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An epidemiological investigation into the association between biomarkers and growth performance in nursery pigs

Mackenzie J Slifierz¹, Robert Friendship¹, Cornelius FM de Lange², Marko Rudar² and Abdolvahab Farzan^{1*}

Abstract

Background: Biomarkers are useful tools in research and clinical practice where they are often used to detect and monitor differences in the physiological state of an animal. The proteins IGF-1, IGFBP-3, GHR, CRP, SAA, Hp, IFN- α , IFN- γ , TNF- α , IL-1 β , IL-6, IL-10, and IL-18 have been proposed as potential biomarkers for monitoring growth in livestock. The objective of this study was to determine whether hepatic gene expression of these proposed biomarkers is associated with growth performance in nursery pigs. Herd information and growth parameters were collected for 168 piglets from 8 commercial farms in southern Ontario. From these pigs, a subset of liver tissue samples ($n = 74$) was used for gene expression analysis of the proposed biomarkers. Multivariable linear regression methods were used to determine whether genetic expression of the proposed biomarkers was associated with growth performance in the nursery.

Results: Modelling the herd information and individual piglet traits in relation to growth performance revealed that the weight at weaning and the age at weaning are significantly associated with nursery performance. Average daily gain (ADG) was significantly associated with hepatic IGFBP-3 and GHR expression in the liver ($P < 0.05$), and tended to be associated with hepatic IGF-1 expression ($P = 0.071$). Similarly, 9-week body weight was significantly associated with hepatic expression of IGFBP-3 and GHR expression ($P < 0.05$), and tended to be associated with hepatic expression of IGF-1 ($P = 0.055$).

Conclusion: The age and weight at which pigs are weaned is an important determinant for nursery performance. Hepatic gene expression of IGF-1, IGFBP-3, and GHR can be useful biomarkers for monitoring growth performance in nursery pigs.

Keywords: Biomarker, Pig, ADG

Background

The insulin-like growth factor (IGF) system is important for development and tissue growth in livestock species. Insulin-like growth factor-1 (IGF-1) is primarily synthesized by the liver and is crucial for stimulating cellular proliferation and differentiation in brain, muscle, and bone tissue [1]. The expression of IGF-1 is mediated by growth hormone (GH), a peptide hormone released by the anterior pituitary gland [2]. The binding of GH to growth hormone receptors (GHR) in hepatic tissue induces expression of IGF-1 [3]. IGF-1 expression is

associated with increased body weight as demonstrated by transgenic over-expression of IGF-1 in mice [4]. The transportation and bioavailability of IGF-1 is facilitated by IGF binding proteins (IGFBPs) which circulate in the blood [5]. The most abundant is IGFBP-3 which binds most circulating IGF-1 [6]. Experiments using a transgenic mouse model have demonstrated that over-expression of IGFBP-3 is associated with growth impediment [7]. In pigs, previous research suggests that serum IGF-1 is positively associated with average daily gain (ADG) and serum IGFBP-3 is negatively associated with ADG [8]. Additional studies have demonstrated that the IGF system is important for postnatal maturation and the development of desirable carcass traits [9-11]. Although these findings demonstrate that the IGF system plays an important role

* Correspondence: afarzan@uoguelph.ca

¹Department of Population Medicine, Ontario Veterinary College, University of Guelph, 50 Stone Road, N1G 2 W1 Guelph, Ontario, Canada
Full list of author information is available at the end of the article

in development, it is not clear to what extent differences in gene expression affect growth performance in pigs, and whether these genes can be used as biomarkers to monitor growth performance across different commercial farms.

There are previous studies which also suggest that growth performance in pigs may be associated with changes in serum concentrations of acute-phase proteins (APP), including C-reactive protein (CRP), haptoglobin (Hp), and serum amyloid A (SAA) [12-15]. It has been proposed that Hp may be a suitable biomarker for monitoring production performance on commercial swine farms [15]. Serum levels of certain cytokines, including interleukin-1 β (IL-1 β), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-18 (IL-18), and tumor necrosis factor- α (TNF- α), have also been associated with body weight in horses and in humans [16-20]. However, the usefulness of these APPs and cytokines as biomarkers for monitoring the growth performance of pigs on commercial swine farms has not been established. The objective of this study was to investigate the association between the hepatic gene expression of potential biomarkers, including IGF-1, IGFBP-3, GHR, CRP, SAA, Hp, IFN- α , IFN- γ , TNF- α , IL-1 β , IL-6, IL-10, IL-18, and growth performance in nursery pigs across diverse commercial farm conditions. A secondary objective of this study was to determine extraneous factors which may influence growth performance in the nursery as such factors are critical for assessing the association between potential biomarkers and nursery performance.

