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Modulation of the somatotropic axis, adiponectin and cytokine secretion during highly pathogenic porcine reproductive and respiratory syndrome virus type 1 (HP-PRRSV-1) infection

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Short title: Endocrine and cytokine response to PRRSV infection

Abstract

Porcine reproductive and respiratory syndrome virus (PRRSV) is known to be clinically responsible for reproductive failure in sows and post-weaning respiratory disease in growing piglets. During the last years, highly pathogenic PRRSV isolates have been discovered. In Italy, a PRRSV-1 subtype 1 strain (namely PR40/2014) characterized by high pathogenicity was isolated and experimental infection was characterized in terms of virological/clinical features and immune modulation (Canelli et al., 2017; Ferrari et al., 2018). The present study was performed in 4-week-old pigs experimentally infected with the highly pathogenic PRRSV1_PR40/2014 (HP-PR40) or with the conventional PRRSV1_PR11/2014 (PR11). The aim was to evaluate the interrelation between plasmatic hormones and cytokines in infected pigs compared to uninfected controls in order to address potential effects on the course of an experimental infection. The time-related changes of growth hormone (GH), insulin-like growth factor-1 (IGF-1), adiponectin, interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) levels appear to be modulated by the infection depending on the PRRSV isolate (HP-PR40 vs. PR11). In particular, in HP-PR40 infected animals, the association between high GH levels and viremia may testify the need to block the anabolic action of GH in order to shift available energy

towards the immune response. This need appeared to be delayed in PR11 animals, given the lower pathogenicity of the isolate. Adiponectin, IL-6 and TNF-α course supports the hypothesis of GH resistance mechanisms to guarantee homeostasis in HP-PR40 animals and underlines the key role of energy availability in events leading to an effective response to the virus.

Keywords: highly pathogenic PRRSV (HP-PRRSV); growth hormone resistance; insulin-like growth factor-1; adiponectin; pro-inflammatory cytokines.

Introduction

Bidirectional communication between the immune and neuroendocrine systems is well known, as immune cells produce hormones and similarly, neuroendocrine cells secrete cytokines and express specific cytokine receptors. This multi-directional communication guarantees the maintenance of homeostasis and, therefore, of health. In particular, it responds to pathogen challenge to re-establish homeostasis (McEwen and Wingfield, 2010). Hormones and cytokines, particularly pro-inflammatory cytokines, are the main players of this coordinated cross-talk. Their action translates into enhancement of innate immunity, support for acquired immunity and control of immune-mediated inflammation, with an efficiency increase of the immune response against infection (Borghetti et al., 2009).

Porcine reproductive and respiratory syndrome (PRRS) is a widespread disease caused by an enveloped, positive-stranded RNA virus (PRRSV) which belongs to the family Arteriviridae. The two well-known genotypes of the virus, type 1 or PRRSV-1 (European) and type 2 or PRRSV-2 (North American), have been recently classified as two viral species within the genus Porartevirus (Adams et al., 2017). The intra-species variability is very high so that PRRSV-1 can be divided into at least four (pan-European subtype 1 and East European subtypes 2, 3 and 4) and PRRSV-2 into at least nine subtypes (Stadejek, et al.,2013). The infection shows three phases identified as acute phase, persistence and extinction (Lunney et al., 2016). In the acute phase, the lung is the preferential site of

55 infection and the typical respiratory disease PRRSV causes in new-born and growing piglets results
56 in severe economic losses. Viremia persists for several weeks despite the presence of circulating
57 antibodies since virus-neutralizing antibodies (VNA) develop very slowly and sometimes maintain
58 very low titres. In fact, the importance of an efficient cellular response has been demonstrated in
59 terms of cytolitic cells and IFN-γ secreting cells, especially during the first weeks after infection
60 (Martelli et al., 2009, 2013). During persistence, the virus replicates in lymphoid organs and
61 replication subsequently declines until the disappearance of the virus. However, the virus may
62 replicate for more than 250 days (Wills et al., 2003).
63 Important breakthroughs in the understanding of PRRSV biology have been obtained in recent years,
64 however, a more comprehensive understanding of the mechanisms of the immune and endocrine
65 system responses to PRRSV infection is needed in order to improve control strategies and design
66 novel vaccines which counteract/dampen PRRSV infections/co-infections and favours balanced
67 integrated responses. A previous study by the authors (Borghetti et al., 2011) showed that time-related
68 changes of hormones such as GH and cortisol, in association with pro-inflammatory and anti-
69 inflammatory cytokines, occur during natural infection by a PRRSV isolate and that vaccination can
70 modulate these responses in terms of a better support for innate immunity.
71 The existence of genetically divergent PRRSV isolates, with varying degree of virulence, makes the
72 understanding of PRRSV immunopathogenic mechanisms more complex. Furthermore, different
73 clinical and virological outcomes have been reported within the known genotypes, suggesting the
74 emergence of highly pathogenic (HP) PRRSV strains (Zhang et al., 2016). In this context, an Italian
75 PRRSV-1 subtype 1 strain (namely PR40/2014) characterized by high pathogenicity was recently
76 isolated and experimental infection was characterized in terms of virological/clinical features and
77 immune modulation (Canelli et al., 2017, 2018; Ferrari et al., 2018).
78 The present experimental study was performed in piglets infected with two European isolates showing
79 different pathogenicity: PRRSV-1_PR40/2014 (HP) and PRRSV-1_PR11/2014 (non-HP), a
80 conventional isolate, both isolated in Italy in 2014. The aim of the study was to compare the endocrine

and immune response to two different strains of PRRSV with different pathogenicity as this feature can cause variations in morbidity and mortality. In particular, the understanding of strain influence on the anti-viral response could improve the control strategies and management of vaccine protocols. Therefore, we evaluated plasma concentrations of growth hormone (GH), insulin-like growth factor-1 (IGF-1), adiponectin, interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) and their association with changes of viremia and antibody concentration. Why did we choose these factors? What connects them? They are all involved in the so called "check and balance system", where the specific effects of each factor (i.e. growth, immune response modulation, metabolism regulation, etc.) are to be read and play a role in body homeostasis.

