Effect of Prostaglandin F$_{2\alpha}$ on Growth of *Mycoplasma bovis* Associated with Bovine Mastitis

Ahmadzadeh A$^1$, Fox LK$^2$, McGuire MA$^1$, and Carnahan KG$^1$

$^1$Department of Animal and Veterinary Science, University of Idaho, 875 Perimeter Drive, MS 2330, Moscow, ID 83844, USA

$^2$College of Veterinary Medicine, Washington State University Pullman, WA 99164-6610, USA

**Corresponding author:** Ahmadzadeh A, Department of Animal and Veterinary Science, University of Idaho, 875 Perimeter Drive, MS 2330, Moscow, ID 83844, USA. Tel: 208 885 7409; Fax: 208 885 6420; E-mail: amin@uidaho.edu

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**Abstract**

*Mycoplasma bovis* (*M. bovis*) is a major mastitis pathogen that has been reported to be refractory to antibiotic treatment. Certain fatty acids have been shown to inhibit the growth of mastitis pathogens such as *Staphylococcus aureus* (*S. aureus*). In vitro experiments were conducted to determine the effects of prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) on growth of *M. bovis*. Five strains of *M. bovis* of bovine origin were selected for the study. Two strains were reference strains (ATCC 25025 and 25523) and the other strains were isolated from diseased cattle. Isolates were cultured and suspended in saline to achieve an optical density of 0.2 at 520 nm, a suspension of approximately 1 × 10$^{8}$ CFU/ml. Subsequently, *M. bovis* suspensions were incubated in culture media containing PGF$_{2\alpha}$ (dinoprost tromethamine) at final concentrations of 0 (control), 2, 4, and 8 mg/ml, for 8 h at 37°C. A sample from each treatment group was obtained and cultured on agar plates for 10 d and bacterial growth assessed as CFU/ml. The entire experiment was repeated four times using duplicate tubes per PGF$_{2\alpha}$ concentrations for each strain. Data were analyzed by ANOVA and the model included the effect of treatment, strain, and their interaction. Treatment affected (P<0.01) *M. bovis* growth, and mean CFU/ml decreased with concentrations of PGF$_{2\alpha}$ at 4 and 8 mg/ml but not 2 mg/ml (43.6, 42.1, 24.3, 7.8 [±1.1] for 0, 2, 4, 8 mg/ml, respectively). However, an effect of treatment by strain interaction on mean CFU/ml was detected (P<0.05), indicating that the effect of PGF$_{2\alpha}$ on bacterial growth was not consistent across strains. Overall, the 2 mg/ml PGF$_{2\alpha}$ decreased CFU/ml in only one strain compared with control, whereas 4 and 8 mg/ml PGF$_{2\alpha}$ decreased CFU/ml in all strains compared with control. These *in vitro* results provide evidence, for the first time, that the fatty acid PGF$_{2\alpha}$, in the form of dinoprost tromethamine, has inhibitory effects on growth of *M. bovis*, and this bacteriostatic effect appears to be strain and dose dependent.

**Keywords:** *Mycoplasma*, Fatty acids; Prostaglandin F$_{2\alpha}$; Bacteriostatic

**Introduction**

Mastitis causes the greatest economic loss to the dairy industry. It has been estimated that costs associated with mastitis for the US dairy industry exceed $2 billion per year [1,2]. Antibiotic treatments and other mastitis management strategies have improved the control of contagious pathogens; nonetheless, mastitis caused by microorganisms is still a major problem, even in well-managed dairy farms [3,4].

Mycoplasmas are highly contagious organisms and can cause various diseases in humans and animals [5]. In dairy cows, *Mycoplasma bovis* (*M. bovis*) is known to be the most common and virulent bovine Mycoplasma species in the United States [6]. The financial losses of *M. bovis* infections, as a result of loss of weight gain and carcass value and other associated diseases, are estimated to be approximately $108 million per year with infection rates of up to 70% in a herd [7]. *Mycoplasma bovis* is characteristically refractory to Beta lactam antibiotics because it does not possess a cell wall. Furthermore, evidence is accumulating that antibiotics, including tetracycline, tilmicosin and spectinomycin, are not effective for the treatment of *M. bovis* [8]. Thus, finding alternative yet effective treatments against *M. bovis* is vital.

Extensive research has shown that the various fatty acids have antimicrobial effects and may be used as an inhibitory agent against bacteria [9-11]. Kelsey et al. [12] demonstrated that linoleic acid inhibited growth of two different mastitis strains of *Staphylococcus aureus* (*S. aureus*). Prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) is synthesized via a metabolic pathway commencing with arachidonic acid which is previously derived from linoleic acid [13]. Results from a recent study in our laboratory [14,15] indicate that the fatty acid PGF$_{2\alpha}$, in the form of dinoprost tromethamine, inhibits inhibitory effects on growth of *S. aureus*, and may have similar inhibitory effects on growth of *M. bovis* associated with bovine mastitis. Thus, the objective was to determine the effect of PGF$_{2\alpha}$ on *M. bovis* growth *in vitro*.

