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# Feeding of pregnant sows with mycotoxin contaminated diets has no effect on fetal and maternal hepatic transcription of genes of the insulin-like growth factor system

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► **To cite this version:**

Ute Tiemann, Klaus-Peter Brüssow, Sven Dänicke, Jens Vanselow. Feeding of pregnant sows with mycotoxin contaminated diets has no effect on fetal and maternal hepatic transcription of genes of the insulin-like growth factor system. *Food Additives and Contaminants*, 2008, 25 (11), pp.1365-1373. 10.1080/02652030802112619 . hal-00577392

**HAL Id: hal-00577392**

**<https://hal.science/hal-00577392>**

Submitted on 17 Mar 2011

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**Feeding of pregnant sows with mycotoxin contaminated diets has no effect on fetal and maternal hepatic transcription of genes of the insulin-like growth factor system**

Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2007-410.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	17-Mar-2008
Complete List of Authors:	Tiemann, Ute; Research Institute for the Biology of Farm Animals, Reproductive Biology Brüssow, Klaus-Peter; Research Institute for the Biology of Farm Animals, Reproductive Biology Dänicke, Sven; Federal Agriculture Research Center (FAL) Vanselow, Jens; Research Institute for the Biology of Farm Animals, Molecular Biology
Methods/Techniques:	Molecular biology - PCR
Additives/Contaminants:	Mycotoxins - fusarium
Food Types:	Animal feedingstuffs

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3 1 **Feeding of pregnant sows with mycotoxin contaminated diets has no effect**  
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6 2 **on foetal and maternal hepatic transcription of genes of the insulin-like**  
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9 3 **growth factor system**  
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43 17 Running title: Effects of mycotoxins on hepatic expression of IGF genes  
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## 25 **Abstract**

26 The insulin-like growth factor (IGF) system regulates foetal and placental growth. It can be  
27 influenced by dietary factors, but little is known about effects of mycotoxin feeding on  
28 hepatic levels of the IGF system. We aimed to determine (1) the normal foetal and maternal  
29 hepatic transcription of major IGF genes at distinct stages of pregnancy and to find out, (2) if  
30 the mycotoxins deoxynivalenol (DON) and zearalenone (ZON) affect transcript  
31 concentrations. Pregnant sows were fed with naturally contaminated diets from days 35 to 70  
32 (experiment 1), or from days 75 to 110 (experiment 2), or with control diets. Foetal hepatic  
33 IGF transcripts were determined at days 70 and 110, maternal transcripts at day 70 by qPCR.  
34 Highest levels of IGF-I transcripts were found in sows. Contrary, IGF-II was predominantly  
35 expressed in foetuses at day 70. Expression of IGF-IR and IGFBP-3 decreased, whereas that  
36 of IGFBP-2 increased towards term. IGF-IIR and IGFBP-1 expression was similar in both,  
37 foetuses and full-term piglets. IGF-IIR showed reduced levels in the maternal liver. Exposure  
38 of pregnant sows to DON and ZON contaminated diets significantly reduced the maternal and  
39 tended to reduce the weight gain of piglets in experiment 2 but had no effects on hepatic  
40 levels of IGF transcripts in both experiments. This suggests that mycotoxin contaminated  
41 diets can induce growth depression in pigs during pregnancy without affecting hepatic  
42 transcription of major IGF genes.

43  
44 **Keywords:** Deoxynivalenol, Zearalenone, IGF-system, foetus, neonatal pig, liver

## 47 **Introduction**

48 Mycotoxins are biologically active secondary fungal metabolites found as contaminants of  
49 feedstuffs, which exert toxic effects on animals and human beings (Fink-Gremmels 1999).  
50 Among farm animals, pigs are most susceptible to deoxynivalenol (DON), resulting in  
51 guidance values for critical dietary concentrations of 0.9 mg DON/kg for all pig categories; of  
52 0.1 mg zearalenone (ZON)/kg for piglets and gilts, and of 0.25 mg ZON/kg for sows and  
53 fattening pigs (The Commission of the European Communities 2006). The presence of DON  
54 in swine feedstuffs decreases feed intake, causes feed refusal, and induces occasional  
55 vomiting (Conkova et al. 2003; Dänicke 2002; Diekman and Green 1992). Besides these  
56 effects, reproductive alterations have been observed in humans and pigs (Alm et al. 2006;  
57 Hussein and Brasel 2001; Tiemann and Dänicke 2007).