Materials and methods

Animals and experimental design

The study was conducted in a biosafety level 2 (BSL-2) facility. Seventeen, 4 week-old, conventional mixed sex pigs from a PRRSV-free herd were included in the study. These animals, allocated in three different rooms of the experimental facility, were checked to confirm PRRSV-negativity by quantitative real-time PCR (qtRT-PCR) according to Martelli et al. (2013), randomized by random numbers table (obtained by Microsoft Excel) and assigned to three different groups:

Group 1: 7 pigs, intra-nasally (IN) infected with PRRSV1_PR40/2014 (HP-PR40);

Group 2: 7 pigs, IN infected with PRRSV1_PR11/2014 (PR11);

Group 3: 3 pigs, IN inoculated with medium only (non-infected / negative control).

For both group 1 and group 2, a dose of 10^5 TCD_{50} PRRSV/pig in 2 ml (1 ml/nostril) was IN inoculated. Working stock for both viruses was the 3rd passage on porcine alveolar macrophages (PAMs), confirmed by a PRRSV-specific staining on cells and by qtRT-PCR (Martelli et al., 2013), titrated and tested negative for other relevant viruses (porcine circovirus type 2, PCV2, and swine influenza virus, SIV). Plasma and serum samples were collected on the day of inclusion (-6) and on days 0, 3, 7, 10, 14, 17, 21, 28 and 35 post-infection (pi). On day 35 pi the animals surviving the

experiment were euthanized, according to standard protocols for the humane treatment of experimental animals. The experimental study was approved by the Ethical Committee and by the Ministry of Health in Italy (171/2016-PR), according to the European and National regulations on experimental infection studies and animal welfare.

Clinical monitoring and gross anatomo-pathological lesions

Daily rectal temperature, average daily weight gain (ADWG) and general conditions, and the appearance of respiratory symptoms such as coughing, nasal discharge, abnormal breath were recorded and scored for severity. Technicians involved in this monitoring were blinded. Clinical signs and gross anatomical lesions were previously described (Canelli et al., 2017). In summary, control animals did not exhibit significant clinical signs or gross lesions. Mortality rate was similar in the two infected groups. The severity of the clinical conditions made it necessary to euthanize four pigs per group during the study. All the other pigs survived until the end of the experimental period.

No remarkable lung lesions were observed in the negative control group while interstitial pneumonia was evident in infected groups, independently from the isolate. In particular, animals showed multifocal to coalescing areas of atelectasis, congestion and alveolar and interlobular edema. The incidence of lesions was higher in the HP-PR40 group compared to the PR11 group. Atrophy of the thymus was detected in both groups, with an almost complete atrophy of the cervical part of the thymus in the HP-PR40 group.

Interstitial pneumonia with lymphocytes and monocyte/macrophage septal infiltrations was observed at different degrees of severity in both groups.

In both groups, thymus showed atrophy of germinal centres and lymphocytopenia of lobular medulla and reduced T cell subpopulation. In lymph nodes, B cells were very scarce and located in the germinal centers of mildly activated follicles while T cells were located all around the follicles. These patterns were present in the HP-PR40 group with a higher severity respect to PR11 pigs. Negative controls did not show any microscopic lesions.

Viremia and virus re-isolation

RNA was extracted from serum by using Trizol LS (Invitrogen) following the manufacturer's instructions. Serum virus RNA copy number was evaluated by using a quantitative real time RT-PCR (qtRT-PCR) as previously described (Martelli et al., 2013).

Viruses used for experimental infection were re-isolated from sera of infected pigs with the highest copy numbers detected using qtRT-PCR, by one-passage cultivation on PAMs and adapted to MARC-145 cells. The MARC-145 adapted viruses were confirmed as homologous with the original strains of the infection by ORF5 and ORF7 sequencing.

Hormone and cytokine assays

Samples were analyzed in duplicate for hormones and cytokines by ELISA validated for swine. Plasma samples were assayed for growth hormone (GH) by validated ELISA as previously described (Baratta et al., 2002; Saleri et al., 2016). The intra- and inter-assay coefficient of variation (CV) were 3.3% and 6.2%, respectively. The minimal detection limit was 100 pg/ml. Porcine insulin-like growth factor-1 (IGF-1) detection was determined by the porcine IGF-1 ELISA kit (MyBioSource Inc., San Diego, CA, USA). The minimum detectable dose was 0.188 ng/ml. Intra- and inter-assay CV were 4.2% and 7.1%, respectively. A specific commercial kit was used to evaluate adiponectin plasma concentration (porcine ELISA Kit for Adiponectin, Cloud Clone Corp., Houston, TX, USA). Sensitivity was 0.114 ng/ml; intra- and inter-assay CV were 4.2% and 5.4%, respectively. Tumor necrosis factor-α (TNF-α) levels were analyzed by using a specific commercial kit (Quantikine porcine TNF-α, R&D Systems, Abingdon, UK). The intra- and inter-assay CV were 5% and 7%, respectively. The sensitivity was 4 pg/ml. Interleukin-6 (IL-6) concentration was assayed by a competitive commercial kit ELISA (Quantikine porcine IL-6, R&D Systems, Abingdon, UK). The intra- and inter-assay CV were 6.9% and 8%, respectively. The sensitivity was 10 pg/ml.

