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3	Modulation of the somatotropic axis, adiponectin and cytokine secretion during highly
4	pathogenic porcine reproductive and respiratory syndrome virus type 1 (HP-PRRSV-1)
5	infection
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11	
12	Short title: Endocrine and cytokine response to PRRSV infection
13	
14	Abstract
15	Porcine reproductive and respiratory syndrome virus (PRRSV) is known to be clinically responsible
16	for reproductive failure in sows and post-weaning respiratory disease in growing piglets. During the
17	last years, highly pathogenic PRRSV isolates have been discovered. In Italy, a PRRSV-1 subtype 1
18	strain (namely PR40/2014) characterized by high pathogenicity was isolated and experimental
19	infection was characterized in terms of virological/clinical features and immune modulation (Canelli
20	et al., 2017; Ferrari et al., 2018). The present study was performed in 4-week-old pigs experimentally
21	infected with the highly pathogenic PRRSV1_PR40/2014 (HP-PR40) or with the conventional
22	PRRSV1_PR11/2014 (PR11). The aim was to evaluate the interrelation between plasmatic hormones
23	and cytokines in infected pigs compared to uninfected controls in order to address potential effects
24	on the course of an experimental infection. The time-related changes of growth hormone (GH),
25	insulin-like growth factor-1 (IGF-1), adiponectin, interleukin-6 (IL-6) and tumor necrosis factor- $\alpha$
26	(TNF- $\alpha$ ) levels appear to be modulated by the infection depending on the PRRSV isolate (HP-PR40
27	vs. PR11). In particular, in HP-PR40 infected animals, the association between high GH levels and
28	viremia may testify the need to block the anabolic action of GH in order to shift available energy

29	towards the immune response. This need appeared to be delayed in PR11 animals, given the lower
30	pathogenicity of the isolate. Adiponectin, IL-6 and TNF- $\alpha$ course supports the hypothesis of GH
31	resistance mechanisms to guarantee homeostasis in HP-PR40 animals and underlines the key role of
32	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.

36

### 37 Introduction

Bidirectional communication between the immune and neuroendocrine systems is well known, as 38 immune cells produce hormones and similarly, neuroendocrine cells secrete cytokines and express 39 specific cytokine receptors. This multi-directional communication guarantees the maintenance of 40 41 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-42 inflammatory cytokines, are the main players of this coordinated cross-talk. Their action translates 43 into enhancement of innate immunity, support for acquired immunity and control of immune-44 mediated inflammation, with an efficiency increase of the immune response against infection 45 46 (Borghetti et al., 2009).

Porcine reproductive and respiratory syndrome (PRRS) is a widespread disease caused by an 47 enveloped, positive-stranded RNA virus (PRRSV) which belongs to the family Arterividae. The two 48 49 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 50 et al., 2017). The intra-species variability is very high so that PRRSV-1 can be divided into at least 51 four (pan-European subtype 1 and East European subtypes 2, 3 and 4) and PRRSV-2 into at least nine 52 subtypes (Stadejek, et al., 2013). The infection shows three phases identified as acute phase, 53 persistence and extinction (Lunney et al., 2016). In the acute phase, the lung is the preferential site of 54

infection and the typical respiratory disease PRRSV causes in new-born and growing piglets results 55 56 in severe economic losses. Viremia persists for several weeks despite the presence of circulating antibodies since virus-neutralizing antibodies (VNA) develop very slowly and sometimes maintain 57 very low titres. In fact, the importance of an efficient cellular response has been demonstrated in 58 terms of cytolitic cells and IFN- $\gamma$  secreting cells, especially during the first weeks after infection 59 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 replicate for more than 250 days (Wills et al., 2003). 62