Methods

Experimental design and sampling

The use of animals in this study was approved by the Animal Care Committee at the University of Guelph. Eight swine farms in southern Ontario were recruited to participate in a collaborative field study. On each farm, seven sows were randomly enrolled in the study and three piglets (small, medium, and large size) were selected from each sow at weaning and ear tagged. In total 168 piglets (21 piglets per farm) were included. All male piglets recruited in this study were castrated. A questionnaire was administered and information about the enrolled sows (farrowing date, parity, litter size, numbers of live born, stillborn), farm management (pig flow, number of gilts, sows, nursery pigs, and grower-finishers, and type of farrowing room floor), health status (recent diseases, mortality in different stage of production), vaccination, and in-feed drug use was collected. Piglets were weighed at weaning and once again at 5 weeks post-weaning. Fecal and blood samples were collected at weaning, 2 weeks post-weaning, and 5 weeks post-weaning.

At 5 weeks post-weaning the piglets were transported to the University of Guelph where they were euthanized. Tissue samples collected immediately from the liver,

intestinal lymph node, ileum, and spleen were placed immediately into RNAlater and allowed to sit at 4°C for 24 h before being stored at -80°C. Samples were stored at -80°C for no longer than 4 weeks before being processed for gene expression analysis. Separate portions of the ileum and colon were collected and nasal swabs, blood, and fecal samples were collected from each pig prior to euthanasia.

Calculating growth performance

Growth performance was determined by assessing the post-weaning ADG and the 9 week body weight (9-wk BW) of each nursery piglet. ADG was calculated as the difference between the weaning weight and the 5-wk post-weaning weight, divided by the number of days between each weighing. Body weights were standardized to 9 weeks of age as there was considerable variation in piglet age within each farm and between farms. Body weights were standardized to 9 weeks of age as the mean age at the second weighing was 59.5 d.

RNA Extraction

Seventy-four pigs from 4 representative farms with different health status and production parameters were included for gene expression analysis. RNA was extracted from liver tissue using a Qiagen RNeasy Mini Kit (Qiagen). Approximately 30 mg of liver tissue was added to 600 μ l of RLT buffer in a RNase-Free tube and homogenized for 30–40 sec using a tissue homogenizer (PowerGen 125, Fisher Scientific). RNA was then extracted from the homogenate as outlined by the manufacturer's protocol without any modifications (Qiagen). The concentration of the eluted RNA was determined using the Thermo Scientific NanoDrop 8000 Spectrophotometer (Fisher Scientific), and RNA was re-extracted from samples with signs of degradation or contamination.

All RNA samples were treated with an Amplification Grade DNase I kit (Sigma-Aldrich) to remove any genomic DNA. 24 μ l of nucleic acid-free water, 3 μ l of reaction buffer, 3 μ l of Amplification Grade DNase I, and 3 μ g of total RNA (1 unit/ μ l) were added together and centrifuged for 10 seconds to collect the reaction at the bottom of the tube. The mixtures were incubated at room temperature for 15 min. 3 μ l of the Stop Solution (50 mM EDTA) was added and the mixtures were incubated at 70°C for 10 min. The mixtures were then chilled on ice before being stored at -80°C.

The purity and concentration of RNA was reassessed after DNase digestion using the Thermo Scientific NanoDrop 8000 Spectrophotometer (Fisher Scientific). The integrity of all the RNA samples was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies) before and after DNase treatment to ensure there was no indication of degradation. The mean RNA Integrity Number

(RIN) was 8.5, and any samples with RIN < 8.0 were repeated as recommended by Fleige and Pfaffl [16].

