

# **CONCENTRATIONS OF HAPTOGLOBIN IN BOVINE PLASMA DETERMINED BY ELISA OR A COLORIMETRIC METHOD BASED ON PEROXIDASE ACTIVITY**

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# Background

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Our laboratory determines plasma concentrations of haptoglobin using a low-cost colorimetric procedure that measures haptoglobin-hemoglobin complexing by estimating differences in peroxidase activity (CPPA).

Results are based on absorption readings, given that the CPPA method does not contain a standard curve.

Conversely, commercially available ELISA methods generate results based on standards with known haptoglobin concentrations.

Therefore, the objective of this study was to determine if the CPPA method generates results compatible with a commercial ELISA kit.



# Materials and Methods

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Nine Angus steers were vaccinated against *Mannheimia haemolytica* to stimulate an acute-phase protein response.

Blood samples were collected prior to vaccination (d 0), and on d 1, 3, 5, 7, and 10

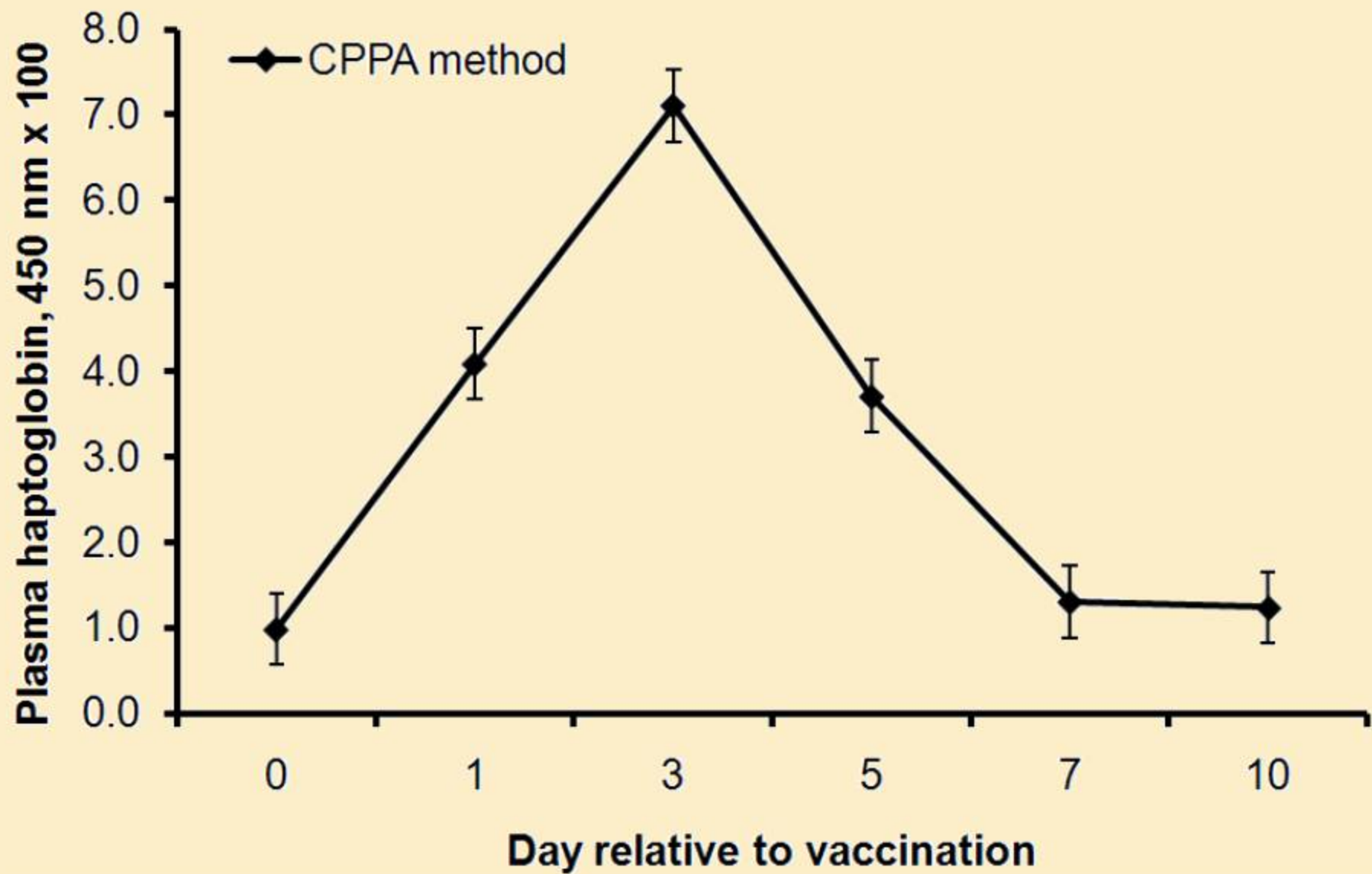
Samples were centrifuged for plasma collection and stored in triplicates at  $-80^{\circ}\text{C}$  on the same day of collection.