Statistical analysis

Immunological and hormonal data were analyzed by ANOVA using a mixed model with group, sampling and the interaction between group and sampling as fixed factor. The basal values recorded before PRRSV infection were used as a covariate. Experimental data were presented as mean ±

159 standard error of mean (SEM). Statistical significance was reached for $P<0.05$. Differences among
160 groups were considered significant if $P<0.05$, and as a trend to significance when $0.05 \leq P<0.10$.
161 ANOVA was performed by applying the GLM procedure SAS 9.4 (2014).
162
163 **Results**
164 *Viremia and virus re-isolation*
165 Comprehensive data relative to the course of viremia are reported in Canelli et al. (2017). For reasons
166 of completeness and clarity, we provide here a description of virological data. Briefly, at 0 dpi all
167 animals were PRRSV-negative. PRRSV was not detected in sera from control group animals. In the
168 HP-PR40 and PR11 groups, viremia started at 3 dpi and peaked at 7 and 10 days after inoculation,
169 respectively. Uninfected control animals remained negative throughout the study.
170 After 10 dpi, viremia started to decrease in both infected groups. Survived animals showed values
171 lower than one cDNA Log_{10} copies/µL at the end of the study period. HP-PR40 pigs showed a higher
172 viremia for the whole duration of the study.
173 The phylogenetic analyses performed after sequencing the isolates at the viremic peak directly from
174 serum and the isolates used for infection confirmed, in all cases, a homology >98% for ORF5 and
175 >99.5% for ORF7.
176 *Hormone and cytokine plasma levels*
177 The plasma concentrations of GH over the period of study are shown in Figure 1. On day 0, mean
178 plasma levels in control, HP-PR40 and PR11 groups were 7.45±0.12, 6.89±0.32 and 7.41±0.67 ng/ml,
179 respectively. From day 7 until day 21 pi, plasmatic GH significantly increased ($P<0.05$) in HP-PR40
180 pigs, with statistically significant differences observed compared to control and PR11 pigs.
181 Thereafter, GH levels decreased to reach basal levels at 35 dpi. In control and PR11 groups, plasma
182 GH levels were consistent with physiological variations of the hormone. Plasma concentration of
183 IGF-1 (Figure 2) on day 0 was 78.6±7.76 (control), 79.4±6.9 (HP-PR40) and 92.4±13.2 ng/ml (PR11).
184 In the HP-PR40 group, a significant decrease ($P<0.05$) in plasma levels was observed from day 7 to

185 day 21 pi as compared to PR11 and control groups. The PR11 group did not show significant
186 differences as compared to the control group. The adiponectin profiles in the three groups are shown
187 in Figure 3. No significant differences were detected in plasma levels on day 0 among groups: mean
188 values were 8.7±0.2 (control), 5.9±0.5 (HP-PR40) and 7.1±0.3 (PR11) µg/ml. Adiponectin levels in
189 the HP-PR40 group showed significantly higher concentrations ($P<0.05$) in correspondence of the
190 viremia peak and until day 14 pi, as compared to the control group and the PR11 group. PR11 pigs
191 showed a significant increase in adiponectin levels starting after the vanishing of viremia (17 dpi)
192 until the end of the experimental period. Tumour necrosis factor-α plasma concentration (Figure 4)
193 at the start of the study was 65.3±6.6 (control), 57.4±4.7 (HP-PR40) and 53.7±6.5 (PR11). In the HP-
194 PR40 group, a significant increase ($P<0.05$) was observed at 3 and 7 dpi. The PR11 group showed a
195 similar trend, even if the significant increase ($P<0.05$) in TNF-α levels was at 14 and 17 dpi. The
196 results regarding IL-6 are shown in figure 5. In pigs inoculated with the PR11 isolate (PR11 group),
197 IL-6 showed a significant increase ($P<0.05$) in correspondence of the viremic peak (day 7 pi),
198 followed by a decrease to basal levels. Animals infected with the PR40 strain (HP-PR40 group)
199 showed inhibition of the IL-6 response from day 7 pi to the end of the study; the reduction appeared
200 to be significant at 7, 14, 17, and 21 dpi as compared to the control group and the PR11 group
201 ($P<0.05$).
202
203 **Discussion**
204 Infection by PRRSV in piglets is characterized by fever, anorexia and respiratory disease and in
205 infected new-born and growing pigs, the typical consequence of PRRSV infection is a respiratory
206 disease due to interstitial pneumonia. Gross lesions observed following PRRSV infection are
207 dependent on the virus isolate, genetics of the infected pig, stress factors and other complicating
208 agents, particularly bacteria and interactions with other viruses (e.g. PCV2). Frequently, innate and
209 acquired immune responses are inefficient to early and efficiently eliminate the virus because PRRSV
210 is able to suppresses type 1 interferons from infected cells and innate cytokine secretion (Loving et

al., 2015). Furthermore, the cytokine response can be influenced by the virus strain, in fact, available evidence suggest that different strains can induce different cytokine release patterns (Park et al., 2008; Silva-Campa et al., 2010). For this reason, we chose to evaluate the dynamics of response to two Italian isolates of PRRSV differing in pathogenicity, starting from a study approach based on the bidirectional communication between the immune and neuroendocrine systems. It has become accepted that energy availability is strongly linked to the integrated response of the immune and neuroendocrine systems (Ashley and Demas, 2017).

It is known that classical hormones modulate immunity (Taub, 2008; Borghetti et al., 2009; Dantzer, 2018) and pro-inflammatory cytokines can act with local and systemic hormonal effects (Elenkov, 2008). In fact, GH, that is essential in growth regulation, belongs to the large group of class 1 helical cytokines and mainly influences the immune responses. GH is a potent anabolic hormone: several of its effects are mediated by IGF-1 which is mainly produced by the liver.

The first result that stands out from this study is that GH levels increased in the HP-PR40 and PR11 groups as compared to controls. Numerous studies have shown that inflammatory states lead to a state of hepatic GH resistance. The reduced GH sensitivity may be accompanied by decreased hepatic GH receptor (GH-R) expression, which in turn leads to GH resistance. This condition is characterized by normal or elevated levels of GH associated with decreased IGF-1 levels (Soendergaard et al., 2017), which results in an altered hepatic response to GH. Physiologically, GH resistance occurs in any status where it is essential to limit energy expenditure by modulating the anabolic actions of GH. In humans, chronic diseases, malnutrition and systemic inflammation can lead to GH resistance (Soendergaard et al., 2017) whereas in swine no data are available on this phenomenon. In the present study, infected animals (HP-PR40 and PR11 groups) showed higher levels of GH (compared to uninfected controls), associated with a marked delay in average daily weight gain. It is also interesting to observe that the higher GH levels in the HP-PR40 group occurred simultaneously with the viremic peak. Therefore, these results would suggest a state of GH resistance. There are two particularly interesting aspects about the results on GH secretion. First, the viral strain seems to influence the

response: the highly pathogenic strain (HP-PR40) is characterized by severe clinical signs in growers as compared to PR11 (Canelli et al., 2017). Therefore, the correspondence between high GH levels and viremia may indicate the need to block the anabolic action of GH, in order to shift available energy towards the immune response. This need could be delayed in PR11 animals, given the lower pathogenicity and clinical impact of the strain. In fact, in this group, the increase of GH levels was subsequent to the viremic peak. Secondly, the significant decrease in plasma levels of IGF-1 during viremia would support the presence of GH resistance in the HP-PR40 group. In HP-PR40 infected animals, the response may be directly linked to the inflammatory response and to the levels of pro-inflammatory cytokines, namely TNF-α and IL-1, specifically induced by viral infection (unpublished data).