Important breakthroughs in the understanding of PRRSV biology have been obtained in recent years, 63 however, a more comprehensive understanding of the mechanisms of the immune and endocrine 64 system responses to PRRSV infection is needed in order to improve control strategies and design 65 novel vaccines which counteract/dampen PRRSV infections/co-infections and favours balanced 66 integrated responses. A previous study by the authors (Borghetti et al., 2011) showed that time-related 67 changes of hormones such as GH and cortisol, in association with pro-inflammatory and anti-68 inflammatory cytokines, occur during natural infection by a PRRSV isolate and that vaccination can 69 modulate these responses in terms of a better support for innate immunity. 70

The existence of genetically divergent PRRSV isolates, with varying degree of virulence, makes the understanding of PRRSV immunopathogenic mechanisms more complex. Furthermore, different clinical and virological outcomes have been reported within the known genotypes, suggesting the emergence of highly pathogenic (HP) PRRSV strains (Zhang et al., 2016). In this context, an Italian PRRSV-1 subtype 1 strain (namely PR40/2014) characterized by high pathogenicity was recently isolated and experimental infection was characterized in terms of virological/clinical features and immune modulation (Canelli et al., 2017, 2018; Ferrari et al., 2018).

The present experimental study was performed in piglets infected with two European isolates showing
different pathogenicity: PRRSV-1\_PR40/2014 (HP) and PRRSV-1\_PR11/2014 (non-HP), a
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 81 82 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. 83 Therefore, we evaluated plasma concentrations of growth hormone (GH), insulin-like growth factor-84 1 (IGF-1), adiponectin, interleukin-6 (IL-6) and tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) and their 85 86 association with changes of viremia and antibody concentration. Why did we choose these factors? 87 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.) 88 are to be read and play a role in body homeostasis. 89

90

#### 91 Materials and methods

#### 92 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:

- 98 Group 1: 7 pigs, intra-nasally (IN) infected with PRRSV1 PR40/2014 (HP-PR40);
- 99 Group 2: 7 pigs, IN infected with PRRSV1 PR11/2014 (PR11);
- 100 Group 3: 3 pigs, IN inoculated with medium only (non-infected / negative control).
- 101 For both group 1 and group 2, a dose of  $10^5$  TCD<sub>50</sub> PRRSV/pig in 2 ml (1 ml/nostril) was IN
- 102 inoculated. Working stock for both viruses was the 3<sup>rd</sup> passage on porcine alveolar macrophages
- 103 (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.

111 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.

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.

No remarkable lung lesions were observed in the negative control group while interstitial pneumonia

Interstitial pneumonia with lymphocytes and monocyte/macrophage septal infiltrations was observedat 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.

132 Viremia and virus re-isolation

RNA was extracted from serum by using Trizol LS (Invitrogen) following the manufacturer's 133 134 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). 135

Viruses used for experimental infection were re-isolated from sera of infected pigs with the highest 136 copy numbers detected using qtRT-PCR, by one-passage cultivation on PAMs and adapted to 137 MARC-145 cells. The MARC-145 adapted viruses were confirmed as homologous with the original 138 139 strains of the infection by ORF5 and ORF7 sequencing.

140 Hormone and cytokine assays

141 Samples were analyzed in duplicate for hormones and cytokines by ELISA validated for swine. 142 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 143 3.3% and 6.2%, respectively. The minimal detection limit was 100 pg/ml. Porcine insulin-like growth 144 145 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 146 4.2% and 7.1%, respectively. A specific commercial kit was used to evaluate adiponectin plasma 147 concentration (porcine ELISA Kit for Adiponectin, Cloud Clone Corp., Houston, TX, USA). 148 Sensitivity was 0.114 ng/ml; intra- and inter-assay CV were 4.2% and 5.4%, respectively. Tumor 149 150 necrosis factor- $\alpha$  (TNF- $\alpha$ ) levels were analyzed by using a specific commercial kit (Quantikine 151 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 152 153 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. 154

155 Statistical analysis

Immunological and hormonal data were analyzed by ANOVA using a mixed model with group, 156 157 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  $\pm$ 158 7

standard error of mean (SEM). Statistical significance was reached for P<0.05. Differences among</li>
groups were considered significant if P<0.05, and as a trend to significance when 0.05≤P<0.10.</li>
ANOVA was performed by applying the GLM procedure SAS 9.4 (2014).