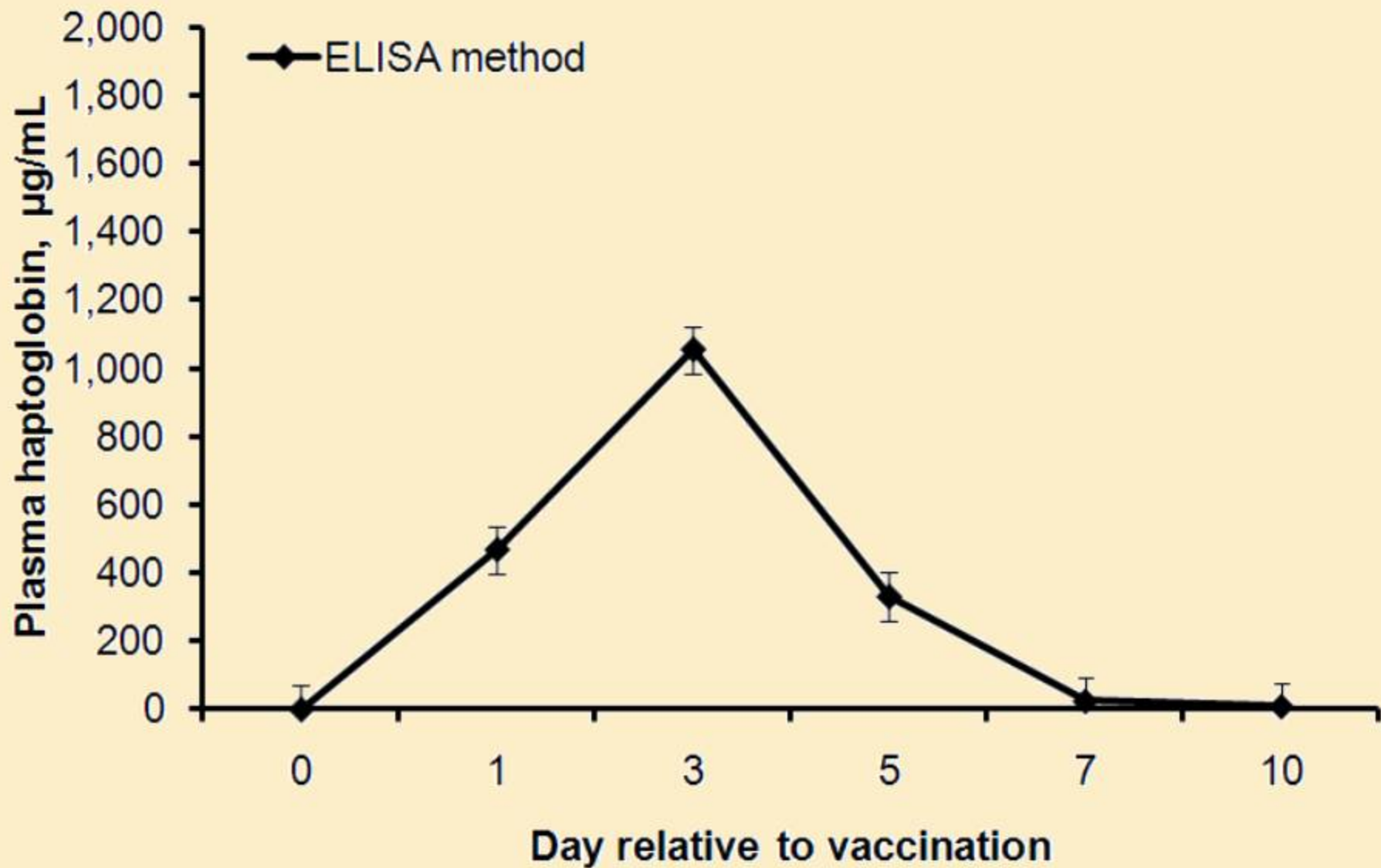
Each sample was analyzed for haptoglobin by