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2  
3 59 The data reported here were collected in the context of a large study analyzing the transfer of  
4  
5 60 DON and ZON from sows to their foetuses during pregnancy and their influence on  
6  
7 61 physiological parameters in sows and foetuses/piglets (Dänicke et al. 2007; Goyarts et al.  
8  
9 62 2007; Tiemann et al. 2008). Feeding pregnant sows with a *Fusarium* toxin contaminated  
10  
11 63 triticale diet (4.42 mg DON and 0.048 mg ZON per kg diet) in the period from day 35 to 70 of  
12  
13 64 gestation, DON, ZON and their metabolites were detected foetally on day 70 (Goyarts et al.  
14  
15 65 2007), but neither the live weight of sows nor the foetus weight was significantly reduced.  
16  
17 66 Furthermore, no teratogenic or embryolethal effects could be observed in experimental  
18  
19 67 groups. Histopathological evaluation of tissues from sows of the treated group revealed  
20  
21 68 changes in liver and spleen tissues, whereas no significant changes were observed in these  
22  
23 69 tissues in their piglets (Tiemann et al. 2008). However, pregnant sows which were fed a  
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25 70 *Fusarium* toxin contaminated diet containing 9.57 mg DON and 0.36 mg ZON per kg  
26  
27 71 between the gestation days 75 to 110, reduced their mean feed intake. As a result of the initial  
28  
29 72 maternal feed intake depression, the mean live weight of foetuses and the live weight gain in  
30  
31 73 mothers was reduced. However, macroscopic lesion in any organs of sows and piglets were  
32  
33 74 not observed (Dänicke et al. 2007).

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35 75  
36  
37 76 The IGF axis is highly responsive to the nutritional status (Ketelslegers et al. 1995; Simmen et  
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39 77 al. 1998; Thissen et al. 1994). In sheep, reduced growth correlates with lower IGF-I  
40  
41 78 expression in the liver, but not in skeletal muscles, where no changes occurred (Pell et al.  
42  
43 79 1993). The liver is generally thought to be the major production site for IGFs (Jones and  
44  
45 80 Clemmons 1995; Ohlsson et al. 2000). In adult rats, IGF-I transcripts were found in many  
46  
47 81 tissues, but had highest concentrations in the liver (Murphy et al. 1987). The IGF-system is  
48  
49 82 important in regulating foetal and placental growth (DeChiara et al. 1990; deVrijer et al. 2006;  
50  
51 83 Han and Hill 1992; Liu et al. 1991). It acts via endocrine as well as via autocrine/paracrine  
52  
53 84 mechanisms and has metabolic and anabolic functions (Ashworth et al. 2001; Jones and  
54  
55 85 Clemmons 1995). Functionally, the IGF-system comprises the ligands, IGF-I and -II, their  
56  
57 86 receptors, and the IGF-binding proteins (IGFBP). The various IGFBPs are modulators of the  
58  
59 87 biological activity of IGFs and each IGFBP can act in either a paracrine or autocrine manner  
60  
61 88 to elicit its own set of biological responses (McCusker and Clemmons 1992).

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63 89  
64  
65 90 During the present study, firstly, the hepatic expression of major genes of the IGF-system  
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67 91 during pregnancy as reflected by transcripts for IGF-I, IGF-II, their receptors IGF-IR and  
68  
69 92 IGF-IIR and the binding proteins IGFBP-1, 2, and 3 was recorded in foetal livers at days 70

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3 93 and 110 and in maternal livers at day 70 of gestation. Secondly, to find out if the hepatic  
4  
5 94 expression of the IGF system is affected by mycotoxin contaminated diets, sows were fed  
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7 95 with diets naturally contaminated with *Fusarium* toxins during days 35 to 70 and 75 to 110 of  
8  
9 96 pregnancy and the respective levels of IGF transcripts were determined.

