

The *in vivo* effects of a Mycophenolic Acid (MPA) treatment on the cytokine expression in sheep leukocytes, using an efficiency corrected relative quantification model in real-time RT-PCR

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INTRODUCTION

Mycophenolic acid (MPA) is an active secondary metabolite from *Penicillium roqueforti*. It is frequently found in contaminated silages for animal feed. MPA is known to have immune suppressive effects and is therefore used in human medicine as an anti-proliferative agent and for immune suppression after organ transplantation. In sheep immune system may be modulated by MPA contaminated silage and cause appetite lost, ketosis, paralysis and abortion.

In the present study the *in vivo* effects of a long term MPA administration (9 weeks) were investigated on cytokine mRNA expression in sheep leukocytes.

AIM

To characterize the long term MPA influence on the cytokine mRNA expression in white blood cells (WBC).

- Tumor necrosis factor alpha (TNF α)
- Interleukins (IL1 α , IL1 β , IL2, IL6)

MATERIAL AND METHODS

- Eighteen healthy sheep (Merino-Landschaf x Schwarzkopfschaf) were used in the experiment. Nine sheep were orally treated with 300 mg MPA/day/sheep, and nine animals served as untreated control.
- For quantification we validated and applied a recently describe method using an efficiency corrected relative quantification model using REST© (*Relative Expression Software Tool*)
- Expression data were normalised by the non-regulated mRNA expression of the housekeeping gene β -actin.
- Relative expression ratio results are tested for significance by *Pair wise Fixed Reallocation Randomisation Test*©
- REST© mathematical algorithms is based on RT-PCR efficiency correction and the mean crossing point (CP) deviations (Δ CP) between the treatment (sample group) and control group, according to equation:

$$R = \frac{(E_{\text{target gene}})^{\Delta CP_{\text{target gene}} (\text{MEAN control} - \text{MEAN MPS})}}{(E_{\beta\text{-actin}})^{\Delta CP_{\beta\text{-actin}} (\text{MEAN control} - \text{MEAN MPS})}}$$

RESULTS

No effects were found on leucocytes total RNA contents. Each factor exhibited an individual mRNA expression pattern. qRT-PCR relative mRNA quantification showed:

- significant 4-fold TNF α up-regulation after 1 week ($p < 0.05$) in the MPA treated group;
- continuous 6-fold IL1 α down-regulation ($p < 0.01$) between week 2 and 5;
- IL1 β showed an unsteady mRNA regulation and finally a 2-fold down-regulation at the end of the trial;
- IL 2 and IL 6 showed constant expression mRNA pattern;

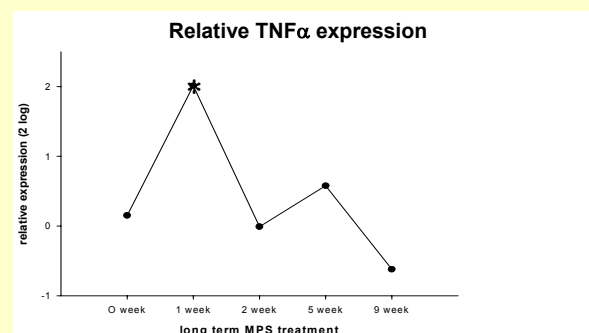
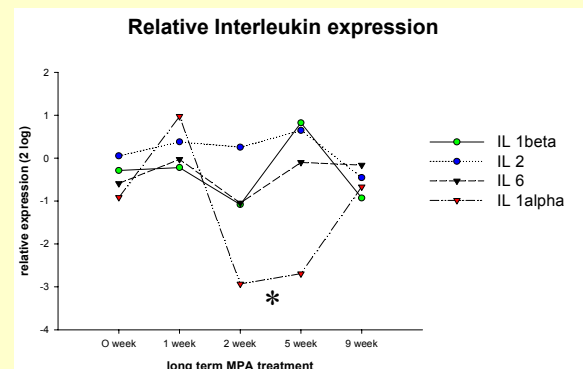
Table 1:

Mean expression strength of TNF α , IL1 α , IL1 β , IL 2 and IL6 in sheep WBC. Calculation is based on CP data of animals in the experiment ($n = 2 \times 9$), normalized via the internal housekeeping gene expression (β -actin) and converted in x-fold expression compared to the lowest expression level in IL 6 (\Rightarrow 1.0-fold).

FACTORS in WBC	FACTOR's [CP \pm std.dev]	β -aktin CP [CP \pm std.dev]	Δ CP	$\Delta\Delta$ CP	E $\Delta\Delta$ CP
IL 1 α	30,48 \pm 0,87	18,48 \pm 2,3	12,00	+ 5,07	33,59
IL 1 β	29,45 \pm 1,73		10,97	+ 6,10	68,59
IL 2	33,12 \pm 1,07		14,64	+ 2,43	5,38
IL 6	35,55 \pm 3,76		17,07	\pm 0,00	1,00
TNF α	26,55 \pm 1,19		8,07	+ 9,00	512

Figure 1 & 2:

Influence of long time MPA treatment on TNF α , IL1 α , IL1 β , IL 2 and IL6 mRNA expression level in WBC ($n = 9$) in comparison to untreated control ($n = 9$). Expression changes through MPA are shown in logarithmic scale (2 log).



CONCLUSION

MPA may have immuno-suppressive effects in white blood cells. TNF α is highly regulated in early MPS treatment phase. Both IL 1 subtypes (IL1 α and IL1 β) are down-regulated, responsible for the initial B-cell activation and proliferation and therefore a down-regulation might have severe immuno suppressive effects.

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