The growth hormone is mainly secreted by the anterior pituitary, but the pituitary is not the only site of production. Weignent et al. (1988) first showed that immune cells not only express GH receptors but also express and secrete GH. In humans, immune cells mainly express two GH genes, which are also expressed in the anterior pituitary (Melen et al. 1997; Kooijman et al., 2000). It has been demonstrated that GH mainly stimulates the immune response and is directly involved in thymus activity and involution (Verburg et al., 2017). It is known that GH regulation in the immune and endocrine systems is very different. Systemic GH binding to its lymphocyte receptors would cause an up-regulation of secretion of local GH. It could act in an autocrine/paracrine fashion on immune cells themselves to produce further amounts of GH (positive feedback) or to induce the production of cytokines. GH and cytokines use the same JAK-STAT molecular pathway and a direct positive effect of GH on TNF-α secretion in humans was demonstrated (Bozzola et al., 2003). Some of the key inflammatory cytokines, such as TNF-α and IL-6, negatively influence the expression of GH receptors (Soendergaard et al., 2017).

In HP-PR40 animals, TNF-α levels peaked at 3 dpi, before the viremic peak; this cytokine levels then decreased during viremia to reach control values at 10 dpi. In PR11 animals, TNF-α levels increased starting after the end of the viremic peak (14 dpi). The courses of GH and TNF-α appear rather similar

among the animal groups. The difference between HP-PR40 and PR11 groups was, as for GH, the start of increase: at viremia for HP-PR40 and after viremia for PR11. In our opinion, since TNF-α directly inhibits GH-R expression in the liver, these results support the hypothesis of GH resistance. The different time of the TNF-α response in the groups lead us to hypothesize that the virus pathogenicity elicits different mechanisms in the organism. It is known that PRRSV down-regulates TNF-α secretion in the early phase of infection, to bypass the induction of apoptosis in infected cells (Lopez-Fuertes, 2000). This mechanism may be interrupted in HP-PR40 pigs to safeguard energy to guarantee a more efficacious reaction to a strain with high pathogenicity. However, the trend of TNF-α in PR11 infected pigs was in line with the results on this cytokine we observed under field conditions, where PRRSV infection did not activate an early and efficient inflammatory and innate immune response but, contrarily, induced a down-regulation/delayed response of pro-inflammatory and immune cytokines (Borghetti et al., 2011).

In this regard, we also underline the different behaviour of IL-6 in the two infected groups. The animals infected by the HP-PR40 strain showed, from 7 dpi to the end of the study period, an inhibition of the IL-6 response. In PR11 pigs, the IL-6 peak occurred in correspondence of the viremia peak, followed by a decrease in plasma levels. IL-6 acts by two pathways (Rose-John, 2012), dependent on two different receptor forms: the classical IL-6 signalling begins with the binding to the membrane-bound receptor expressed only on the hepatocytes and some epithelial cells. The complex IL-6/IL-6 receptor associates with the protein gp130 and activates the JAK/STAT pathway. The protein gp130 is ubiquitously expressed in the cells, but cells which express only gp130 cannot respond to IL-6. In fact, in these cells the binding of IL-6 to a soluble form of IL-6 receptor can activate gp130 (trans-signalling pathway). In this way, all cells can be responsive to IL-6. The classical signalling pathway mediates the anti-inflammatory actions of IL-6 such as the inhibition of epithelial cell apoptosis and the induction of hepatic acute phase response (APR). Trans-signalling is also involved in IL-6 pro-inflammatory activities. The rise of IL-6 levels at 7 dpi in response to a "conventional virus" but not in response to the high pathogenicity strain, leads us to hypothesize that

289 the trans-signalling pathway was blocked. This supports the hypothesis of a different energy
290 management depending on the virulence and on the indirect role of IL-6 in the induction of regulatory
291 molecules of the suppressor of cytokine signalling (SOCS)-family (Denson et al., 2003).
292 Also the results on the adiponectin concentration are in agreement with this scenario. In our study,
293 the trend of adiponectin in infected groups could testify that the neuroendocrine response is linked
294 with the immune response, and specifically with the inflammatory cytokines, namely TNF-α.
295 Adiponectin levels in the control group are significantly different only on day 17. No differences
296 were observed in TNF-α levels. The levels of adiponectin are linked to the adipose tissue content.
297 During growth, the levels physiologically change showing physiological fluctuations. In fact, our
298 results are consistent with data reported by Ramsay et al. (2010). Several clinical data reported in
299 obese humans show a negative correlation between adiponectin and TNF-α (Kern et al., 2003; Liu et
300 al., 2016), suggesting a direct role of adiponectin on macrophage activity to suppress pro-
301 inflammatory cytokine production and to exert a protective effect. In fact, in both infected groups,
302 the plasmatic peak of adiponectin is after the decrease in TNF-α levels, subsequent to the TNF-α
303 peak. Adiponectin includes multiple complexes and has a structure similar to the complement factor
304 C1q (Scherer et al., 1995). In mice, TNF-α inhibits the multimerization of adiponectin *in vitro* and *in*
305 *vivo* (He et al., 2016): adiponectin multimerization would be a more efficient mechanism in the
306 adipocyte regulation of adiponectin production and secretion. We can hypothesize the involvement
307 of a similar mechanism also in our animals, when PRRSV infection imposes a different use of energy
308 availability. In addition, it is well known that adiponectin has an anti-inflammatory activity (Esmaili
309 et al., 2014) through suppression of differentiation and classical activation of M1 macrophages (M1)
310 by downregulating pro-inflammatory cytokines, i.e TNF-α and IL-6 (Ajuwon et al., 2005; Ohashi et
311 al. 2010). Macrophages exhibit adiponectin receptors 1 (ADIPO-R1) and 2 (ADIPO-R2) even if the
312 exact role of these receptors in anti-inflammatory adiponectin effects has yet to be clarified
313 (Yamaguchi et al., 2008; Hui et al., 2015). However, we know that the mediators involved in the
314 immune response are the key modulators in the regulation of energy and therefore in homeostasis.