Primer design and efficiency

Primer designs and efficiencies are listed in Table 1. Primers for IL-1 β , IL-10, IFN- α , and IFN- γ were previously designed by Collado-Romero et al. [17]. All other primer sequences were designed and screened for homology using the NCBI Primer-BLAST program. The cDNA sequences used to design the primers were obtained from the GenBank database. Primers were designed to span known exon-exon junctions. Primer efficiency and the coefficient of determination (R^2) were determined using the StepOnePlus™ Real-Time PCR System (Applied Biosystems).

Analysis of gene expression with RT-qPCR

Relative gene expression was assessed using a real time quantitative polymerase chain reaction (RT-qPCR) assay. The synthesis of cDNA and the RT-qPCR assay were completed by the Advanced Analysis Centre's Genomic Facility at the University of Guelph. For synthesis of cDNA, 1000 ng of total RNA was reverse transcribed into cDNA using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems). A total reaction volume of 20 μ l was used for reverse transcription; 2 μ l of reverse transcript buffer, 0.8 μ l (100 mM) dNTP, 2 μ l random priming oligonucleotides, 1 μ l of MultiScribe™ Reverse Transcriptase (50 U/ μ l) (Applied Biosystems), 4.2 μ l of nucleic acid-free water, and 10 μ l (1000 ng) of total RNA. The reverse transcription assay was run at

Table 1 Primers used for RT-qPCR of hepatic biomarkers

Gene symbol	Gene name	Accession number ^a	Primers (5' - 3')	T _m (°C)	Amplicon length (bp)	R ²	Primer efficiency (%)
<i>IL1B</i>	Interleukin-1 β	NM_214055	F: GGCCGCCAAGATATAACTGA ^b R: GGACCTCTGGGTATGGCTTTC ^b	59	70	0.991	100
<i>IL6</i>	Interleukin-6	NM_214399	F: CCCTGAGGCAAAAGGAAAGA R: CGTGGACGGCATCAATCTCA	60	212	0.998	95.2
<i>IL10</i>	Interleukin-10	L20001	F: CAGATGGGCGACTTGTTG ^b R: ACAGGGCAGAAATTGATGAC ^b	57	219	0.993	91.7
<i>IL18</i>	Interleukin-18	NM_213997	F: GCTGCTGAACCGGAAGACAA R: AAACACGGCTTGATGTCCCT	60	192	0.996	95.3
<i>IFNA</i>	Interferon- α	AB257591	F: GACCTGCCTCAGATCCACAG R: ATGGCTTGAGCCTTCTGGAC	60	158	0.986	82.3
<i>IFNG</i>	Interferon- γ	X53085	F: CAAAGCCATCAGTGAACCTCATGA ^b R: TCTCTGGCCTTGGACATAGTCT ^b	60	100	0.985	98.7
<i>TNFA</i>	Tumor necrosis factor- α	NM_214022	F: CCTCTTCTCCTCCTCCTG ^b R: CCTCGGCTTTGACATTGG ^b	57	194	0.997	100
<i>CRP</i>	C-reactive protein	NM_213844	F: TGCCAGACAGACATGATCG R: GGTCGGTATAGACACGCAGG	60	131	0.999	100
<i>HP</i>	Haptoglobin	NM_214000	F: TGAATGTGAAGCAGTGTGCG R: CGAGGTGAGGTTATGGTGGG	59	133	0.996	96
<i>SAA</i>	Serum amyloid A	EF362780	F: TGATCAGCGATGCCAGAGAG R: CTTGAGTCTCCACTCCGTG	60	85	0.998	87.8
<i>IGF1</i>	Insulin-like growth factor-1	JX827417	F: TCTTCTACTTGGCCCTGTGCTT R: CCAGCTCAGCCCCACAGA	61	81	0.998	97.7
<i>IGFBP3</i>	Insulin-like growth factor binding protein-3	NM_001005156	F: GGATCCACATCCCCAACT R: CCCCCTTCTGCTTT	60	80	0.990	97.3
<i>GHR</i>	Growth hormone receptor	JF276446	F: CTCCACAGGGCCTCGTACTC R: GCTCACATAGCCACACGATGA	60	80	0.999	89.2
<i>GAPDH</i>	Glyceraldehyde 3-phosphate dehydrogenase	NM_001206359	F: ACACACCAGCATCTCCTGACT R: CGAGGCAGGTCTCCCTAAGC	61	80	0.998	100

^aAccessed via GenBank.

^bDesigned by Collado-Romero et al. [17].