162

### 163 Results

164 Viremia and virus re-isolation

Comprehensive data relative to the course of viremia are reported in Canelli et al. (2017). For reasons of completeness and clarity, we provide here a description of virological data. Briefly, at 0 dpi all animals were PRRSV-negative. PRRSV was not detected in sera from control group animals. In the HP-PR40 and PR11 groups, viremia started at 3 dpi and peaked at 7 and 10 days after inoculation, 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

lower than one cDNA Log<sub>10</sub> copies/µL at the end of the study period. HP-PR40 pigs showed a higher
viremia for the whole duration of the study.

The phylogenetic analyses performed after sequencing the isolates at the viremic peak directly from
serum and the isolates used for infection confirmed, in all cases, a homology >98% for ORF5 and
>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 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, 178 179 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. Thereafter, GH levels decreased to reach basal levels at 35 dpi. In control and PR11 groups, plasma 181 GH levels were consistent with physiological variations of the hormone. Plasma concentration of 182 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). 183 184 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 in Figure 3. No significant differences were detected in plasma levels on day 0 among groups: mean 187 values were 8.7±0.2 (control), 5.9±0.5 (HP-PR40) and 7.1±0.3 (PR11) µg/ml. Adiponectin levels in 188 the HP-PR40 group showed significantly higher concentrations (P<0.05) in correspondence of the 189 viremia peak and until day 14 pi, as compared to the control group and the PR11 group. PR11 pigs 190 191 showed a significant increase in adiponectin levels starting after the vanishing of viremia (17 dpi) until the end of the experimental period. Tumour necrosis factor- $\alpha$  plasma concentration (Figure 4) 192 193 at the start of the study was  $65.3\pm6.6$  (control),  $57.4\pm4.7$  (HP-PR40) and  $53.7\pm6.5$  (PR11). In the HP-PR40 group, a significant increase (P<0.05) was observed at 3 and 7 dpi. The PR11 group showed a 194 similar trend, even if the significant increase (P<0.05) in TNF- $\alpha$  levels was at 14 and 17 dpi. The 195 results regarding IL-6 are shown in figure 5. In pigs inoculated with the PR11 isolate (PR11 group), 196 197 IL-6 showed a significant increase (P<0.05) in correspondence of the viremic peak (day 7 pi), followed by a decrease to basal levels. Animals infected with the PR40 strain (HP-PR40 group) 198 showed inhibition of the IL-6 response from day 7 pi to the end of the study; the reduction appeared 199 to be significant at 7, 14, 17, and 21 dpi as compared to the control group and the PR11 group 200 (P<0.05). 201

202

## 203 Discussion

Infection by PRRSV in piglets is characterized by fever, anorexia and respiratory disease and in infected new-born and growing pigs, the typical consequence of PRRSV infection is a respiratory disease due to interstitial pneumonia. Gross lesions observed following PRRSV infection are dependent on the virus isolate, genetics of the infected pig, stress factors and other complicating agents, particularly bacteria and interactions with other viruses (e.g. PCV2). Frequently, innate and acquired immune responses are inefficient to early and efficiently eliminate the virus because PRRSV 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 221 cytokines and mainly influences the immune responses. GH is a potent anabolic hormone: several of 222 its effects are mediated by IGF-1 which is mainly produced by the liver.

