Transcription of IL-6 and IFN-γ in chicken lymphocytes stimulated with synbiotics *in vitro*.

Anna SŁAWIŃSKA [1], Maria SIWEK [1], Jadwiga BRZEZIŃSKA[1], Joanna ŻYLIŃSKA [2], Jacek BARDOWSKI[2], Johaness BLUIJSSEN[3], Marek BEDNARCZYK [1],

[1]*University of Technology and Life Sciences, Animal Biotechnology Department, Mazowiecka 28, 84-085, Bydgoszcz, Poland .*
[2]*Department of Microbial Biochemistry, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5a, 02-106 Warszawa, Poland*
[3]*Adam Mickiewicz University, Department of Human Molecular Genetics, Institute of Molecular Biology and Biotechnology, Umultowska, 89 61-614 Poznań, Poland*

63th Annual Metting EAAP Bratislava, 27 – 31 August 2012

Introduction

Synbiotic is a substance obtained by combining prebiotics (e.g. raffinose family oligosaccharides, RFOs) and probiotics (e.g. LAB – lactic acid bacteria). Synbiotic supplements in chicken diet stimulate gut microflora and immune system through gut-associated lymphoid tissue.

The Goal : To assess the reaction of the chicken lymphocytes to *in vitro* stimulation with synbiotics on the molecular level

Materials & Methods

Chicken splenocytes & stimulation

- Chicken lymphocytes were isolated from 7 spleens, rinsed in PBS and centrifuged for 5min at 1000 rpm.
- In the next step splenocytes were separated by Histopaque-1077 density gradient centrifugation.
- Lymphocyte culturing was performed in 24-well plates by applying 0.5 ml of cell suspension containing 10^6 viable cells in RPMI medium with 10%FBS, 350ul NaCl (2M), 2-ME and 0.25 ml of a substance that stimulates an immune response.
- Splenocytes were stimulated for 2hrs, 4hrs, and 6hrs.
- Five experimental groups were defined for in vitro culture: S1: Synbiotic 1 (RFO + *Lactococcus lactic subsp. lactis*), S2: Synbiotic 2 (RFO + *Lactococcus lactic subsp. cremoris*), S3: Synbiotic 3 (commercial synbiotic Duolac), P: prebiotic (RFO) and C: cell culture medium (negative control).

RT-PCR reaction & analysis

- Splenocytes were preserved in RNA later.
- RNA was isolated by Trizol (Life Technologies) followed up by purification with an EURx kit (EURx Gdansk Poland)
- Two genes coding chicken cytokines linked to activation of the immune system were used :
  - IL-6 (gen ID 395337) and IFN-γ (gen ID 396054)
- Expression level was defined based on relative quantification of the target gene compared to the reference gene (ACTB, gen ID 396526).
- qRT-PCR reaction was performed in duplicates
- Standard curves were based on a cDNA from C group in serial dilutions as follow: 1, 0.5, 0.25, 0.125 i 0.0625
- Results were analyzed with Standard Curve Method, and Control group was used as a calibrator