25°C for 5 min, 37°C for 120 min, 85°C for 5 min, and cooled at 4°C.

GAPDH and RLP4 were evaluated as potential reference genes for the present study based on validation and reliable performance in previous research [10,18-20]. The stability of these reference genes in the liver tissue samples was measured using RT-qPCR and analyzed using BestKeeper software [21]. The RT-qPCR cycle quantification (C_q) values of GAPDH and RLP4 had standard deviations of 0.34 and 0.49, respectively. GAPDH was chosen as the only endogenous reference gene to be used for internal normalization of gene expression data in this study due to its very low variability ($C_q = 18.17 \pm 0.34$) for the present samples and its reliable performance as a sole reference gene in previous studies [10,20].

A single StepOnePlus™ Real-Time PCR System (Applied Biosystems) was used for all RT-qPCR assays in this study. The RT-qPCR assay was completed using duplicates of each sample. Each 20 μ l reaction consisted of 7.5 μ l of 2 \times PerfeCta SYBR Green FastMix ROX (Quanta BioScience), 5 μ l of template cDNA, 1.9 μ l of molecular grade water, and 0.6 μ l of primer. The final primer concentration in each reaction was 200 nM. The RT-qPCR assay conditions were 95°C for 30 s followed by 40 cycles of 95°C for 15 s and 60°C for 30 s. A melting curve was performed to confirm specificity of the amplicon.

Statistical analysis

Linear regression methods were used to model production parameters and gene expression (ΔC_q) in relation to growth performance. First, the C_q values of each biomarker were normalized using the C_q values of the endogenous reference gene GAPDH as described by the following equation:

$$\Delta C_q = [C_q \text{ of target gene}] - [C_q \text{ of reference gene}] \quad (1)$$

The normalized ΔC_q values already exist as the log transformed product of the expression ratios that are conventionally calculated using the $2^{-\Delta \Delta C_t}$ method [22], thereby fulfilling the assumption of linearity. The linearity of the ΔC_q data was confirmed by assessing a smoothed locally weighted scatterplot. The ΔC_q values were modelled as the magnitude of gene expression for each target gene.

One multivariable random-effect generalized least squares (GLS) model and two multivariable fixed-effect least squares dummy variable (LSDV) models were created in STATA 10.0 (Stata Corporation, College Station, TX). The GLS model was designed to evaluate the association of production characteristics with 9-wk BW across the 8 farms. The two LSDV models were designed

to determine the association of biomarker expression with ADG and 9-wk BW across 4 farms. Extraneous variables that were used for model building include the farm, parity of the sow, stillbirths per litter, live births per litter, sex of the piglet, age at weaning, weight at weaning, and disease status. Each model was manually designed using a parsimonious approach. All variables were first screened individually with univariable linear regression. Variables with a liberal p-value of ≤ 0.20 were included in the initial model. All variables regardless of inclusion were evaluated for confounding. A variable was determined to be a confounder if its inclusion in the model changed the coefficient of any predictor by $\geq 20\%$. Two-way interactions were generated for all variables in the initial model and interactions were retained in the model if they were statistically significant ($P < 0.05$). Any variable from the initial model was only retained in the final model if it was statistically significant ($P < 0.05$), if the variable behaved as a confounder, or if the variable was a component of a statistically significant interaction term. If two independent variables presented colinearity ($R^2 > 0.8$) then the less significant variable was excluded. Clustering at the farm level was accounted for by modelling each farm as a random-effect in the GLS model and as a fixed-effect in the LSDV models. Homoskedasticity and normal distribution of standardized residuals was assessed graphically for the GLS model. The assumptions of constant variance and normal distribution of standardized residuals was evaluated for each LSDV model using the Cook-Weisberg test and the Shapiro-Wilk test, respectively. Diagnostic analysis revealed no outliers or extremely influential observations. However, observations for two pigs were excluded from the model due to missing data. Effects with a $P < 0.05$ were considered statistically significant and non-significant effects with a $P < 0.10$ were considered to be suggestive of an association. All descriptive statistics are reported as the mean \pm standard deviation.

Results

The mean performance parameters for each farm are presented in Table 2. The average weight at 5-wk post-weaning (59.5 \pm 5.5 d) was 19.6 \pm 4.3 kg. From weaning to 5-wk post-weaning, a total mortality and culling rate of 2.4% (4/168) was observed. Table 3 summarizes the sow characteristics of each farm.