223 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 224 of hepatic GH resistance. The reduced GH sensitivity may be accompanied by decreased hepatic GH 225 receptor (GH-R) expression, which in turn leads to GH resistance. This condition is characterized by 226 normal or elevated levels of GH associated with decreased IGF-1 levels (Soendergaard et al., 2017), 227 228 which results in an altered hepatic response to GH. Physiologically, GH resistance occurs in any 229 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 230 231 (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 232 uninfected controls), associated with a marked delay in average daily weight gain. It is also interesting 233 to observe that the higher GH levels in the HP-PR40 group occurred simultaneously with the viremic 234 235 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 236

237 response: the highly pathogenic strain (HP-PR40) is characterized by severe clinical signs in growers 238 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 239 energy towards the immune response. This need could be delayed in PR11 animals, given the lower 240 pathogenicity and clinical impact of the strain. In fact, in this group, the increase of GH levels was 241 242 subsequent to the viremic peak. Secondly, the significant decrease in plasma levels of IGF-1 during 243 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-244 245 inflammatory cytokines, namely TNF-α and IL-1, specifically induced by viral infection (unpublished data). 246

The growth hormone is mainly secreted by the anterior pituitary, but the pituitary is not the only site 247 of production. Weignent et al. (1988) first showed that immune cells not only express GH receptors 248 249 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 250 demonstrated that GH mainly stimulates the immune response and is directly involved in thymus 251 activity and involution (Verburg et al., 2017). It is known that GH regulation in the immune and 252 endocrine systems is very different. Systemic GH binding to its lymphocyte receptors would cause 253 254 an up-regulation of secretion of local GH. It could act in an autocrine/paracrine fashion on immune 255 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 256 257 of GH on TNF- $\alpha$  secretion in humans was demonstrated (Bozzola et al., 2003). Some of the key inflammatory cytokines, such as TNF- $\alpha$  and IL-6, negatively influence the expression of GH 258 receptors (Soendergaard et al., 2017). 259

In HP-PR40 animals, TNF- $\alpha$  levels peaked at 3 dpi, before the viremic peak; this cytokine levels then 260 decreased during viremia to reach control values at 10 dpi. In PR11 animals, TNF- $\alpha$  levels increased 261 starting after the end of the viremic peak (14 dpi). The courses of GH and TNF- $\alpha$  appear rather similar 262 11

among the animal groups. The difference between HP-PR40 and PR11 groups was, as for GH, the 263 264 start of increase: at viremia for HP-PR40 and after viremia for PR11. In our opinion, since TNF- $\alpha$ directly inhibits GH-R expression in the liver, these results support the hypothesis of GH resistance. 265 The different time of the TNF- $\alpha$  response in the groups lead us to hypothesize that the virus 266 pathogenicity elicits different mechanisms in the organism. It is known that PRRSV down-regulates 267 268 TNF- $\alpha$  secretion in the early phase of infection, to bypass the induction of apoptosis in infected cells 269 (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-270 271  $\alpha$  in PR11 infected pigs was in line with the results on this cytokine we observed under field 272 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 273 274 and immune cytokines (Borghetti et al., 2011).

275 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 276 inhibition of the IL-6 response. In PR11 pigs, the IL-6 peak occurred in correspondence of the viremia 277 peak, followed by a decrease in plasma levels. IL-6 acts by two pathways (Rose-John, 2012), 278 dependent on two different receptor forms: the classical IL-6 signalling begins with the binding to 279 280 the membrane-bound receptor expressed only on the hepatocytes and some epithelial cells. The 281 complex IL-6/IL-6 receptor associates with the protein gp130 and activates the JAK/STAT pathway. 282 The protein gp130 is ubiquitously expressed in the cells, but cells which express only gp130 cannot 283 respond to IL-6. In fact, in these cells the binding of IL-6 to a soluble form of IL-6 receptor can 284 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 285 epithelial cell apoptosis and the induction of hepatic acute phase response (APR). Trans-signalling is 286 287 also involved in IL-6 pro-inflammatory activities. The rise of IL-6 levels at 7 dpi in response to a 288 "conventional virus" but not in response to the high pathogenicity strain, leads us to hypothesize that

the trans-signalling pathway was blocked. This supports the hypothesis of a different energy management depending on the virulence and on the indirect role of IL-6 in the induction of regulatory molecules of the suppressor of cytokine signalling (SOCS)-family (Denson et al., 2003).