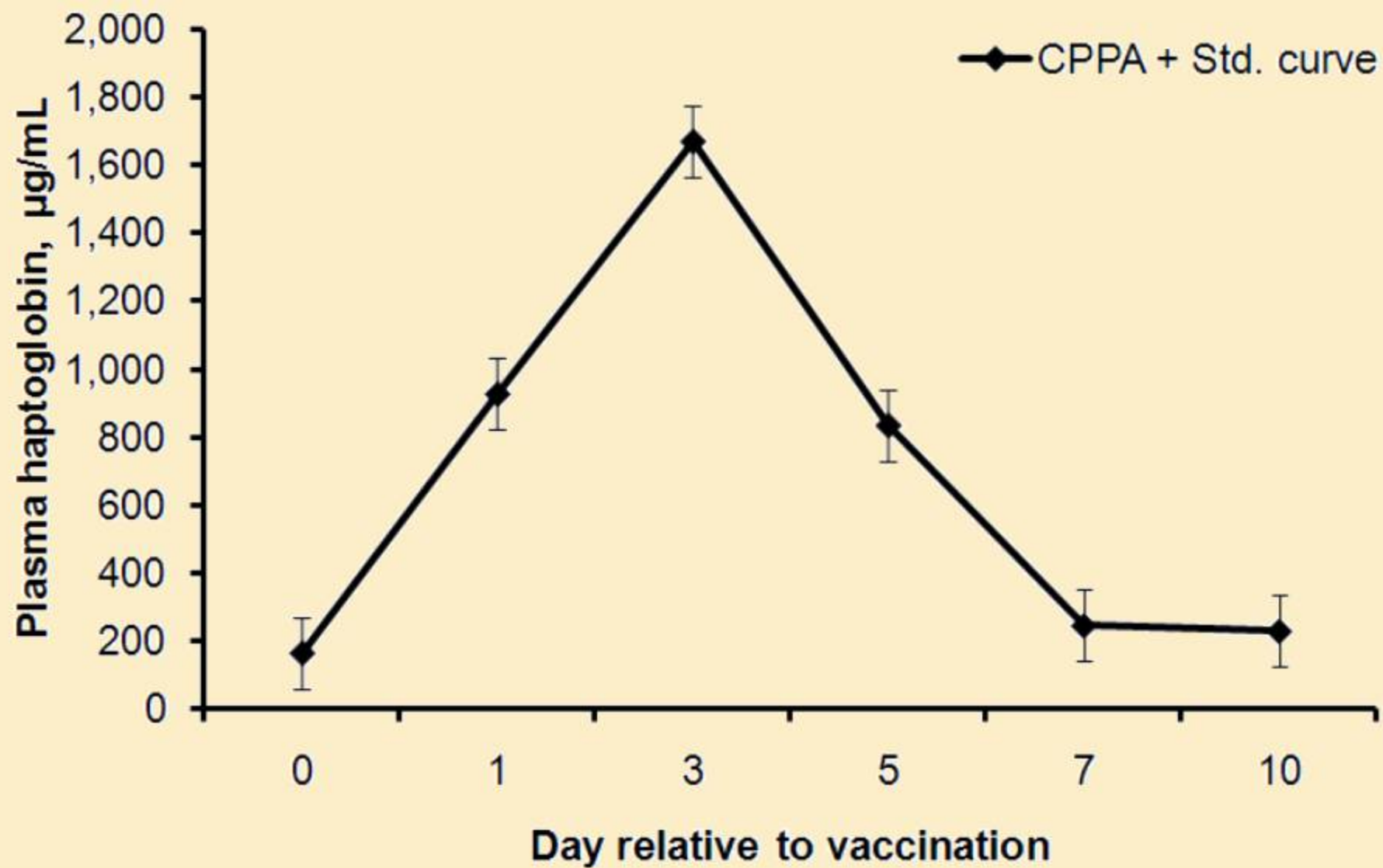
1. CPPA procedure.
2. commercial ELISA kit
3. using the CPPA procedure that included a standard curve based on a sample with haptoglobin concentration determined by the ELISA kit.