10 97

## 11 98 **Materials and methods**

### 12 99 *Experimental design and diets*

13  
14 100 Experiments were performed with pregnant gilts (German Landrace). Sows were kept  
15  
16 101 individually without bedding on a partially slatted floor. Water and food was provided *ad*  
17  
18 102 *libitum*. Animals were fed on a wheat diet, blended with batches of triticale or wheat that were  
19  
20 103 partly naturally contaminated with *Fusarium* toxins. In experiment 1, the contaminated diet  
21  
22 104 contained 15% of *Fusarium* toxin contaminated triticale which resulted in a dietary DON and  
23  
24 105 ZON concentration of 4.42 and 0.048 mg/kg, respectively (Tab. 1). This diet was fed from  
25  
26 106 days 35 to 70 of pregnancy. In experiment 2, 40% of a *Fusarium* toxin contaminated wheat  
27  
28 107 was included in the diet of the experimental group which resulted in dietary DON and ZON  
29  
30 108 concentrations of 9.57 and 0.358 mg/kg, respectively (Tab. 1). This diet was fed from days 75  
31  
32 109 to 110 of pregnancy. The diet for the control groups in both experiments also contained 15%  
33  
34 110 and 40% of triticale and wheat, respectively. However, the DON and ZON concentrations of  
35  
36 111 control diets were 0.15 and 0.004 mg/kg, respectively, in experiment 1, and 0.210 and 0.004  
37  
38 112 mg/kg diet in experiment 2. Both, according to diet composition (commonly used feedstuffs)  
39  
40 113 and because of the very low toxin levels, these control diets are in the range of commonly  
41  
42 114 used complete diets for pigs (Meng et al. 2006). Analysis of wheat and triticale by GC-MS for  
43  
44 115 a detailed characterization of the trichothecene mycotoxin pattern revealed that further B-  
45  
46 116 trichothecene mycotoxins such as 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol and  
47  
48 117 nivalenol, and of the A-trichothecene mycotoxin HT-2 toxin were all lower than the detection  
49  
50 118 limits.

51 119

52 120 Detailed information on the composition of the diet was also described by Goyarts et al.  
53  
54 121 (2007) for experiment 1 and by Dänicke et al. (2007) for experiment 2. The experiments were  
55  
56 122 terminated by delivering the foetuses by Caesarean section. Liver samples from foetuses  
57  
58 123 (n=10)/full-term piglets (n=10) were collected on days 70 and 110, from sows (n=5) only on  
59  
60 124 days 70 of pregnancy. The samples were cut into pieces of about 0.5 cm diameter,  
125 immediately transferred to RNAlater solution (Qiagen, Hilden, Germany), incubated for 24  
126 hrs at 4°C and subsequently stored at -20°C until RNA preparation.

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5 128 *Mycotoxin determination*  
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7 129 DON content of diets was analyzed by HPLC with DAD (diode array detection) after a clean-  
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9 130 up with IAC (immunoaffinity column, DONprep™, R-Biopharm Rhone Ltd., Darmstadt,  
10  
11 131 Germany) according to a slightly modified procedure of R-Biopharm Rhone. The detection  
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13 132 limit was 0.03 mg/kg and the recovery approximately 89%. Further trichothecenes in wheat  
14  
15 133 and triticale were analysed by the Institute of Animal Nutrition of the University of  
16  
17 134 Hohenheim, Germany, using a GC-MS method (Dänicke et al. 2005).

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19 136 *Caesarean section and collection of physiological samples*

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21 137 Both experiments were terminated after exposure to the experimental and control diets for 35  
22  
23 138 days. At both time points, pregnant sows were anaesthetized by an intravenous injection with  
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25 139 ketamin/xylazin (15 mg/kg Ursotamin® and 1.5 mg/kg Xylazin®, Serumwerk Bernburg,  
26  
27 140 Germany). Following anaesthesia, the fetuses/full-term piglets were delivered by Caesarean  
28  
29 141 section. The fetuses/full-term piglets were euthanized after cutting the umbilical cord by  
30  
31 142 injecting T61® (embutramide/mebezoniumiodide/tetracainhydrochloride, Intervet  
32  
33 143 Unterschleißheim, Germany) via the umbilical vein. Fetuses/full-term piglets were weighed  
34  
35 144 and the head-rump-length was measured. Thereafter, livers were dissected and weighed. After  
36  
37 145 euthanizing the sows with T61, livers were dissected and weighed too.

37 146  
38  
39 147 The present study was approved by the Committee for Animal Use and Care of the  
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41 148 Ministerial Agricultural Department of Mecklenburg-Vorpommern, Germany, according to  
42  
43 149 the German Law for Animal Protection (TierSchG).