Also the results on the adiponectin concentration are in agreement with this scenario. In our study, 292 the trend of adiponectin in infected groups could testify that the neuroendocrine response is linked 293 294 with the immune response, and specifically with the inflammatory cytokines, namely TNF- $\alpha$ . 295 Adiponectin levels in the control group are significantly different only on day 17. No differences were observed in TNF- $\alpha$  levels. The levels of adiponectin are linked to the adipose tissue content. 296 297 During growth, the levels physiologically change showing physiological fluctuations. In fact, our results are consistent with data reported by Ramsay et al. (2010). Several clinical data reported in 298 obese humans show a negative correlation between adiponectin and TNF-α (Kern et al., 2003; Liu et 299 300 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, the plasmatic peak of adiponectin is after the decrease in TNF- $\alpha$  levels, subsequent to the TNF- $\alpha$ 302 peak. Adiponectin includes multiple complexes and has a structure similar to the complement factor 303 Clq (Scherer et al., 1995). In mice, TNF-α inhibits the multimerization of adiponectin in vitro and in 304 vivo (He et al., 2016): adiponectin multimerization would be a more efficient mechanism in the 305 306 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 availability. In addition, it is well known that adiponectin has an anti-inflammatory activity (Esmaili 308 309 et al., 2014) through suppression of differentiation and classical activation of M1 macrophages (M1) by downregulating pro-inflammatory cytokines, i.e TNF-α and IL-6 (Ajuwon et al., 2005; Ohashi et 310 al. 2010). Macrophages exhibit adiponectin receptors 1 (ADIPO-R1) and 2 (ADIPO-R2) even if the 311 exact role of these receptors in anti-inflammatory adiponectin effects has yet to be clarified 312 313 (Yamaguchi et al., 2008; Hui et al., 2015). However, we know that the mediators involved in the immune response are the key modulators in the regulation of energy and therefore in homeostasis. 314

Homeostasis is strongly linked to energy balance and availability. The high levels of adiponectin in
correspondence of the rise of GH could support the need of the GH resistance mechanism to guarantee
homeostasis in diseased animals.

318

### 319 Conclusions

In summary, this study suggests that the time-related changes of peripheral GH, adiponectin, IL-6 and TNF- $\alpha$  levels appear to be modulated by the PRRSV strain (HP-PR40 vs. PR11) and underline the key role of energy availability in events leading to an effective response to the virus. A more thorough understanding of the pathways and molecules regulating the interface of the immune and endocrine response would be necessary and a better evaluation of this integrated response to a specific viral infection such as PRRS may provide additional knowledge on the pathogenesis and on the 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

- Italy (171/2016-PR), according to the European and National regulations on experimental infectionstudies and animal welfare.
- studies and annual
- 336

# 337 Software and data repository resources

- 338 None of the data were deposited in an official repository.
- 339

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## 467 Figure captions

Figure 1 Course of GH plasma levels in infected (PR11 and HP-PR40 groups) and control (C group) pigs in the post-exposure period. Data shown as mean values  $\pm$  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.

473

Figure 2 Course of IGF-1 plasma levels in infected (PR11 and HP-PR40 groups) and control (C group) pigs in the post-exposure period. Data shown as mean values  $\pm$  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.

479

Figure 3 Course of adiponectin plasma levels in infected (PR11 and HP-PR40 groups) and control (C group) pigs in the post-exposure period. Data shown as mean values  $\pm$  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.

485

Figure 4 Course of TNF- $\alpha$  plasma levels in 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.

492	Figure 5 Course of IL-6 plasma levels in PR11 and PR40 infected (PR11 and HP-PR40 groups) and
493	control (C group) pigs in the post-exposure period. Data shown as mean values $\pm$ SEM. Asterisk (*)
494	indicates a statistically significant difference (P<0.05) between infected (groups PR11 and HP-PR40)
495	and control pigs. Different letters indicate a statistical difference (P<0.05) among time points within
496	the same group.
497	



500 Figure 1





Figure 5