

# Production and application of a polyclonal antibody against purified bovine adiponectin

M. Mielenz, B. Mielenz, C. Kopp, J. Heinz, S. Häussler, H. Sauerwein

Institute of Animal Science, Physiology & Hygiene Unit, University of Bonn, Germany.

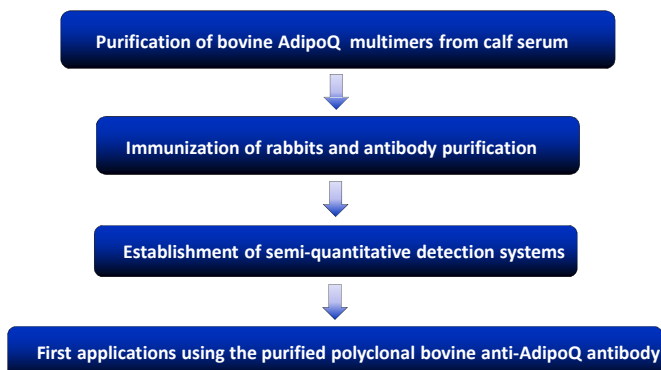
## Introduction

- The highly abundant adipose-tissue derived glycoprotein adiponectin (AdipoQ) is related to glucose- and fatty acid metabolism and exhibits anti-inflammatory properties [1].
- Bovine AdipoQ (~ 28 kDa) contains 240 amino acids and is mostly found as high molecular weight (HMW) multimers in serum [2, 3].
- Very recently, a reduction of AdipoQ mRNA in subcutaneous (s.c.) adipose tissue (AT) from tailhead was observed in dairy cows during the transition period [4].
- Data about AdipoQ in human milk is associated with early postnatal growth and leaner body proportionality of the infant [5].

## Objectives

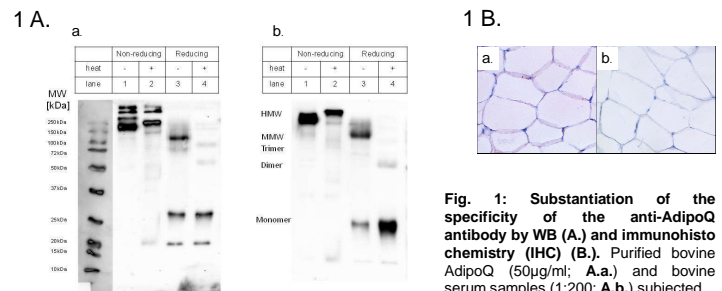
- Establishment of a bovine species-specific polyclonal anti-AdipoQ antibody
- Generation of basic data about AdipoQ protein expression in cattle

## Materials and methods

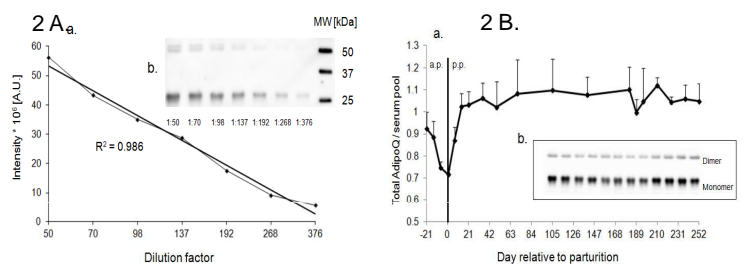


## Results

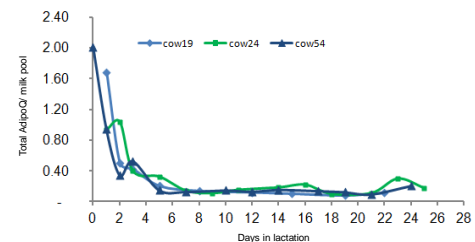
- Substantiation of the specificity of the anti-AdipoQ antibody by reducing and denaturing conditions was completed (Fig. 1A).
- As expected, AdipoQ was detected in bovine adipocytes by IHC (Fig. 1B).
- AdipoQ was detected by Western blot (WB) in bovine serum, in differentiated but not in undifferentiated bovine preadipocytes and in bovine milk.
- After establishment of a semi-quantitative WB (Fig. 2A), the time course of serum AdipoQ concentrations during a lactational cycle was characterized (Fig. 2B).
- AdipoQ concentrations in milk can be assessed via the WB; exemplarily, the concentrations in milk from 3 individual cows during the first 3 weeks of lactation are shown in Fig. 3.



**Fig. 1: Substantiation of the specificity of the anti-AdipoQ antibody by WB (A.) and immunohistochemistry (IHC) (B.).** Purified bovine AdipoQ (50µg/ml; **A.a.**) and bovine serum samples (1:200; **A.b.**) subjected to reducing and denaturing conditions. Mostly, monomeric AdipoQ (~28 kDa) is visible using reducing and denaturing conditions but also dimers (~56 kDa) in contrast to less/no HMW (≥ 300 kDa) and middle molecular weight (MMW, ~130 kDa) AdipoQ in lane 4 of Fig. A.a. and Fig. A.b. A band of ~18 kDa produced by treatment may represent the globular AdipoQ domain. **B.a.:** Positive staining of AdipoQ in retroperitoneal bovine AT by IHC, localized in the cytoplasmic rim of the adipocytes; **B.b.:** Negative control omitting the anti-AdipoQ antibody. Magnification: 200fold, bar represents 20 µm.



**Fig. 2: Semi-quantification of AdipoQ throughout the transition period and during lactation in dairy cows by WB.** **A.a.:** Demonstration of the methodological reliability by showing the linearity of a serum dilution series. **A.b.:** Corresponding digital image of a WB. For quantification the intensity of dimeric and monomeric AdipoQ were totaled and related to the values obtained from a standard serum pool; **B.a.:** Semi-quantitative data for AdipoQ (means ± SEM) in serum from pluriparous cows (n = 6) from day 21 ante partum up to the 36<sup>th</sup> week of lactation post partum. **B.b.:** Corresponding digital image of one WB, each sample and standards were analyzed as duplicates.



## Conclusions

- A species-specific polyclonal antibody against bovine anti-AdipoQ was generated.
- The detection of reduced AdipoQ serum concentrations around calving partum indicates a relation to the reduced insulin sensitivity and inflammatory conditions at that time.
- The presence of AdipoQ in colostrum and mature milk supports its role as bioactive component of cow milk.

## Acknowledgement

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## References

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