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46 151 *RNA preparation, cDNA synthesis and quantitative PCR (qPCR)*

47  
48 152 Total RNA was prepared with the RNeasy mini kit (Qiagen, Hilden, Germany). Briefly, tissue  
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50 153 chunks, previously conserved in RNAlater (Qiagen), were transferred into a guanidine iso-  
51  
52 154 thiocyanate containing buffer and homogenized with an Ultra Turrax (IKA instruments,  
53  
54 155 Germany). Subsequently one volume of 70% ethanol (v/v) was added to the homogenized  
55  
56 156 lysates and the RNA was extracted from the samples by adsorption to silica gel spin columns.  
57  
58 157 After three washing steps and elution in 30 µl deionised, RNase free water (washing buffers  
59  
60 158 and water provided by the kit), RNA was quantified in a GeneQuant II instrument  
159  
160 159 (Pharmacia, Freiburg, Germany). Quality of RNA was monitored from randomly selected  
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160 160 samples by denaturing agarose (1%) gel electrophoresis.

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5 162 Gene-specific primers for cDNA synthesis and amplification of porcine IGF-I, IGF-II, IGF-  
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7 163 IR, IGF-IIR, and IGFBP-1, 2, 3 transcripts were designed according to published mRNA  
8  
9 164 sequences (Tab. 2). For cDNA synthesis, 0.5 µg total RNA was reversely transcribed in a  
10  
11 165 25µl reaction volume using M-MLV reverse transcriptase, RNase H Minus, Point Mutant  
12  
13 166 (Promega, Mannheim, Germany) with gene-specific primers. The freshly synthesized cDNA  
14  
15 167 samples were cleaned with the High Pure PCR Product Purification Kit (Roche, Mannheim,  
16  
17 168 Germany) and eluted in 50 µl elution buffer. The identity of products generated with different  
18  
19 169 primer pairs was controlled by sequencing.

20  
21 170  
22 171 For qPCR, routinely, 0.25 and 0.5µl of the cDNA samples were amplified with the  
23  
24 172 LightCycler-FastStart DNA Master<sup>PLUS</sup> SYBR Green I Kit (Roche) in 10µl total reaction  
25  
26 173 volume. Amplification and quantification of products were performed in a LightCycler® I  
27  
28 174 instrument (Roche) under the following cycling conditions: Pre-incubation at 95°C for 10',  
29  
30 175 followed by 45 cycles denaturation at 95°C for 15'', annealing at 60°C for 10'', extension at  
31  
32 176 72°C for 10'' and single point fluorescence acquisition at 83°C for 6''. The melting peaks of  
33  
34 177 all samples were routinely determined by melting curve analysis in order to ascertain that only  
35  
36 178 the expected products had been generated. Additionally, the length of all PCR products was  
37  
38 179 monitored by agarose gel electrophoresis analysis (3% agarose, ethidium bromide stained).  
39  
40 180 Cloned PCR products of the respective genes were used to generate external standard curves.  
41  
42 181 Routinely, dilutions of standards covering five orders of magnitude ( $5 \times 10^{-16}$  to  $5 \times 10^{-12}$  g  
43  
44 182 DNA/reaction) were freshly diluted from stocks of 10ng DNA/µl and co-amplified during  
45  
46 183 each run. Copy numbers were calculated relative to the amount of total RNA previously  
47  
48 184 subjected to cDNA synthesis. To normalize for variations between individual LightCycler  
49  
50 185 runs one or two arbitrarily selected samples were co-amplified as calibrators.

51 186  
52 187 *Statistical analysis*

53 188 The data were analyzed using the Student-Newman-Keuls test following one-way analysis of  
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55 189 variance (ANOVA) using the SigmaStat Statistical Analysis System (Jandel Scientific, San  
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57 190 Rafael, CA). The data represent means ± SEM. All results with  $P < 0.05$  were considered  
58  
59 191 statistically significant. The number of animals used in both of the experiments is described in  
60  
192 the figures.

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194



## 195 **Results**

### 196 *Hepatic transcripts of IGF genes show very different levels during ontogenesis*

197 Hepatic transcript levels of different IGF genes were found at very different expression levels  
198 covering several orders of magnitude. IGF-II transcripts exceeded levels of  $4 \times 10^7$  copies/ $\mu$ g  
199 RNA in foetal livers at day 70, whereas foetal expression of IGF-I was more than three orders  
200 of magnitude lower (Fig. 1). Most of the transcripts, IGF-II, IGF-IR, IGF-IIR, and IGFBP-2,  
201 showed highest hepatic concentrations during foetal life. However, two of the transcripts  
202 showed an opposite ontogenetic regulation. Particularly, levels of IGF-I transcripts in livers of  
203 pregnant sows, exceeded foetal levels by 14 to 17 fold. But also IGFBP-3 showed  
204 significantly increased levels in adult animals. The levels of some transcripts significantly  
205 decreased during foetal development as observed in case of IGF-II, IGF-IR, and IGFBP-3.  
206 However, IGFBP-2 transcript concentrations significantly increased towards term. Transcript  
207 levels of IGF-I and IGF-IIR did not show significant differences between both foetal stages  
208 but had significantly higher or lower levels in adult sows, respectively. IGFBP-1 transcripts  
209 showed similar levels at all ontogenetic stages but tended to slightly decrease with age.