315 Homeostasis is strongly linked to energy balance and availability. The high levels of adiponectin in
316 correspondence of the rise of GH could support the need of the GH resistance mechanism to guarantee
317 homeostasis in diseased animals.
318
319 **Conclusions**
320 In summary, this study suggests that the time-related changes of peripheral GH, adiponectin, IL-6
321 and TNF-α levels appear to be modulated by the PRRSV strain (HP-PR40 vs. PR11) and underline
322 the key role of energy availability in events leading to an effective response to the virus. A more
323 thorough understanding of the pathways and molecules regulating the interface of the immune and
324 endocrine response would be necessary and a better evaluation of this integrated response to a specific
325 viral infection such as PRRS may provide additional knowledge on the pathogenesis and on the
326 impact of infection on productive performance.
327
328 **Declaration of interest**
329 This study was performed primarily for scientific reasons within a conventional academic framework.
330 The authors declare that there are no conflicts of interest.
331
332 **Ethics statement**
333 The experimental study was approved by the Ethical Committee and by the Ministry of Health in
334 Italy (171/2016-PR), according to the European and National regulations on experimental infection
335 studies and animal welfare.
336
337 **Software and data repository resources**
338 None of the data were deposited in an official repository.
339
340 **References**

1. Ashley NT and Demas GE 2017 Neuroendocrine-immune circuits, phenotypes, and interactions. Horm Behav. 87, 25-34.
2. Baratta M, Saleri R, Mainardi GL, Valle D, Giustina A and Tamanini C 2002. Leptin regulates GH gene expression and secretion and nitric oxide production in pig pituitary cells. Endocrinology 143 (2), 551-557.
3. Borghetti P, Saleri R, Mocchegiani E, Corradi A and Martelli P 2009. Infection, immunity and the neuroendocrine response. Veterinary Immunology and Immunopathology 130(3-4), 141-162.
4. Borghetti P, Saleri R, Ferrari L, Morganti M, De Angelis E, Franceschi V, Bottarelli E and Martelli P 2011. Cytokine expression, glucocorticoid and growth hormone changes after porcine reproductive and respiratory syndrome virus (PRRSV-1) infection in vaccinated and unvaccinated naturally exposed pigs. Comparative Immunology, Microbiology and Infectious Diseases 34, 143–155.
5. Bozzola M, De Benedetti F, De Amici M, Jouret B, Travaglino P, Pagani S, Conte F and Tauber M 2003. Stimulating effect of growth hormone on cytokine release in children. European Journal of Endocrinology 149(5), 397-401.
6. Canelli E, Catella A, Borghetti P, Ferrari L, Ogno G, De Angelis E, Corradi A, Passeri B, Bertani V, Sandri G, Bonilauri P, Leung FC, Guazzetti S and Martelli P 2017. Phenotypic characterization of a highly pathogenic Italian porcine reproductive and respiratory syndrome virus (PRRSV) type 1 subtype 1 isolate in experimentally infected pigs. Veterinary Microbiology 210, 124-133.
7. Canelli E, Catella A, Borghetti P, Ferrari L, Ogno G, De Angelis E, Bonilauri P, Guazzetti S, Nardini R and Martelli P 2018Efficacy of a modified-live virus vaccine in pigs experimentally infected with a highly pathogenic porcine reproductive and respiratory syndrome virus type 1 (HP-PRRSV-1). Veterinary Microbiology 226, 89-96.

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366 8. Dantzer R 2018. Neuroimmune Interactions: from the Brain to the Immune System and
367 ViceVersa. Physiological Reviews 98(1), 477-504.
368 9. Denson LA, Held MA, Menon RK, Frank SJ, Parlow AF and Arnold DL 2003. Interleukin-6
369 inhibits hepatic growth hormone signaling via upregulation of Cis and Socs-3. American
370 Journal of Physiology-Gastrointestinal and Liver Physiology 284(4), G646-G654.
371 10. Elenkov IJ 2008. Neurohormonal-cytokine interactions: implications for inflammation,
372 common human diseases and well-being. Neurochem Int. 52(1-2):40-51.
373 11. Esmaili S, Xu A and George J 2014. The multifaceted and controversial immunometabolic
374 actions of adiponectin. Trends in Endocrinology and Metabolism 25, 444–451.
375 12. Ferrari L, Martelli P, Saleri R, De Angelis E, Cavalli V, Bresaola M, Benetti M and Borghetti
376 P 2013. Lymphocyte activation as cytokine gene expression and secretion is related to the
377 porcine reproductive and respiratory syndrome virus (PRRSV) isolate after in vitro
378 homologous and heterologous recall of peripheral blood mononuclear cells (PBMC) from pigs
379 vaccinated and exposed to natural infection. Veterinary Immunology and Immunopathology.
380 151, 193-206.
381 13. Ferrari L, Canelli E, De Angelis E, Catella A, Ferrarini G, Ogno G, Bonati L, Nardini R,
382 Borghetti P and Martelli P 2018. A highly pathogenic porcine reproductive and respiratory
383 syndrome virus type 1 (PRRSV-1) strongly modulates cellular innate and adaptive immune
384 subsets upon experimental infection. Veterinary Microbiology 216, 85-92.
385 14. He Y, Lu L, Wei X, Jin D, Qian T, Yu A, Sun J, Cui J and Yang Z 2016. The multimerization
386 and secretion of adiponectin are regulated by TNF-alpha. Endocrine 51(3), 456-468.
387 15. Hui X, Gu P, Zhang J, Nie T, Pan Y, Wu D, Feng T, Zhong C, Wang Y, Lam KS and Xu A
388 2015. Adiponectin enhances cold-induced browning of subcutaneous adipose tissue via
389 promoting M2 macrophage proliferation. Cell Metabolism 22, 279–290.

