be affected by subclinical mastitis (4, 16, 18, 21). Most of the cases persist and depress milk production and lower milk quality (3, 4, 21). As reduced milk yield from cows with subclinical mastitis is responsible for the largest losses (3), it is important to appreciate the influence of subclinical mastitis on composition of milk and to control infections of the udder.

Levamisole, the levo isomer of 2,3,5,6-tetrahydro-6-phenylimidazo(2,1-b)thiazole, has influenced host defense by modulating cell-mediated immune response (1, 6, 7, 13, 17, 30, 33, 35), humoral immunity response (17, 23, 31, 33), and enhancement of macrophage and polymorphonuclear cell function (1, 10, 14, 20, 22, 32, 36). Levamisole has only minor toxicity (34). These immunologically enhancing treatments suggested that the compound might be of value in the control of bovine mastitis. Thus, this investigation was to study relationships between the measure of subclinical mastitis and composition of whey proteins in quarter milks and further to evaluate effectiveness of levamisole immunotherapy for bovine mastitis.

MATERIALS AND METHODS
Collection and Culture of Milk Samples
Quarter foremilk samples from 108 Holstein cows free from clinical signs were collected aseptically and immediately cooled. Isolation and identification of microorganisms in freshly collected milk were by routine culture on blood agar plates (9). Bacteria in milk were enumerated by serial dilution in sterile saline and by plating in Trypticase Soy Agar (BBL).

Mastitis Score
Severity of mastitis was measured on quarter foremilk samples by the PL-Tester (PLT; modified California Mastitis Test; Nippon Zenyaku Kogyo Co. Ltd., Koriyama, Japan) (19). Mastitis reaction was expressed as scores 0, trace (T), 1, 2, and 3, with mean leukocyte counts of 88,000, 350,000, 921,000, 2,073,000, and 3,671,000/ml, respectively (19). Test
results were considered to be mastitis-negative for the score of 0 or T, suspicious for 1, and positive for 2 and 3.

**Levamisole Treatment**

Ripercol-L (Lederle Japan, Tokyo, Japan) containing 10% levamisole hydrochloride was dissolved in water and administered orally 7.5 mg/kg body weight to 20 cows affected by subclinical mastitis.

**Preparation of Whey Fraction**

Whole milk was centrifuged at 1,000 x g for 20 min at 4°C and the skim milk recovered. The whey fraction was obtained by precipitating casein isoelectrically at pH 4.6 with HCl and removing it by centrifugation at 2,000 x g for 20 min at room temperature. The upper layer was assayed for protein content by the method of Lowry et al. (15) and stored frozen at -20°C until needed.

**Polyacrylamide-Gel Electrophoresis**

The whey fraction routinely was subjected to electrophoresis by the disc-gel method. The running gel contained 7.0 g acrylamide and 185 mg \( N,N' \)-methylenbisacrylamide in a 100 ml buffer [46 g tris (hydroxymethyl)-aminomethane + 4 ml HCl/1,000 ml] of pH 8.9, and polymerized by the addition of 70 mg ammonium persulphate and 30 \( \mu l \) \( N,N,N',N' \)-tetramethyl-ethylenediamine. Electrophoresis was with an electrode buffer [.6 g tris (hydroxymethyl)-aminomethane + 2.9 g glycine/100 ml] of pH 8.3 The current was held constant at 6 mA/gel. Following electrophoresis, the gel was stained for 1 h in a 1% (wt/vol) solution of amido black 10B in 7% (vol/vol) acetic acid, and the excess stain was removed by leaving it standing in 7% (vol/vol) acetic acid for 48 h. Gels then were scanned at 620 nm with a recording densitometer equipped with an integragraph integrator.

**RESULTS**

**Protein Composition in Whey from Cows with Subclinical Mastitis**

We observed significant differences in relative concentrations of individual whey protein components when milk samples were grouped into five ranges of leukocyte counts (Table 1). The concentration of each compon-
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Figure 1. Effect of levamisole on the PLT score of quarter milk affected by subclinical mastitis.

ent changed with the increasing PLT score, and these changes became clearer with higher scores.

Diagnosis of subclinical mastitis as estimated by the increase in PLT score was followed by a significant decrease in the major whey proteins, β-lactoglobulin and α-lactalbumin (P<.05). However, total whey protein significantly increased and was accompanied by an increase in the relative amounts of serum albumin and immunoglobulin derived from blood.