### 211 *Hepatic transcript levels are not affected by diets naturally contaminated with mycotoxins*

212 In experiment 1 no differences in feed intake were observed between the experimental and  
213 control groups. The live weight gain over the tested gestation period between day 35 and 70  
214 was not significantly influenced by feeding the mycotoxin contaminated diet. The mean fetus  
215 weight was not significantly different between the control group and the group fed with a  
216 *Fusarium* toxin contaminated diet (Goyarts et al. 2007).

218 In experiment 2, the sows fed with the *Fusarium* toxin contaminated diet consumed 38% less  
219 feed than the sows fed with the control diet and the live weight over the tested gestation  
220 period between day 75 and 110 was significantly reduced by 93%. The mean weight of piglets  
221 also tended to be reduced from  $1335.2 \pm 347.0$  g to  $1120.9 \pm 260.2$  g ( $p=0.087$ ) in the control  
222 and experimental group, respectively. The liver weight was not altered in sows. At terminal  
223 necropsy there was no macroscopic lesion observed in any organ of sows and piglets exposed  
224 to the *Fusarium* toxin (Dänicke et al. 2007).

226 The hepatic transcript concentrations of all IGF genes however were not significantly affected  
227 by feeding pregnant sows for 35 days at different stages of gestation with diets naturally  
228 contaminated with *Fusarium* toxins. Instead, the ontogenetic expression profiles of IGF-I,

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3 229 IGF-II, IGF-IR, IGF-IIR, IGFBP-1, IGFBP-2, and IGFBP-3 were virtually identical between  
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5 230 the experimental and respective control groups (Fig. 2).  
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## 8 232 **Discussion**

9  
10 233 We observed that the hepatic expression of IGF-I was remarkably high in pregnant sows. This  
11  
12 234 is in line with data reported by others (Olausson and Sohlstrom 2003). The enhanced IGF-I  
13  
14 235 transcript levels in the maternal liver could act as an endocrine growth factor necessary to  
15  
16 236 promote foetal growth and development (Persson et al. 1997; Sterle et al. 1998). IGFBP-3  
17  
18 237 expression was also significantly higher in the sow compared to foetal levels. It has been  
19  
20 238 shown that levels of IGFBP-3 secretion in humans increase during pregnancy (Giudice et al.  
21  
22 239 1991). The liver seems to be the major source for circulating IGFBP-3 in pig (Dunaiski et al.  
23  
24 240 1999; Lee et al. 1993). Studies suggest that GH may indirectly regulate IGFBP-3 expression  
25  
26 241 by enhanced IGF-I production (Chin et al. 1994; Silha and Murphy 2002). But IGFBP-3  
27  
28 242 levels may also be modified by a pregnancy-specific protease as in some other species  
29  
30 243 (Donovan et al. 1991; Lassarre and Binoux 1994).  
31  
32 244

33 245 Low level expression of IGF-I in foetal livers is consistent with several other reports (Adams  
34  
35 246 et al. 1983; Gluckman et al. 1983; Gluckman and Butler 1983; Liu et al. 1991; Peng et al.  
36  
37 247 1996). This is also in line with the view, that IGF-I is an important factor regulating postnatal  
38  
39 248 but not prenatal growth (Daughaday and Rotwein 1989; Lee et al. 1991; Peng et al. 1996).  
40  
41 249