**Materials and Methods**

**Bacteria strains, experimental design, and growth culture**

Five strains of *M. bovis* from bovine origin were selected for the study. Two strains were reference strains (ATCC 25025 and 25523). The other three strains were isolated from diseased cattle, two from

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milk of cows with clinical mastitis (MKB and CS-UI) and one from a swabbing solution of a cow’s nasal passage with clinical pneumonia (VP-UI). *Mycoplasma bovis* isolates were purified, cultured and suspended in sterile phosphate buffered saline to achieve an optical density of 0.2 at 520 nm, yielding approximately $1 \times 10^7$ to $2 \times 10^8$ CFU/ml as previously described [17]. Subsequently, *M. bovis* suspensions were incubated in culture media containing PGF$_{2\alpha}$ (dinoprost tromethamine) at final concentrations of 0 (control), 2, 4, and 8 mg/ml, for 8 h at 37°C in duplicate, where two sample tubes per treatment concentration per strain of *Mycoplasma* species were tested. After the 8 h incubation a sample from each tube was obtained, serially diluted, and cultured on three modified Hayflick’s agar plates for 10 d. The entire experiment was repeated on 4 different days to account for variation associated with a day effect, categorized as run.

### Statistical Analysis

Data were analyzed by least squares analysis of variance using GLM procedures of SAS and the model included the effect of experimental run (4 runs), treatment (4 doses of PGF$_{2\alpha}$), strain (5 strains), and their interaction.

### Results

Based on CFU/ml, PGF$_{2\alpha}$, in the form of dinoprost tromethamine, had inhibitory effects on *M. bovis* growth; mean CFU/ml decreased (P<0.01) with increasing concentrations of PGF$_{2\alpha}$ and 8 mg/ml of PGF$_{2\alpha}$ being the most inhibitory (Figure 1). Mean CFU/ml (106/ml) was significantly decreased from 43.6 ± 1.1 in control (0 mg/ml PGF$_{2\alpha}$) to 24.3 and 7.8 ± 1.1, in 4 and 8 mg/ml PGF$_{2\alpha}$ treatment (Figure 1).

![Figure 1: Effect of various doses of prostaglandin F$_{2\alpha}$ on *Mycoplasma bovis* growth across all five strains](image1)

However, an effect of treatment by strain interaction on mean CFU/ml was detected (P<0.05) providing evidence that *M. bovis* growth across all PGF$_{2\alpha}$ doses were not similar among strains. Pre-planned contrasts were conducted to compare the mean log CFU/ml values between strains for each treatment. Overall, the 2 mg/ml PGF$_{2\alpha}$ decreased (P<0.05) CFU/ml only in one strain compared with control, whereas 4 and 8 mg/ml PGF$_{2\alpha}$ decreased (P<0.01) CFU/ml in all strains compared with control (Figure 2).

![Figure 2: Effect of various doses of prostaglandin F$_{2\alpha}$ on each strain of *Mycoplasma bovis* growth](image2)

### Discussion

Our results provide evidence, for the first time, that PGF$_{2\alpha}$ in the form of dinoprost tromethamine has inhibitory effects on growth of *M. bovis*. Moreover, the effect of PGF$_{2\alpha}$ on the bacterial growth was not consistent across strains. Nevertheless, if PGF$_{2\alpha}$ were to be considered as a potential treatment for *M. bovis*, then a dose for final concentration and administration routes remains to be determined, using in vivo experiments. The use of intra-mammary treatment of mycoplasma mastitis is questionable and needs further research [18].

The antibacterial effects of fatty acids on pathogens have been studied for years and reviewed [9-12]. Our previous results also showed that [19] PGF$_{2\alpha}$ has inhibitory effects on growth of *S. aureus* and *Streptococcus uberis* in vitro [14,15]. In addition, growth of *S. aureus* and *Streptococcus agalactiae* was inhibited by certain long-chain fatty acids *in vitro* [12].

The mechanism by which PGF$_{2\alpha}$ affected the growth of *M. bovis* cannot be determined from the current study. Based on current evidence in the scientific literature, we postulate that one potential mechanism of action centers on the ability of fatty acids to penetrate the plasma membrane of bacteria, increasing the negative charge of the bacterial membrane surface, and ultimately disrupting cell membrane integrity [20]. Another possibility involves the hindering of bacterial growth via an interaction of the fatty acids at the cell membrane, resulting in a change in membrane permeability [21] or the disruption of transduction cascades leading to cell lysis [22]. Additionally, since PGF$_{2\alpha}$ retains some of its hydrophobic properties from its precursor...
arachidonic acid, it is possible that it is being incorporated into the plasma membrane and may be interfering with membrane fluidity and cellular signaling and transduction cascades similar to mechanisms previously suggested above [21,22]. Mycoplasma are unable to synthesize cholesterol needed to regulate membrane fluidity and must obtain sterols (cholesterol) from their environment to maintain proper fluidity [23,24]. When M. luidlawii were treated with palmitic and steric acids, cell permeability changed, cells appeared irregular, osmotic fragility increased, and cell growth was inhibited [25].

The PGF$_{2\alpha}$ concentrations used in the current in vitro study were relatively high, and therefore its feasibility as a clinical therapeutic agent warrants further investigation. Previous research [12] has shown that linoleic acid and arachidonic acid, precursors of PGF$_{2\alpha}$, are potent bacteriostatic fatty acids and at low doses of these fatty acids can inhibit the growth of several gram positive bacteria. The results of the current research provide an opportunity to examine the effect of these and other fatty acids on M. bovis, which currently has no effective treatment.

Conclusions
These in vitro results provide evidence, for the first time, that PGF$_{2\alpha}$, in the form of dinoprost tromethamine, has inhibitory effects on the growth of M. bovis, and the bacteriostatic effect appears to be strain and dose dependent. The clinical application of PGF$_{2\alpha}$ and its efficacy for treatment of M. bovis requires further investigation. These findings provide further research opportunities to investigate the effect of fatty acids on M. bovis.

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References