Staphylococcus aureus infection caused a significant increase in serum albumin and immunoglobulin and a decrease in β-lactoglobulin and α-lactalbumin despite a PLT score of 0.

Changes in concentrations were least for relative amounts of immunoglobulin. The largest changes occurred for α-lactalbumin.

Efficiency of Levamisole in Treatment of Subclinical Mastitis

Efficacy of levamisole was evaluated for a total of 26 quarters of subclinical mastitis and 35 normal quarters. No adverse reaction from treatment was revealed. The PLT and bacteriological examinations of quarter samples were to substantiate responses to therapy.

Cure rate, defined as PLT score reduction to 0 or 1, was 60% 28 days after treatment (Figure 1). The result of bacteriological examinations (Figure 2) was assessed as “very good” when the invading pathogen was eliminated completely. A “good” response indicated that there was at least a 100-fold decrease in the number of bacteria. Twenty-eight days after treatment, the response was very good in only 8.3% of the mastitic quarters, but a good response was obtained in as many as 66.7% of them. The PLT score was decreased substantially in both of these categories. The score was recorded as “no change” when the pathogen remained without showing a distinct change in the number. A total of 25% of subclinical cases was classified as no change; however, the PLT score of most of these quarters decreased 28 days post-treatment.

Changes in proportion of individual whey proteins and total whey protein are in Figure 3. In the mastitic quarter milks, treatment had little effect on β-lactoglobulin, serum albumin, and immunoglobulin concentrations although there was a moderate increase in α-lactalbumin and a decrease in total whey protein. In normal quarter milks, however, levamisole induced a marked increase in the relative concentration of immunoglobulin (P<.05) by 7 days after treatment, and this content remained elevated even though β-lactoglobulin decreased.

DISCUSSION

Subclinical mastitis caused a rise in non-casein protein concentration of milk. This was mainly caused by increased concentration of serum albumin and immunoglobulin derived from blood. However, concentration of major whey proteins, β-lactoglobulin and α-lactalbumin, decreased in most cases, which agreed with results of previous investigations of milk from cows with clinical and experimentally induced mastitis (2, 8, 9,
Figure 2. Effect of levamisole on bacteriological response of quarter milk affected by subclinical mastitis. VG, very good; G, good; NC, no change but the PLT score decreased (A), or did not decrease (B).

Figure 3. Effect of levamisole on proportion of individual whey proteins and total whey protein of quarter milk: •——•, treated mastitic quarter; o——o, treated normal quarter; ●——●, untreated mastitic quarter.

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21, 26). Effects can be attributed to both inflammatory damage of the mammary secretory tissues and destruction of the blood-milk permeability barriers which restrict and discriminate in transfer of protein from interstitial fluid into milk.

Densitometric quantification of gels revealed considerable variations in relative concentration of individual protein components between quarter samples which showed positive and negative PLT reactions. This variation may have been from differences in severity of inflammation and the nature of the invading pathogen.

When used orally, levamisole was effective for leukocyte count reduction and bacteriological results. Although there was little change in relative amounts of β-lactoglobulin, serum albumin, and immunoglobulin, α-lactalbumin production was enhanced and total whey protein simultaneously was reduced. These findings were evidence that levamisole is of value in treatment of subclinical mastitis.

However, in normal quarter milk, we found a marked increase in relative amounts of immunoglobulin after treatment. This suggests there was increased transport of immunoglobulin to aid udder health.

Levamisole restored the function of phagocytes and T-lymphocytes to normal in immunosuppressed hosts (20, 25, 30, 36); however, B-lymphocytes have not been considered to be influenced directly by this reagent (11, 12, 27, 28). On the other hand, a few reports have described the levamisole-induced increase in number of antibody-forming cells in the spleen (29), and the specific antibody production to the particular bacterial, viral, or cellular immunogen (5, 17, 23, 24, 31, 33). Our results agree with these.

Our observations should prove to be of value in the control of bovine mastitis. Further research is required to determine whether the described phenomena are strictly immunological or aspecific in nature, e.g., mediated through the effect on the phagocytosing capacity of macrophages or neutrophils.

REFERENCES