In the first model, sow parity, live births per litter, stillbirths per litter, sex, age at weaning, and the weight at weaning were used as the independent variables to determine an association with 9-wk BW. There were no additional confounding effects or significant interaction terms detected. The final model revealed that only the weight at weaning and the age at weaning were significantly associated with 9-wk BW (Table 4). This model

Table 2 Description of piglet growth performance by farm

Farm	Age at weaning (days)	Weight at weaning (kg)	9-wk BW (kg)	ADG (g/day)
1 ^a	26.7 ± 1.19	6.19 ± 1.54	21.9 ± 3.24	399 ± 62.4
2 ^a	22.7 ± 3.36	6.41 ± 1.70	17.4 ± 4.31	267 ± 77.0
3 ^a	34.4 ± 7.16	8.93 ± 2.00	21.0 ± 3.47	334 ± 72.4
4	28.3 ± 1.06	6.71 ± 1.88	22.2 ± 4.52	306 ± 65.0
5	30.9 ± 2.92	7.95 ± 1.81	20.2 ± 4.32	360 ± 97.1
6	18.7 ± 0.49	7.04 ± 1.51	22.6 ± 3.01	340 ± 47.5
7	26.1 ± 0.74	8.19 ± 1.82	23.0 ± 4.70	387 ± 143.9
8 ^a	22.4 ± 1.96	5.81 ± 1.30	17.9 ± 3.20	296 ± 55.5
Mean	26.4 ± 5.59	7.16 ± 1.96	20.7 ± 4.31	336 ± 83.2

BW body weight, ADG post-weaning average daily gain.

^aGene expression analysis was performed on liver tissue from the piglets originating from these farms.

explained 55.8% of the variation in 9-wk BW within each herd. The intraclass correlation coefficient (ICC) was 0.668 based on the herd-level and animal-level variance for the 8 farms.

Multivariable analysis revealed ADG was not significantly associated with hepatic gene expression of CRP, SAA, Hp, IFN- α , IFN- γ , TNF- α , IL-1 β , IL-6, IL-10, and IL-18 in the liver ($P > 0.05$), and none of these variables were retained in the final model. The effects of IGFBP-3 expression ($P = 0.016$) and GHR expression ($P = 0.009$) on ADG were statistically significant and the effect of IGF-1 expression ($P = 0.071$) was suggestive (Table 5). The model was able to explain 52.9% of the variation in post-weaning ADG. The weight at weaning was retained in the model because it had a confounding effect. All two-way interactions were not significant and thus excluded from the final model.

Expression of CRP, SAA, Hp, IFN- α , IFN- γ , TNF- α , IL-1 β , IL-6, IL-10, and IL-18 in the liver did not affect 9-wk BW ($P > 0.05$), and all of these variables were

Table 3 Description of sow and litter characteristics by farm

Farm	Number of sows	Sow parity ^a	Live births per litter ^a	Still births per litter ^a
1	650	2.3 ± 1.9	12.6 ± 2.8	0.9 ± 0.6
2	500	3.6 ± 2.3	11.4 ± 2.8	1.1 ± 1.0
3	1000	2.3 ± 3.2	13.9 ± 1.3	0.4 ± 1.4
4	240	2.3 ± 1.2	11.0 ± 0.5	0.6 ± 0.5
5	123	4.9 ± 2.2	12.3 ± 2.0	1.4 ± 1.0
6	650	3.1 ± 1.9	13.4 ± 1.7	1.0 ± 1.1
7	850	3.6 ± 2.2	11.3 ± 2.7	1.0 ± 0.8
8	670	4.1 ± 2.9	12.9 ± 3.2	0.1 ± 0.4
Mean	585	3.3 ± 2.43	12.3 ± 2.46	0.8 ± 1.0

^abased on the seven randomly selected sows per farm used in this study.

Table 4 Characteristics associated with the weight of pigs at 9 weeks of age (model 1)

Variable	Coefficient ^a	SE	95% CI	P value
Weight at weaning	1.670	0.128	1.419, 1.920	<0.001
Age at weaning	-0.226	0.069	-0.361, -0.090	0.001
Sex	0.524	0.424	-0.307, 1.356	0.217
Parity of sow	0.072	0.097	-0.117, 0.262	0.455
Stillbirths per litter	0.290	0.248	-0.197, 0.777	0.243
Live births per litter	-0.102	0.093	-0.285, 0.081	0.274

SE standard error, CI confidence interval.

