

## Applied Microbiology 2019: *In vitro* antagonistic combinatory effect of palm oils rich in lauric acid with oxacillin towards *Staphylococcus aureus*- Klara Lalouckova- Institute of Animal Science

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Due to their increasing antibacterial resistance and potential transmission to other cows, calves and the environment, various pathogens causing mastitis in dairy cattle are of serious concern, particularly through the milking process. Alternative approaches to antimicrobial use are therefore greatly needed in the treatment or control of mastitis in dairy cattle. It is known that the antibacterial effect of medium-chain fatty acids (MCFAs) is important for various pathogens but there is limited knowledge on the role of MCFAs on mastitis-causing pathogens. Furthermore, no evidence of the antimicrobial effects of MCFA-rich palm oils, such as coconut, palm kernel and tucuma oil, can be found in current literature. The objective of this study was to evaluate the *in vitro* antibacterial effect of MCFA-rich palm oils on bovine mastitis-causing strains (*Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, and *Streptococcus uberis*) following cleavage of exogenous lipase from *Mucor javanicus*. All tested palm oils had antibacterial activity against eight tested bacterial strains in the range 64–8192  $\mu\text{l}$  / ml with Str. The *agalactiae* are the most sensitive and the *S. Aureus* to be the most re resistant species. The results of the present study show that palm oils rich in MCFAs can serve as an alternative to pre-dip and post-dip treatment in bovine mastitis control, but further *in vivo* studies are needed to confirm the findings for their possible applications.

The traditional approach to the treatment of mastitis in cattle is the use of antibiotics, but this treatment has several disadvantages, including a low cure rate, an increase in resistance and the presence of antibiotic residues in milk. Intramammary antibiotic therapy is a common practice in the treatment or control of mastitis in dairy cattle herds. Commonly used drugs include betalactams, macrolides and lincosamide. Cure rates scarcely reach 50 percent in S-causing mastitis. *Aureus* strains with currently available therapy. Modern concepts for the prevention and treatment of mastitis include non-steroidal anti-inflammatory drugs and intramammary teat seals.

Sigma-Aldrich (USA) purchased coconut (*C. nucifera*) oil and palm kernel (*E. guineensis*) oil, while tucuma (*A. vulgare*) oil was obtained from Natural Sweet Botanicals (USA). The oils used in this experiment have been prepared according to previous research. The oils were briefly dissolved in dimethyl sulfoxide (DMSO) and emulsified with Tween 80 (both Sigma-Aldrich) to ensure sufficient dispersion into an emulsion at a final concentration of 819 200  $\mu\text{g}$  / ml. The final solvent concentration in the samples tested did not exceed 1 percent; therefore, it was not possible to influence the bacterial viability. Reasonable amounts of previously obtained 100% higher

concentrations emulsions were finally diluted to a final concentration of 8192  $\mu\text{g}$  / ml in tryptic soy broth (TSB) (Oxoid, UK) or TSB enriched with yeast extract (Oxoid). Based on its lipolytic activity at 2,73 mg / ml in culture media, this oil-medium emulsion was further supplemented with a lipase from *Mucor javanicus* (Sigma-Aldrich). The solution consisting of the oil tested, the correct culture medium selected according to the strain tested and the lipase from M. In a water bath heated to 37 ° C for 1 h, *javanicus* was then shaken to release MCFAs from the triglycerides and to promote their antibacterial action. Penicillin G (Sigma-Aldrich) has been used in bacterial crops as a growth control. In each microtiter plate a row of wells filled with medium only and a row of bacteria in medium without palm oil emulsion were included as a negative control and positive control of bacterial growth, respectively.

The minimum inhibitory concentrations (MICs) shown in Table 2 show the susceptibility of all Gram-positive bovine mastitis-causing pathogenic strains to selected palm oils in the 64 (*S. aureus*)–8192  $\mu\text{g}$  / ml (Str. *agalactiae*) range. Str was the most sensitive of the bacterial strains tested according to the lowest measured modal MIC value. CCM 6178 *agalactiae* (64  $\mu\text{g}$  / ml for the palm kernel oil) accompanied by Str. DSM 6784, and Str. *agalactiae* *Uberis* DSM 20569 (Tucuma and Palm Kernel Oil: 128  $\mu\text{g}$  / ml, respectively). Palm kernel oil (average MIC 1720  $\mu\text{g}$  / ml), followed by tucuma oil (average MIC 1776  $\mu\text{g}$  / ml) and coconut oil (average MIC 3040  $\mu\text{g}$  / ml), was shown to be the most effective in inhibitory activity for each oil against all strains. The most sensitive species of the bacteria tested was Str. *Agalactiae* with an average MIC value of 331  $\mu\text{g}$  / ml for all oils tested, followed by Str. *Uberis* (1045  $\mu\text{g}$  / ml), Str. *Dysgalactic* (1707  $\mu\text{g}$  / ml) and *S. Atreus* (4323  $\mu\text{g}$  / ml). Its Str. *Agalactiae* was also the most sensitive species (mean MIC 0.00037  $\mu\text{g}$  / ml) and *S. Aureus* was the most resistant species (average MIC 0.00138  $\mu\text{g}$  / ml) to antibiotic control as shown by penicillin G. The species' susceptibility pattern to palm oils was similar to that of penicillin G, except for the switch between the susceptibility of Str. *Dysgalacticiae* and Str. *Uberis*, man. The potential health risk of foodborne transmission of antibiotic-resistant animal pathogenic bacterial strains has resulted in the need for new alternative antibacterial sources in the dairy sector. In this study, the antibacterial effect of MCFA-rich palm oils, namely coconut, palm kernel, and tucuma oil (after cleavage with *Mucor javanicus* lipase) was observed in eight bovine mastitis-causing pathogens *in vitro*. The results offer an alternative approach to the prevention of mastitis in cattle herds, since MCFA-rich oils are valuable antibacterial remedies suitable for use in the preparation and treatment of teats.