42 250 In contrast to data of others (Peng et al. 1996) we clearly detected IGF-IR transcripts in foetal  
43  
44 251 livers, however with quite lower levels in full term fetuses compared to the earlier stage. This  
45  
46 252 discrepancy is most likely due to the enhanced sensitivity of qPCR used during the present  
47  
48 253 study, compared to Northern blot analysis used by Peng et al. (1996).  
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51 255 Most of the IGF genes however showed higher levels of expression in foetal compared to  
52  
53 256 adult liver tissue. Particularly IGF-II, which showed highest levels of expression in fetuses at  
54  
55 257 day 70 is considered to have a major role in foetal growth (Butler and LeRoith D. 2001;  
56  
57 258 DeChiara et al. 1990; DErcole 1996; Kampman et al. 1993; Martin et al. 2005). Hepatic  
58  
59 259 expression levels of IGF-II and of IGF-IR were significantly down-regulated in full term  
60  
260 piglets compared to earlier foetal stages. IGF-II is considered as a dominant regulator of  
261 foetal adrenal growth during mid-gestation, due to high expression and regulation by ACTH  
262 (Coulter et al. 1996). A marked rise of cortisol and triiodothyronine concentrations close to

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3 263 term has been shown to play an important role for the maturation of the GH/IGF-system  
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5 264 activating expression of growth factors and their receptors. Down-regulation of the hepatic  
6  
7 265 IGF-II gene toward term has been previously shown to be dependent on the prepartum  
8  
9 266 cortisol surge (Li et al. 1996; Li et al. 1993). The authors found that the prepartum rise in  
10  
11 267 foetal plasma cortisol was responsible for down-regulating IGF-II gene expression in the  
12  
13 268 foetal ovine liver close to term. Additionally, it was demonstrated by others (Forhead et al.  
14  
15 269 1998) that increased concentrations of both, cortisol and triiodothyronine, appear necessary to  
16  
17 270 induce down-regulation of hepatic IGF-II transcripts in foetal sheep close to term. The pre-  
18  
19 271 term down-regulation of hepatic IGF-II gene expression is part of a general ontogenetic  
20  
21 272 change along the somatotrophic axis. At birth, IGF synthesis switches from a local GH-  
22  
23 273 independent production of predominantly IGF-II *in utero* to the adult GH-dependent  
24  
25 274 production of endocrine IGF-I (Gluckman 1995). After birth, IGF-II expression is decreased  
26  
27 275 in most tissues (Sara and Hall 1990). This is completely in line with the data of our present  
28  
29 276 study.

30 277  
31 278 Foetal levels of IGFBP-2 transcripts increased from day 70 towards term, which is consistent  
32  
33 279 with earlier reported data in pig (Lee et al. 1993) and sheep (Delhanty and Han 1993),  
34  
35 280 indicating that hepatic IGFBP-2 transcripts and serum IGFBP-2 levels increase during the  
36  
37 281 second half of gestation. This suggests that IGFBP-2 derived from the foetal liver is an  
38  
39 282 important endocrine factor during late gestation.

40 283  
41 284 In experiment 2, we observed that mothers showed a significant decrease in feed intake after  
42  
43 285 feeding the mycotoxin-contaminated diet from day 75 to 110 of gestation. The mean weight  
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45 286 of piglets tended to be reduced in the experimental group (Dänicke et al. 2007). However, this  
46  
47 287 was not paralleled by any significant effects on the hepatic expression profiles of IGF-I, IGF-  
48  
49 288 II, IGF-IR, IGF-IIR, or IGFBP- 1, 2, 3 genes. Based on these results, we suggest that the  
50  
51 289 transcription of hepatic genes of the IGF system was not associated with the observed  
52  
53 290 tendency of growth restriction in the mycotoxin group on day 110. This is in line with  
54  
55 291 previous studies demonstrating that a moderate food restriction during pregnancy did not  
56  
57 292 reduce the expression of hepatic mRNA for IGF-I and IGF-II in rats (Monaco and Donovan  
58  
59 293 1996) and guinea pigs (Olausson and Sohlstrom 2003). However, we can not exclude the  
60  
294 possibility that levels of individual IGF proteins could be changed directly by mycotoxin  
295 actions or indirectly by mycotoxin induced food refusal through post-transcriptional or even  
296 post-translational mechanisms.

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5 298 From the present data we conclude that diets naturally contaminated with mycotoxins do not  
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7 299 affect hepatic transcript concentrations of major IGF genes even if growth depression can be  
8  
9 300 observed.

10 301  
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12 302 **Acknowledgments**  
13  
14 303 The authors are grateful for support through a grant from the Deutsche  
15  
16 304 Forschungsgemeinschaft (Ti 189/5-3; DA 558/3-3) and from the technical assistance of Mrs.  
17  
18 305 M. Anders, P. Reckling, and V. Tesch.