^aThe predicted change in body weight (kg) at 9 weeks of age if the corresponding variable is increased by one unit (ie. the model predicts a decrease of 0.226 kg in body weight at 9 weeks of age if a pig is weaned one day later).

excluded from the final model. However, the effects of IGFBP-3 expression ($P = 0.012$) and GHR expression (0.016) were significantly associated with 9-wk BW. The effect of IGF-1 expression ($P = 0.055$) was suggestive of an association (Table 6). The model was able to explain 69.4% of the variation in the 9-wk BW. The weaning age and the weight at weaning were retained in the model because both variables had a confounding effect. All two-way interactions were excluded from the model as none were statistically significant.

Discussion

The weight at weaning and the age at weaning both contributed significantly to growth performance in nursery pigs. The weight at weaning was positively associated with body weight during the nursery period which is consistent with results from previous studies [23-25]. Additional research has demonstrated that pigs with higher weaning weights reach market weight 9–15 days earlier [23,26]. These results support the importance of pre-weaning interventions that improve weight at weaning and subsequent performance in the nursery.

Growth performance was negatively associated with age at weaning which implies that pigs weaned at an earlier age will reach a greater body weight during the nursery stage. This finding is in agreement with previous research which indicates that late weaning is disadvantageous for post-weaning growth performance [24]. Research by Main et al. determined that weaning at 21.5 d is optimal for nursery performance in multisite production

Table 5 Predicted change in ADG given a 2-fold increase in gene expression (model 2)

Gene	Change in ADG (g)	SE	95% CI	P value
IGF-1	12.85	6.990	-1.11, 26.82	0.071
IGFBP-3	-33.75	13.65	-61.03, -6.48	0.016
GHR	41.76	15.57	10.65, 72.87	0.009

ADG post-weaning average daily gain, SE standard error, CI confidence interval.

Table 6 Predicted change in 9-wk BW given a 2-fold increase in gene expression (model 3)

Gene	Change in 9-wk BW (kg)	SE	95% CI	P value
IGF-1	0.547	0.280	-0.012, 1.105	0.055
IGFBP-3	-1.409	0.545	-2.497, -0.320	0.012
GHR	1.580	0.638	0.305, 2.854	0.016

BW body weight, SE standard error, CI confidence interval.

systems [27]. In the present study, 80.4% of the piglets were >21.5 d old at weaning which is indicative of the negative association observed between weaning age and post-weaning growth performance.

The sex of the piglet was not associated with growth performance in the nursery. These findings consistently agree with previous research [23,24]. Growth performance of gilts and barrows appears to be significantly different only during the grower-finisher phase of production [8,23]. Paredes et al. demonstrated that sex can be significantly associated with nursery performance in different data sets although the authors attributed this consequence to differences in the proportion of intact males [25]. In the present study, all male piglets had been castrated prior to weaning.

Litter characteristics were not associated with nursery growth performance. The parity of the sow, stillbirths per litter, and live births per litter showed no effect on 9-wk BW. There are conflicting reports of the effects of sow parity on post-weaning growth performance. A study by Smith et al. suggests that piglets born to primiparous sows have a growth disadvantage compared to higher parity sows [28]. However, the authors noted that the mean post-weaning weights varied greatly among parties and there was no discernible pattern of growth performance. Other researchers have concluded that sow parity does not influence growth performance at the nursery stage [25]. In addition, previous findings have demonstrated that the number of live births per litter is not associated with post-weaning growth performance [25], although the number of live births does appear to be associated with birth weight [29]. One reason for lack of association between sow characteristics and growth performance in this study may have resulted from selecting three piglets of small, medium, and large size from each sow.