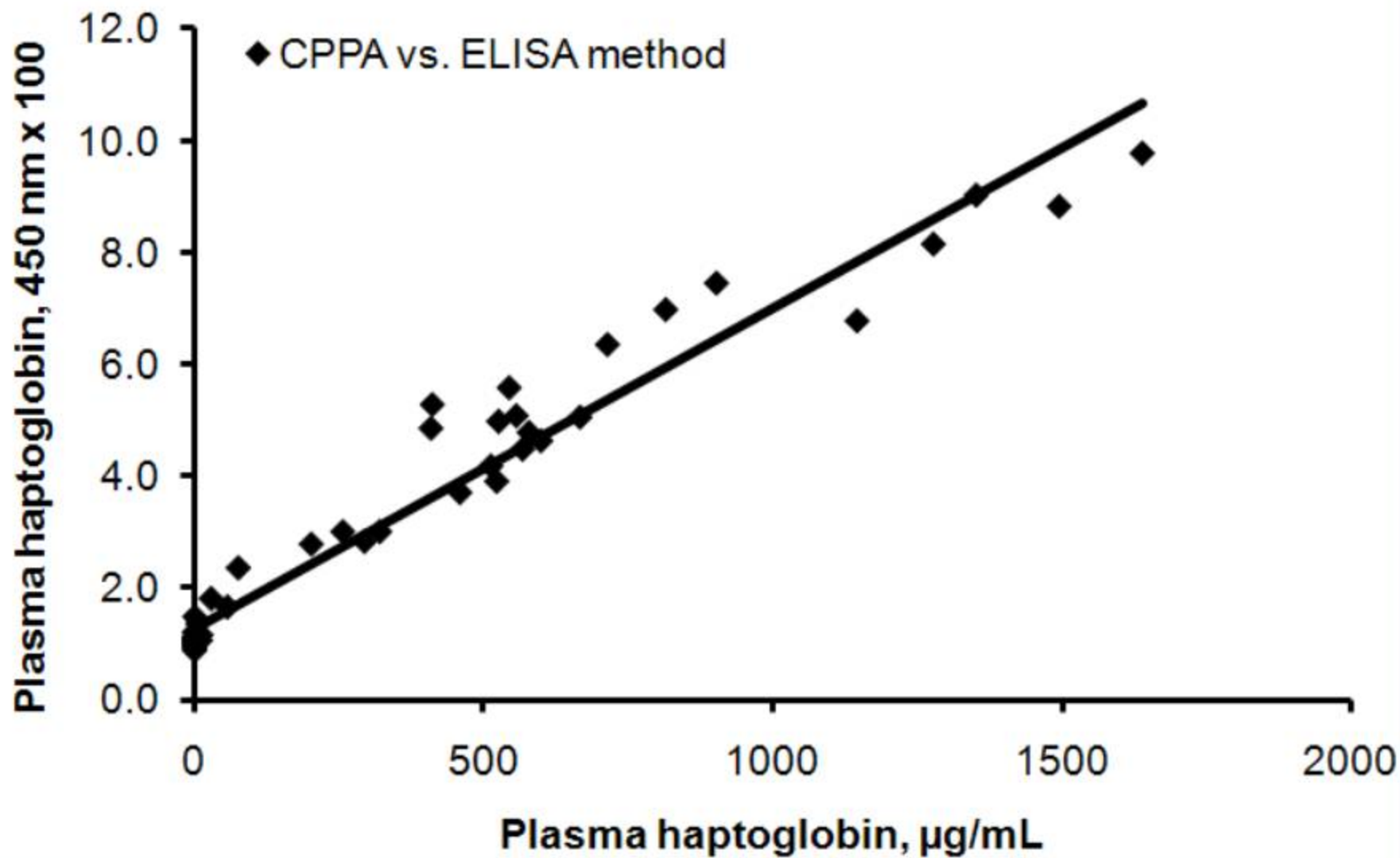


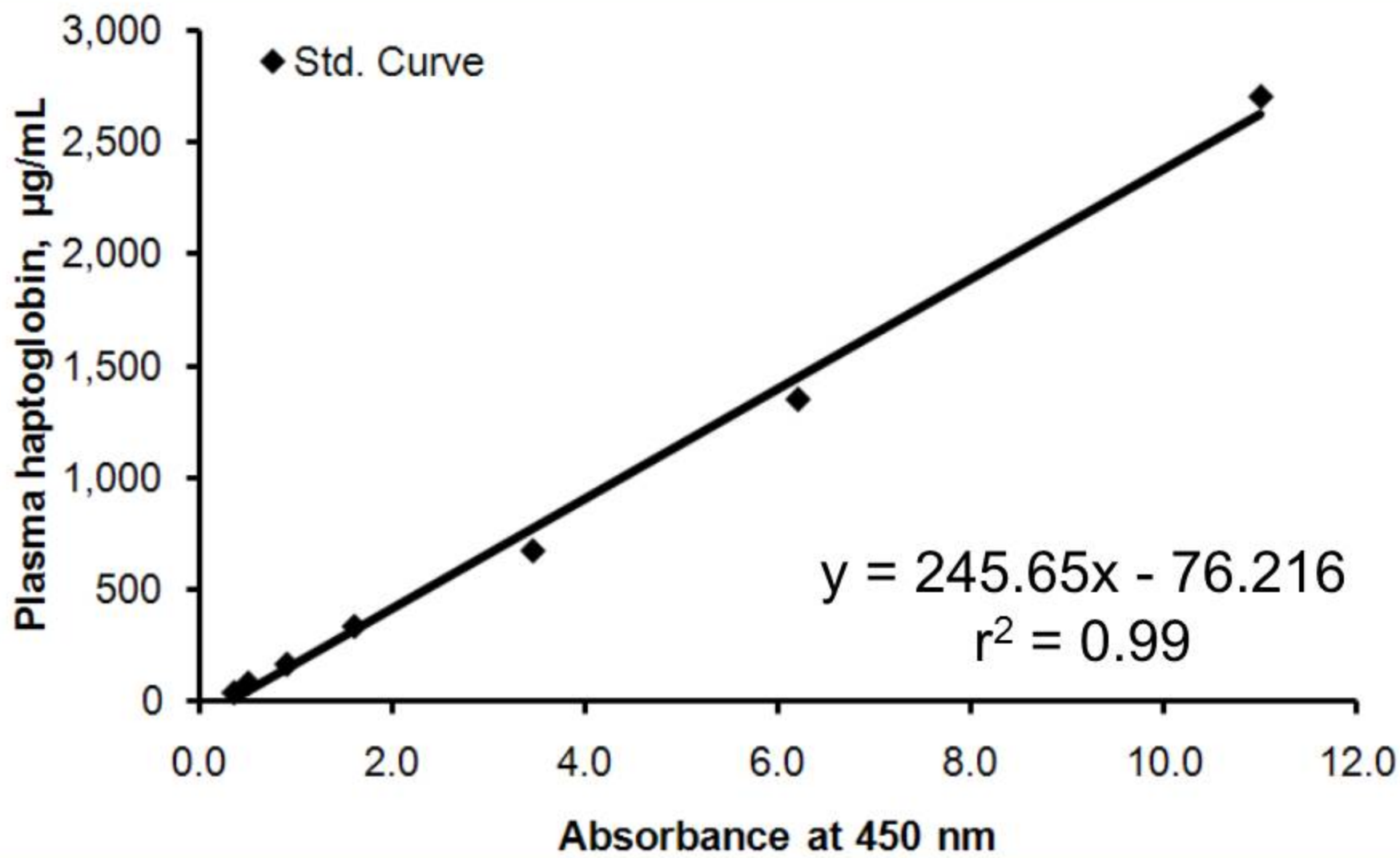














# Results

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A similar day effect was detected for all assays types, with plasma haptoglobin peaking on d 3 relative to vaccination and returning to baseline on d 7.

A linear standard curve ( $r^2 = 0.99$ ) was generated when a sample with elevated and known haptoglobin concentration (via ELISA method) was serially diluted with a sample from the same calf with reduced haptoglobin content and incorporated into the CPPA method.

A strong correlation was detected among results yielded by the CPPA and ELISA methods ( $P < 0.01$ ;  $r = 0.98$ ). The same correlation coefficient was generated with the CPPA with standard curve was compared with the ELISA method given that the standard curve generated was linear.

However, the values generated by the CPPA procedure with standard curve differed ( $P < 0.01$ ) when compared to those generated by ELISA.

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# Conclusion

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Assessing concentrations of haptoglobin in bovine plasma using the CPPA method yields results highly correlated to ELISA.

Inclusion of a standard curve into the CPPA method did not result in values similar to the ELISA method, probably due to assay sensitivity.

Therefore, the CPPA method can be adopted to evaluate plasma haptoglobin concentrations in cattle when absolute values are not required.



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