Technical Literature

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Effect of a lipid-based low pKa anti-pathogenic product (R2™) on mitigating PRRSV in feed evaluated by quantitative RT-PCR and pig bioassay

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Introduction

In recent years, contaminated feed has been confirmed as a vehicle for transmission of porcine epidemic diarrhea virus (PEDV). It was also demonstrated that a number of additional viruses, such as Seneca Valley virus, feline calicivirus, bovine herpesvirus-1, porcine sapelovirus, and porcine reproductive and respiratory syndrome virus (PRRSV), could maintain infectivity in feed ingredients even after 37-day transport, emphasizing the importance to reduce the potential transmission of these viruses via contaminated feed. In the current study, an *in vitro* experiment and an *in vivo* pig bioassay were conducted to evaluate the ability of a fat-based anti-pathogenic product to mitigate or inactivate PRRSV present in feed. The product used in the test was R2 from Feed Energy Company. R2 provides a source of essential fatty acids along with having proven feed biosecurity benefits.

Methods

The *in vitro* experiment included 3 treatment groups with 3 replicates per group: Treatment 1 (NC) – untreated complete feed spiked with phosphate buffered saline (PBS, pH 7.4); Treatment 2 (PC) – untreated complete feed spiked with PRRSV MN184 (10^4 TCID50/g); Treatment 3 (Product) – complete feed treated with the R2 product one day before spiking with PRRSV MN184 (10^4 TCID50/g). At 0h, 1d, 4d, 7d, 14d and 21d post spiking, an aliquot of feed was taken from each replicate in each treatment group and processed into PBS to generate 20% feed homogenates. All of the feed homogenates were tested by a PRRSV quantitative real-time RT-PCR (RT-qPCR). Seven groups of feed homogenates from Treatment 1 (NC)-1d, Treatment 2 (PC)-1d, Treatment 3 (Product)-1d, Treatment 2 (PC)-4d, Treatment 3 (Product)-4d, Treatment 2 (PC)-7d, and Treatment 3 (Product)-7d were selected for pig bioassay. Twenty-one (10-day-old) PRRSV pigs were divided into 7 groups with 3 pigs per group in individual rooms with similar average weight per group. After 2-day acclimation, piglets were intramuscularly injected with 2ml and intranasally inoculated with 4ml (2ml per nostril) of feed homogenates. All pigs were necropsied on 7 days post inoculation (DPI). Serum samples collected on 0, 4, and 7 DPI and lung samples collected on 7 DPI were tested by PRRSV RT-qPCR. Formalin-fixed lung samples were subject to histopathology and immunohistochemistry (IHC) examinations.

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Results

Feed homogenates from the Treatment 1 (NC) remained PRRSV PCR negative throughout the 0-21d *in vitro* experiment. For the Treatment 2 (PC), all replicates of feed homogenates collected during the *in vitro* experiment were PRRSV PCR positive with an average Ct of 22.1 at 0h and 22.3-23.9 during 1d-21d. For the Treatment 3 (Product), all replicates of feed homogenates collected during the *in vitro* experiment were PRRSV PCR positive with an average Ct of 23.3 at 0h and 25.3-26 during 1d-21d. For pigs inoculated with feed homogenates from "Treatment 1 (NC)-1d", "Treatment 3 (Product)-1d", "Treatment 3 (Product)-4d" and "Treatment 3 (Product)-7d", serum samples collected on 0, 4, and 7 DPI and lung samples collected on 7 DPI were all PRRSV PCR negative, indicating that feed homogenates in these groups did not contain infectious PRRSV. In contrast, for pigs inoculated with feed homogenates from "Treatment 2 (PC)-1d", "Treatment 2 (PC)-4d" and "Treatment 2 (PC)-7d", serum samples collected on 4 and 7 DPI and lung samples collected on 7 DPI were all PRRSV PCR positive, indicating that feed homogenates in these groups contained infectious PRRSV. Histopathology and IHC examinations on the lung samples supported the above results.

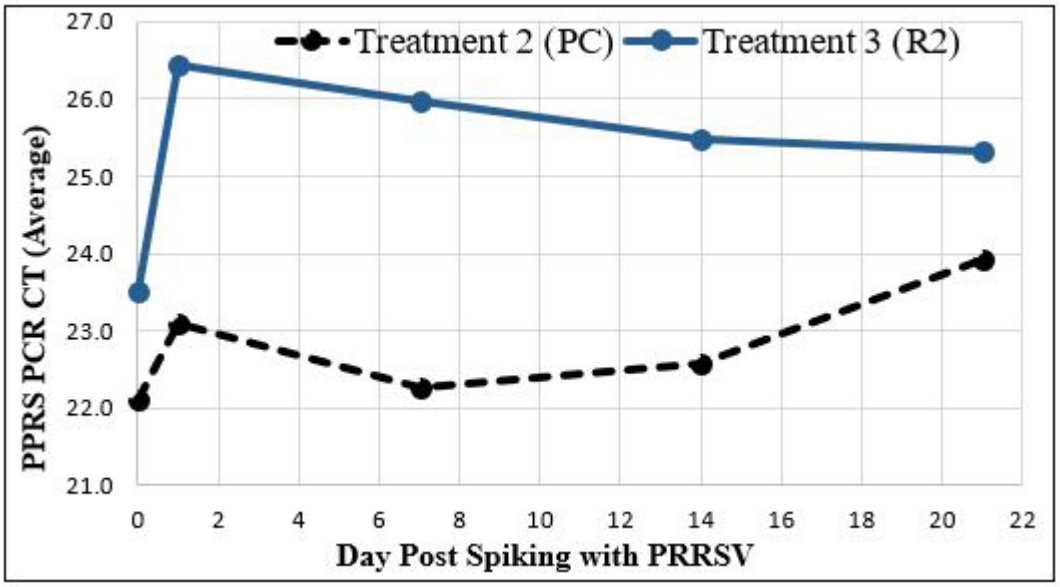

Figure 1: Results of in vitro experiments testing the effects of treatment on PPRSV in feed at room temperature as examined by PRRSV RT-qPCR. The higher the Ct value, the less quantity of PRRSV RNA genetic material is detected.

Conclusions and Discussion

The results from the positive control group in this study provide evidence that PRRSV can survive in feed and result in infection of pigs, emphasizing that feed contaminated with PRRSV can be a risk factor for PRRSV transmission. Pre-treatment of feed with the R2 product can effectively render PRRSV, subsequently introduced into feed, non-infectious. Thus, the R2 product is capable of inactivating PRRSV present in feed and reducing the risk of PRRSV transmission via contaminated feed. This study also suggests that PCR testing in the *in vitro* experiment is not reliable, but pig bioassay is more reliable, to assess the infectivity of the PRRSV-contaminated feed samples.

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References

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