19 306  
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20 **Figure captions**  
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24 462 Figure 1: Normal hepatic transcript concentrations of genes of the IGF system in fetuses/full-  
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26 463 term piglets at days 70 and 110 and sows at day 70 of gestation. The sows were fed with a  
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28 464 control diet. Each bar represents the mean ( $\pm$  SEM) transcript abundance of IGF genes. Bars  
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30 465 denoted by different letters represent significantly different means ( $P < 0.05$ ).  
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33 467 Figure 2: Hepatic transcript concentrations of genes of the IGF system in fetuses (B and C)  
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35 468 and sows (A) fed with a control or *Fusarium* contaminated diet on the 35<sup>th</sup> to the 70<sup>th</sup> and the  
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37 469 75<sup>th</sup> to the 110<sup>th</sup> day of gestation. Each bar represents the mean ( $\pm$  SEM) transcript  
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39 470 concentrations of IGF genes of each experimental and control group. Bars denoted by  
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41 471 different letters represent significantly different means ( $P < 0.05$ ).  
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Table 1. Composition of the experimental diets (g/kg)

	Experiment 1		Experiment 2	
	CON	MYCO	CON	MYCO
<i>Components:</i>				
Control triticale	150	0		
Contaminated triticale	0	150		
Control wheat	250	250	400	0
Contaminated wheat			0	400
Barley	284.3	284.3	284.3	284.3
Dried chips	100	100	100	100
Soybean meal	175	175	175	175
Soy oil	10	10	10	10
Vilomin 19730 <sup>a</sup>	30	30	30	30
L-lysin HCL	0.7	0.7	0.7	0.7
<i>Calculated composition:</i>				
Dry matter	875	875	884	877
Crude protein	167	167	159	162
Deoxynivalenol (mg/kg) <sup>b</sup>	0.15	4.42	0.21	9.57
Zearalenone (µg/kg) <sup>b</sup>	3.5	48.3	4	358

CON= control group; MYCO=*Fusarium* toxin contaminated group

<sup>a</sup>Premix provided per kg diet: Ca, 6.3 g; P, 2.1 g; lysine, 0.6 g; Na, 1.8 g; Mg, 0.3 g; vitamin A, 15000 IE; vitamin D<sub>3</sub>, 1500 IE; vitamin E, 45 mg; Cu, 22.5 mg; Se, 0.36 mg

<sup>b</sup> Measured by HPLC-methods

Table 2. Primers used for cDNA synthesis and qPCR.

Genes	Forward primers	Reverse primers	Primers for cDNA synthesis	size (bp)	Acc. Nos.
<i>Igf1</i>	GCACATCACATCCTCTTCGCATC	AGCTCCGGAAGCAGCACTCATC	ATCCTGCCAGTGGCATGTCATTC	212	NM_214256
<i>Igf1r</i>	GACTGGTCCCTGATCCTGGATG	CTCGGGGTGGCAGCACTCGTG	GCACTCGCCATCATGGATCAC	237	NM_214172
<i>Igf2</i>	GCCTTGGCCTCGTGCTGCTATG	CAGCTACGGAAGCAGCACTCTTC	AGGTGTCATAGCGGAAGAACTTG	176	NM_213883
<i>Igf2r</i>	GCCTCCAGATCTCTTCTGGAATTC	GCTCTCTGTCAAACACGTAGCAC	AAGGGACAGGCACGCAACTGTG	232	AF339885
<i>Igfbp1</i>	TCCTGGACAGTTTCCACCTGATG	ATAGAATCCGTTCTTGTTCAGTTC	CGTGGAGCCCAGGATCTTCTTC	236	AB053605
<i>Igfbp2</i>	CCCGAGCAGGTTGCAGACAATG	GCGGCGGCCGCAGCTTCTTG	GTTCCAGGACCTGGTCCAATC	235	NM_214003
<i>Igfbp3</i>	GAGCACGGACACCCAGAATTC	GAAGCCCTTCTTGTTCGAGTTAG	GAGGGGCTGCCCGTACTTATC	157	J05228

