RELATIVE QUANTIFICATION OF Fc RECEPTOR (FcRn) AND POLYMERIC IMMUNOGLOBULIN RECEPTOR (pIgR) mRNA IN SHEEP USING REVERSE TRANSCRIPTION REAL-TIME PCR (real-time RT-PCR)

A Dzidic, A Mohr[1], K Meyer[1], J Bauer[1], HHD Meyer & MW Pfaffl

Institute of Physiology and [1] Institute of Animal Hygiene,
Center of Life and Food Science, Technical University of Munich,
Freising-Weihenstephan, Germany

andzidic@wzw.tum.de

BACKGRUND

Real-time transcription followed by polymerase chain reaction (real-time RT-PCR) is the most suitable method for the detection and quantification of mRNA. Herein low abundant Ig receptor mRNA was quantified using SYBR Green I fluorescence dye by real-time RT-PCR on the LightCycler (Roche Diagnostics).
The Fc receptor (FcRn) is responsible for the specific trans-membrane immunoglobulin (IgG) transport while the polymeric Ig receptor (pIgR) recognizes the dimeric IgA and pentameric IgM.

AIM

Quantification of two Ig receptor mRNA expression levels under the influence of the *P. roqueforti* mycotoxin - **Mycophenolic Acid (MPA).**

MATERIAL AND METHODS

- Nine healthy Merino Landschaf x Schwarzkopfschaf sheep were treated over 9 week treatment with 300 mg MPA/day/sheep, and nine animals served as untreated control,

- Relative quantification of low abundant mRNA using an eff
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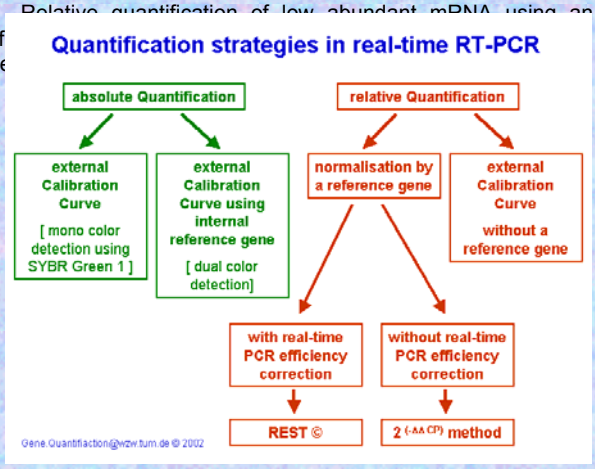


- Mathematical algorithms is based on RT-PCR efficiency and the mean crossing point (CP) deviations (Δ CP) between the treatment (sample group) and control group, according to equation:

$$R = \frac{(E_{target})^{\Delta CP target\ (MEAN\ control - MEAN\ sample)}}{(E_{ref})^{\Delta CP ref\ (MEAN\ control - MEAN\ sample)}}$$

- New software REST© (Relative Expression Software Tool) for group-wise comparison and statistical analysis of relative expression results in real-time RT-PCR,

- Relative expression ratio results are tested for significance by *Pair wise Fixed Reallocation Randomisation Test©*

- http://www.wzw.tum.de/gene-quantification/

RESULTS

Each tissue exhibited an individual expression pattern of FcRn and pIgR mRNA. Both receptor types were highly expressed in liver > kidney > and gastrointestinal tract. In spleen, thymus and two lymph nodes medium to low expression levels were determined.
FcRn mRNA showed tendency of down-regulated by MPA in liver (p=0.06). In ileum (p=0.05) and liver (p=0.05) a significant up-regulation for pIgR mRNA expression was observed

Tables:

Mean tissue specific expression level of FcRn mRNA (1a) and pIgR mRNA (1b). Calculation is based on CP data of animals in the experiment (n = 18), normalized via the internal housekeeping gene expression (β-Actin) and conversed in x-fold expression compared to the lowest expression level in lymph nodes (= 1.0-fold).

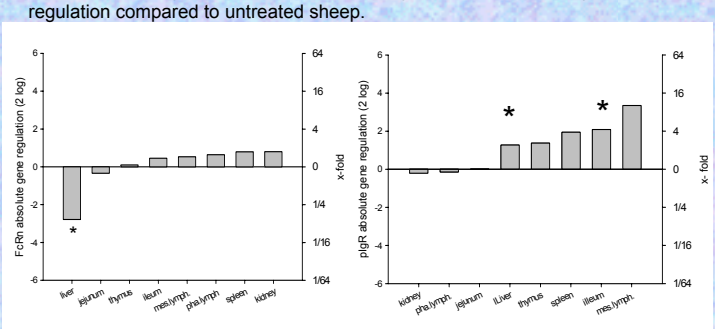
Table 1a:

Tissue	FcRn [CP±std.dev]	β-Actin [CP±std.dev.]	Δ CP	ΔΔ CP	E ΔΔ CP x-fold
Ileum	28.88 ± 2.17	20.34 ± 2.16	8.54	+2.59	5.05
Jejunum	28.71 ±1.51	20.73 ± 2.47	7.98	+3.15	6.94
Kidney	22.09 ±5.66	17.60 ± 1.71	5.52	+5.61	17.8
Liver	28.09 ±3.67	23.79 ± 2.30	4.30	+6.83	117.7
Spleen	28.56 ±3.10	19.58 ± 5.11	8.98	+2.15	2.97
Thymus	30.94 ±3.21	21.45 ± 2.64	9.49	+1.64	2.38
Pharyngeal lymph node	30.06 ±2.55	18.93 ± 5.13	**11.13**	**±0.00**	**1.00**
Mesenterial lymph node	27.89 ±3.19	18.17.± 7.36	9.71	+1.42	2.14

Table 1b:

Tissue	pIgR [CP±std.dev]	β-Actin [CP±std.dev.]	Δ CP	ΔΔ CP	E ΔΔ CP x-fold
Ileum	30.90 ±3.11	20.34±2.16	10.56	+6.19	30.8
Jejunum	30.63 ±2.28	20.73 ±2.47	9.91	+6.84	46.0
Kidney	27.74 ±1.65	17.60 ±1.71	10.14	+6.61	56.3
Liver	31.61 ±2.27	23.79 ±2.30	7.83	+8.92	87.1
Spleen	33.78 ±4.57	19.58 ±5.11	14.20	+2.55	5.14
Thymus	36.93 ±4.67	21.45 ± 2.64	15.48	+1.27	1.46
Pharyngeal lymph node	31.88 ±.3.30	18.93 ± 5.13	12.95	+3.80	11.7
Mesenterial lymph node	34.93 ±.3.55	18.17 ±7.16	**16.75**	**±0.0**	**1.00**

Figure 1a and 1b:
Influence on MPA treatment (300 mg MPS/day/sheep) on Ig receptors FcRn mRNA (2a) and pIgR mRNA (2b) expression level in treated ovine tissues (n = 9) in comparison to untreated control (n = 9). Expression changes through MPA (up- or down-regulation) are shown in logarithmic scale (2-log) and x-fold regulation compared to untreated sheep.



CONCLUSION

MPA may have immuno-suppressive effects in liver by low level FcRn expression and therefore a low IgG serum-to-bile transport is expected. Although MPA showed stimulatory effects of pIgR expression in liver and ileum. Consequently, a good IgA and IgM transport in the tissues is given.