16. Kern PA, Di Gregorio GB, Lu T, Rassouli N and Ranganathan G 2003. Adiponectin expression from human adipose tissue: relation to obesity, insulin resistance, and tumor necrosis factor-alpha expression. Diabetes 52(7), 1779-1785.
17. Kooijman R, Gerlo S, Coppens A and Hooghe-Peters EL 2000. Growth hormone and prolactin expression in the immune system. Annals of the New York Academy of Sciences 917, 534-540.
18. Liu C, Feng X, Li Q, Wang Y, Li Q and Hua M 2016. Adiponectin, TNF-α and inflammatory cytokines and risk of type 2 diabetes: A systematic review and meta-analysis. Cytokine 86, 100-109.
19. López-Fuertes L, Campos E, Doménech N, Ezquerra A, Castro JM, Domínguez J and Alonso F 2000. Porcine reproductive and respiratory syndrome (PRRS) virus down-modulates TNF-alpha production in infected macrophages. Virus Research 69(1), 41-46.
20. Loving CL, Osorio FA, Murtaugh MP and Zuckermann FA 2015. Innate and adaptive immunity against Porcine Reproductive and Respiratory Syndrome Virus. Veterinary Immunology Immunopathology 167, 1-14.
21. Lunney JK, Fang Y, Ladinig A, Chen N, Li Y, Rowland B and Renukaradhya GJ 2016. Porcine Reproductive and Respiratory Syndrome Virus (PRRSV): Pathogenesis and Interaction with the Immune System. Annual Review of Animal Biosciences 4, 129-154.
22. Martelli P, Gozio S, Ferrari L, Rosina S, De Angelis E, Quintavalla C, Bottarelli E and Borghetti P 2009. Efficacy of a modified live porcine reproductive and respiratory syndrome virus (PRRSV) vaccine in pigs naturally exposed to a heterologous European (Italian cluster) field strain: Clinical protection and cell-mediated immunity. Vaccine 27, 3788-3799.
23. Martelli P, Ardigò P, Ferrari L, Morganti M, De Angelis E, Bonilauri P, Luppi A, Guazzetti S, Caleffi A and Borghetti P 2013. Concurrent vaccinations against PCV2 and PRRSV: study on the specific immunity and clinical protection in naturally infected pigs. Veterinary Microbiology 162, 558-571.

24. McEwen BS and Wingfield JC 2010. What is in a name? Integrating homeostasis, allostasis and stress Hormones and Behavior 57, 105-111.
25. Melen L, Hennen G, Dullaart RP, Heinen E and Igout A 1997. Both pituitary and placental growth hormone transcripts are expressed in human peripheral blood mononuclear cells (PBMC). Clinical and Experimental Immunology 110, 336-340.
26. Ohashi K, Parker JL, Ouchi N, Higuchi A, Vita JA, Gokce N, Pedersen AA, Kalthoff C, Tullin S, Sams A, Summer R and Walsh K 2010. Adiponectin promotes macrophage polarization toward an anti-inflammatory phenotype. Journal of Biological Chemistry 285(9), 6153-6160.
27. Park JY, Kim HS and Seo SH 2008. Characterization of interaction between porcine reproductive and respiratory syndrome virus and porcine dendritic cells. Journal of Microbiology and Biotechnology 18(10), 1709-1716.
28. Park PH, McMullen MR, Huang H, Thakur V and Nagy LE 2007. Short-term treatment of RAW264.7 macrophages with adiponectin increases tumor necrosis factor-α (TNF-α) expression via ERK1/2 activation and Egr-1 expression: role of TNF-α in adiponectin-stimulated interleukin-10 production. Journal of Biological Chemistry 282, 21695–21703.
29. Ramsay TG, Stoll MJ and Caperna TJ 2010. Adipokine gene transcription level in adipose tissue of runt piglets. Comparative Biochemistry and Physiology - Part B: Biochemistry & Molecular Biology 155(2), 97-105.
30. Rose-John S 2012. IL-6 trans-signaling via the soluble IL-6 receptor: importance for the pro inflammatory activities of IL-6. International Journal of Biological Sciences 8, 1237-1247.
31. Saleri R, Cavalli V, Martelli P and Borghetti P 2016. Anterior pituitary influence on adipokine expression and secretion by porcine adipocytes. Animal 10(6), 933-938.
32. Scherer PE, Williams S, Fogliano M, Baldini G and Lodish HF 1995. A novel serum protein similar to C1q, produced exclusively in adipocytes. Journal of Biological Chemistry 270, 26746–26749.