Results

Figure 1. Relative expression of INF-γ gene

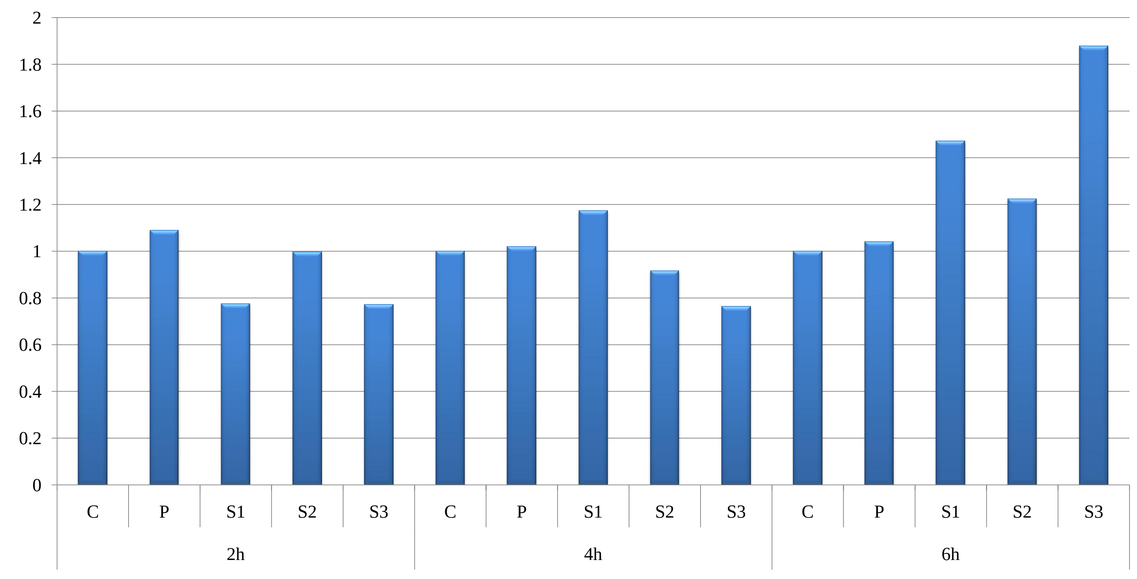
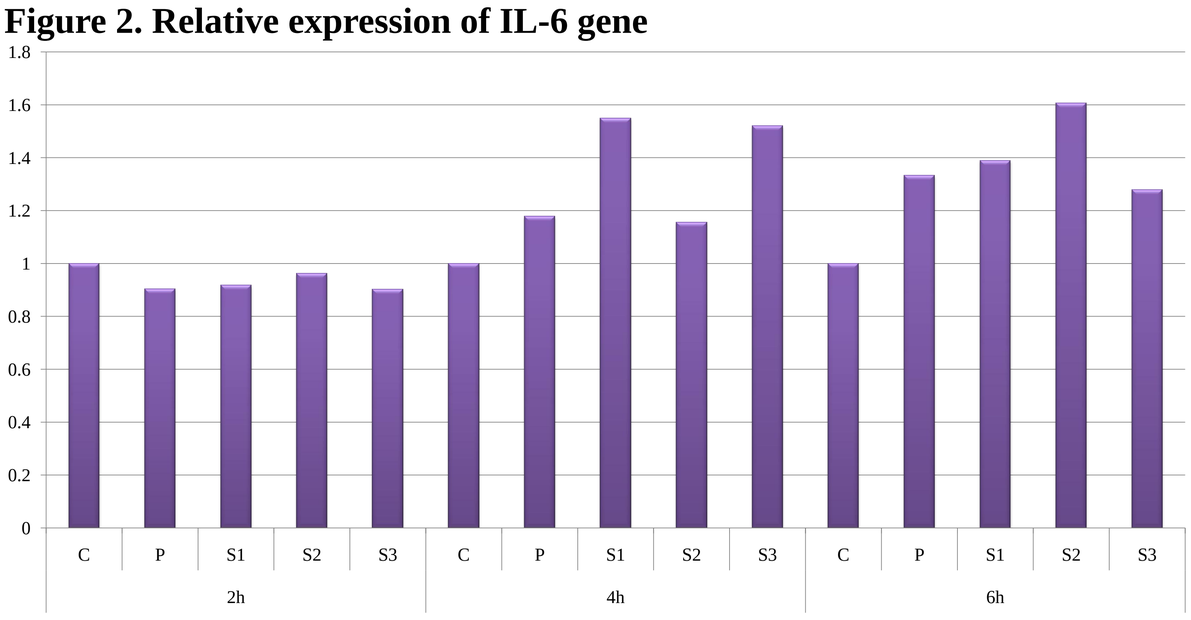


Figure 2. Relative expression of IL-6 gene



X axis: Experimental groups: C – control, P – splenocytes stimulated with prebiotic (RFO), S1 – splenocytes stimulated with Synbiotic 1 (RFO + *Lactococcus lactic subsp. lactis*), S2 – splenocytes stimulated with Synbiotic 2 (RFO + *Lactococcus lactic subsp. cremoris*), S3 – splenocytes stimulated with Synbiotic 3 (commercial synbiotic Duolac). 2h, 4h, 6h – timepoints of the culture stimulation with each substance. Y axis: relative quantity of target gene mRNA (INF-γ or IL6).

Conclusions

1. **Stimulation of splenocytes *in vitro* influences transcription of target genes**
2. **Different synbiotics present diverse impact on chicken lymphocytes *in vitro***
3. **Preliminaray results indicate 4h and 6h time points the most promising for further analysis**

Connected subject:
abstract: 14418
session: 49

ACKNOWLEDGMENTS: The research was supported by the National Science Center (NN311 623938)