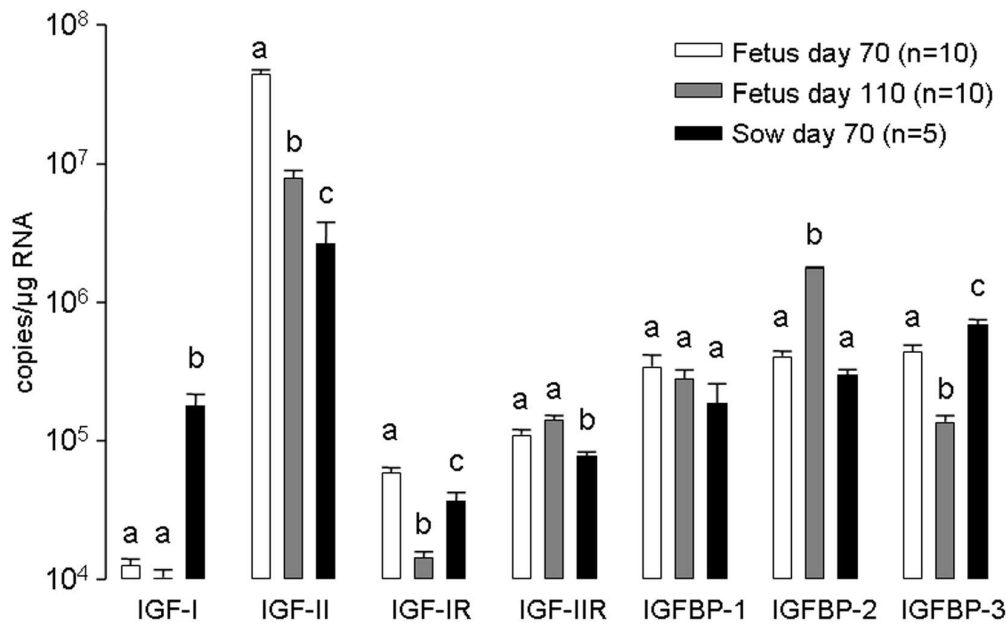


Figure 1: Normal hepatic transcript concentrations of genes of the IGF system in fetuses/full-term piglets at days 70 and 110 and sows at day 70 of gestation. The sows were fed with a control diet. Each bar represents the mean ( $\pm$  SEM) transcript abundance of IGF genes. Bars denoted by different letters represent significantly different means ( $P < 0.05$ ).  
139x86mm (600 x 600 DPI)

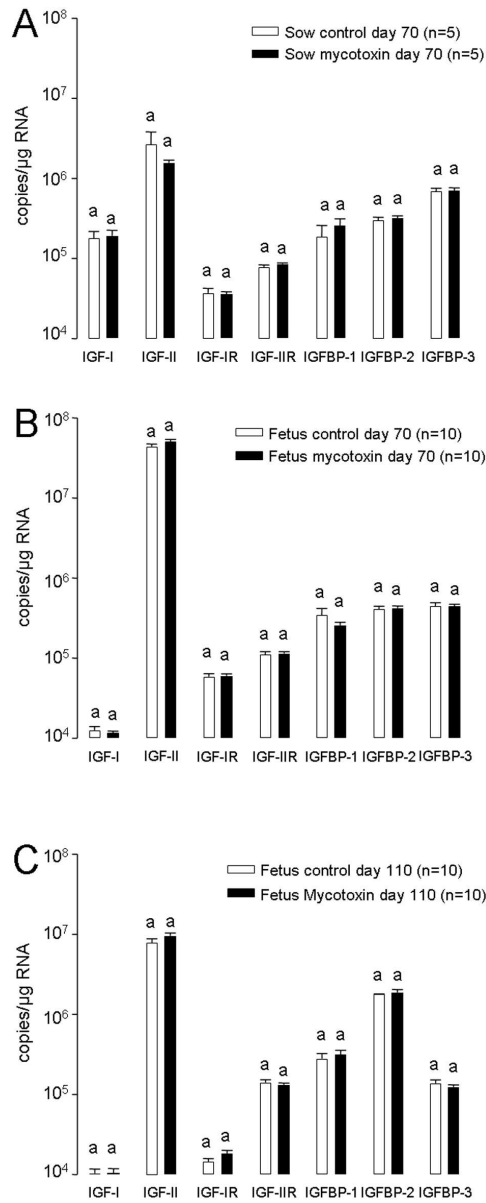


Figure 2: Hepatic transcript concentrations of genes of the IGF system in fetuses (B and C) and sows (A) fed with a control or *Fusarium* contaminated diet on the 35th to the 70th and the 75th to the 110th day of gestation. Each bar represents the mean ( $\pm$  SEM) transcript concentrations of IGF genes of each experimental and control group. Bars denoted by different letters represent significantly different means ( $P < 0.05$ ).

80x199mm (600 x 600 DPI)