441 33. Silva-Campa E, Cordoba L, Fraile L, Flores-Mendoza L, Montoya M and Hernández J 2010.
442 European genotype of porcine reproductive and respiratory syndrome (PRRSV) infects
443 monocyte-derived dendritic cells but does not induce T-reg cells. Virology 396(2), 264-271.
444 34. Soendergaard C, Young JA and Kopchick JJ 2017. Growth Hormone Resistance-Special
445 Focus on Inflammatory Bowel Disease. International Journal of Molecular Sciences 18(5),
446 E1019-E1041.
447 35. Stadejek T, Stankevicius A, Murtaugh MP and Oleksiewicz MB 2103. Molecular evolution
448 of PRRSV in Europe: current state of play. Veterinary Microbiology 165: 21-28.
449 36. Taub DD 2008. Neuroendocrine interactions in the immune system. Cellular Immunology
450 252(1-2), 1-6.
451 37. Verburg-van Kemenade BM, Cohen N and Chadzinska M 2017. Neuroendocrine-immune
452 interaction: Evolutionarily conserved mechanisms that maintain allostasis in an ever-changing
453 environment. Developmental and Comparative Immunology 66, 2-23.
454 38. Weigent DA, Baxter JB, Wear WE, Smith LR, Bost KL and Blalock JE 1988. Production of
455 immunoreactive growth hormone by mononuclear leukocytes. The FASEB Journal 2(12),
456 2812-2818.
457 39. Wills RW, Doster AR, Galeota JA, Sur JH and Osorio FA 2003. Duration of infection and
458 proportion of pigs persistently infected with porcine reproductive and respiratory syndrome
459 virus. Journal of Clinical Microbiology 41, 58–62.
460 40. Yamauchi T, Iwabu M, Okada-Iwabu M, Kadowaki T 2014. Adiponectin receptors: a review
461 of their structure, function and how they work. Best Practice and Research Clinical
462 Endocrinology and Metabolism 28, 15–23.
463 41. Zhang L, Zhou L, Ge X, Guo X, Han J, Yang H 2016. The Chinese highly pathogenic porcine
464 reproductive and respiratory syndrome virus infection suppresses Th17 cells response in vivo.
465 Veterinary Microbiology. 189, 75-85.

466

467 **Figure captions**

468 **Figure 1** Course of GH plasma levels in infected (PR11 and HP-PR40 groups) and control (C group)
469 pigs in the post-exposure period. Data shown as mean values ± SEM. Asterisk (*) indicates a
470 statistically significant difference (P<0.05) between infected (groups PR11 and HP-PR40) and control
471 pigs. Different letters indicate a statistical difference (P<0.05) among time points within the same
472 group.

473

474 **Figure 2** Course of IGF-1 plasma levels in infected (PR11 and HP-PR40 groups) and control (C
475 group) pigs in the post-exposure period. Data shown as mean values ± SEM. Asterisk (*) indicates a
476 statistically significant difference (P<0.05) between infected (groups PR11 and HP-PR40) and control
477 pigs. Different letters indicate a statistical difference (P<0.05) among time points within the same
478 group.

479

480 **Figure 3** Course of adiponectin plasma levels in infected (PR11 and HP-PR40 groups) and control
481 (C group) pigs in the post-exposure period. Data shown as mean values ± SEM. Asterisk (*) indicates
482 a statistically significant difference (P<0.05) between infected (groups PR11 and HP-PR40) and
483 control pigs. Different letters indicate a statistical difference (P<0.05) among time points within the
484 same group.

485

486 **Figure 4** Course of TNF-α plasma levels in infected (PR11 and HP-PR40 groups) and control (C
487 group) pigs in the post-exposure period. Data shown as mean values ± SEM. Asterisk (*) indicates a
488 statistically significant difference (P<0.05) between infected (groups PR11 and HP-PR40) and control
489 pigs. Different letters indicate a statistical difference (P<0.05) among time points within the same
490 group.

491

Figure 5 Course of IL-6 plasma levels in PR11 and PR40 infected (PR11 and HP-PR40 groups) and control (C group) pigs in the post-exposure period. Data shown as mean values ± SEM. Asterisk (*) indicates a statistically significant difference ($P<0.05$) between infected (groups PR11 and HP-PR40) and control pigs. Different letters indicate a statistical difference ($P<0.05$) among time points within the same group.

Figure 1

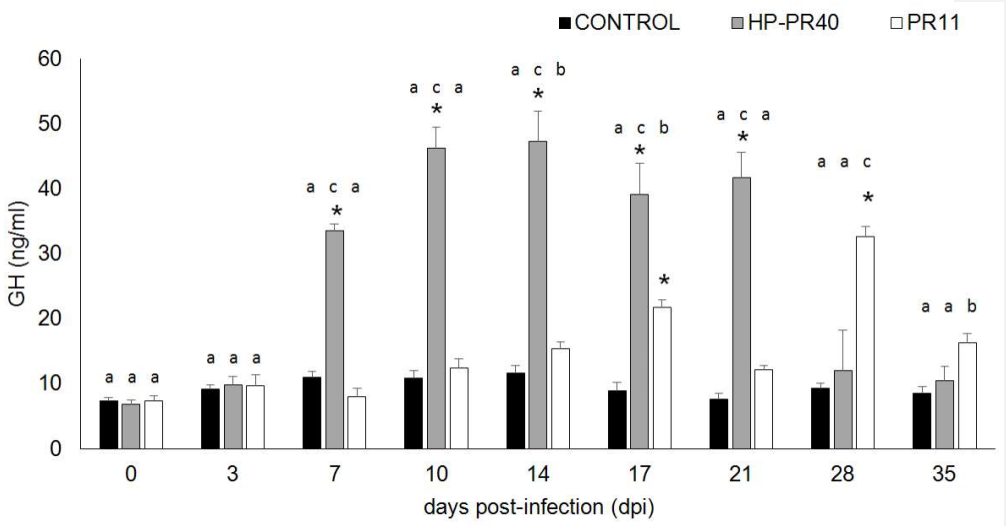


Figure 2

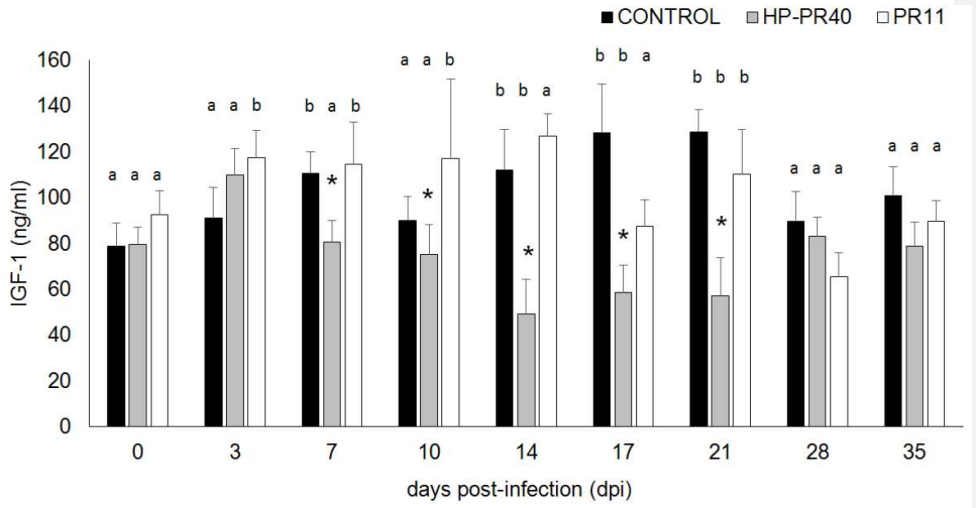


Figure 3

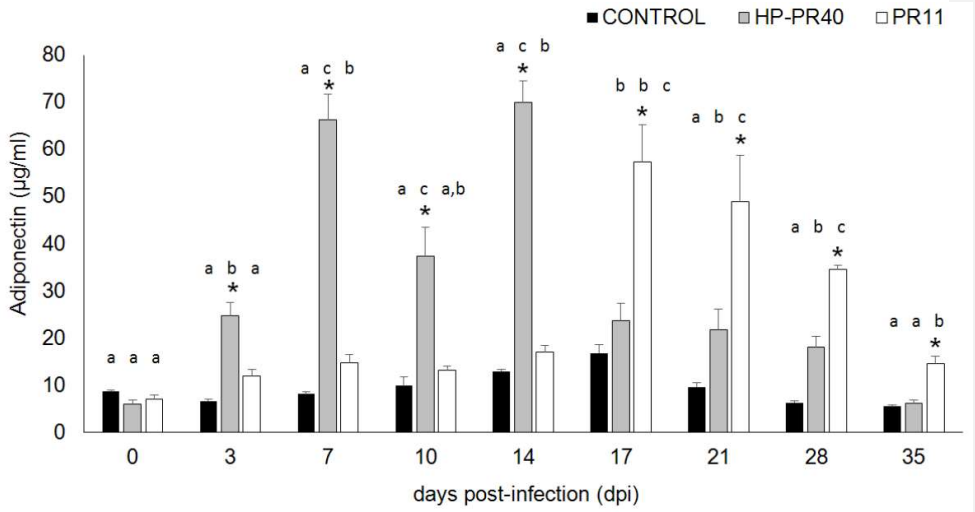


Figure 4

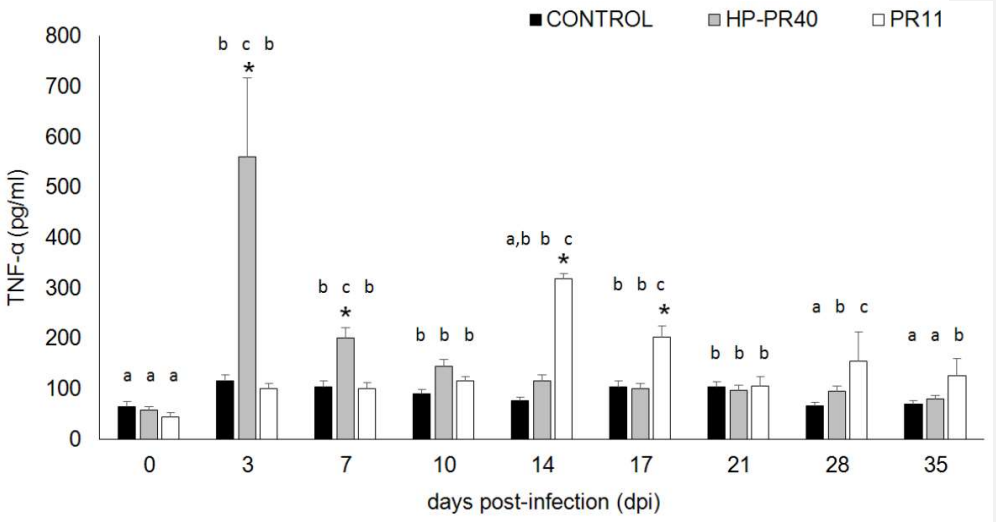
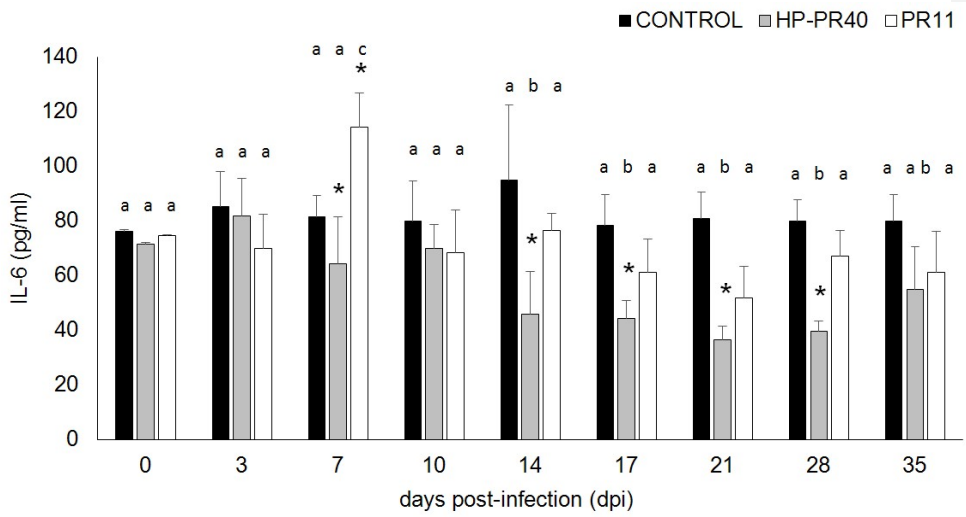


Figure 5


CONTROL
HP-PR40
PR11
IL-6 (pg/ml)
140
120
100
80
60
40
20
0
a a a
a a a
a a c
*
a a a
a b a
*
a b a
*
a b a
*
a b a
*
a ab a
0
3
7
10
14
17
21
28
35
days post-infection (dpi)