Increased expression of IGF-1 in the liver showed a tendency to be positively associated with ADG and 9-wk BW. This is consistent with other studies which have determined that serum IGF-1 concentration is positively associated with growth in pigs and other animals [8,30]. Furthermore, over-expression of IGF-1 at 1.5-fold the normal levels in transgenic mice resulted in a 30% increase in body weight [4]. In this study, a smaller effect was observed in pigs with the model predicting that a 2-

fold increase in IGF-1 would result in a 2.6% increase in body weight at 9 weeks of age for the average nursery pig. The statistical significance of the association between IGF-1 and growth may have been undermined by the large variation in IGF-1 RNA levels. The variation in hepatic IGF-1 expression is likely due to the natural variability observed during this stage of development [1,30]. Additionally, differences in production management, feed composition, and antimicrobial usage may have contributed to the large variation in hepatic IGF-1 expression [31]. It is likely that differences in breed and sex across farms were not a factor in variation as previous studies have demonstrated that IGF-1 levels are not significantly different between breeds [10,32] or between barrows and gilts [30]. Expression of IGF-1 is likely more useful as a growth-associated biomarker for comparing pigs that are raised in the same herd under the same conditions.

IGFBP-3 expression in the liver was negatively associated with ADG and 9-wk BW. The model designed in this experiment predicted that a 2-fold increase in IGFBP-3 expression would reduce 9-wk BW by 6.8% in the average nursery pig. Contrary to the results of our findings, previous research by Owens et al. showed a weak positive correlation between serum IGFBP-3 concentration and ADG [30]. However, other research has been demonstrated that IGFBP-3 is expressed significantly less in gilts bred for enhanced growth performance compared to gilts with diminished growth performance [8], and that hepatic expression of IGFBP-3 is higher in slow-growing breeds than in fast-growing breeds of pigs [10]. Further research using transgenic mice found that a 4.9-7.7-fold increase in IGFBP-3 expression resulted in a 10% reduction in birth weight, moderate post-natal growth retardation, and reduced organ weight [7]. Over-expression of IGFBP-3 in transgenic mice also resulted in a 1.9-2.8-fold increase in circulating IGF-1 levels [7], a finding that can be explained by the concomitant mechanisms that regulate both IGF-1 and IGFBP-3 expression [33,34]. It is likely that neglecting to account for IGF-1 levels during analysis lead Owens et al. to discover a weak positive association between IGFBP-3 and growth performance [30]. Re-modelling the data from our study without IGF-1 confirmed that IGF-1 does have a major confounding effect on the IGFBP-3 coefficient. Overall, the significant negative association between IGFBP-3 and growth performance found in the present study is consistent with findings in other studies [7,8,10].

Expression of GHR in the liver was positively associated with growth performance in nursery pigs. A 2-fold increase in GHR expression appeared to have the greatest effect on the magnitude of growth compared to the same increase in IGF-1 and IGFBP-3 expression. A transgenic experiment with mice demonstrated that deletion of the

GHR gene resulted in decreased circulating IGF-1 and a substantial reduction in body weight [35]. These findings are consistent with the results presented in this paper. Further studies have demonstrated that *in vitro* GHR expression of swine hepatocytes is significantly influenced by glucose and amino acid concentrations [36], and *in vivo* experiments with pigs indicate that dietary protein concentration is positively associated with GHR expression in the liver, skeletal muscle, and adipose tissue [37]. In light of the results from the present study, assessing expression of GHR may be useful for evaluating the effects of different dietary regimens on growth performance at the molecular level.

ADG and 9-wk BW were not associated with hepatic expression of the APPs assessed in this study. A previous experiment by Saco et al. demonstrated that Hp is a suitable biomarker for assessing the effects of an immunomodulating feed additive on production parameters [15]. However, Saco et al. were unable to exclusively identify an association between Hp and growth performance [15]. Additional research by Pineiro et al. demonstrated differential serum concentrations of CRP and Hp between two groups of pigs subjected to different feeding regimens [14]. Although the ADG was significantly different between groups, Pineiro et al. attributed the differences in APP levels to stress caused by disorderly feeding [14]. In this study it is likely that no significant association was found between APP expression and growth performance because APP levels are influenced considerably by pathogen colonization and stress [14,38]; two factors which are not necessarily indicative of growth performance. The results of the present study indicate that CRP, Hp, and SAA are not suitable biomarkers for evaluating the production performance of nursery pigs on commercial farms.

The expression of IFN- α , IFN- γ , TNF- α , IL-1 β , IL-6, IL-10, and IL-18 in the liver was not significantly associated with growth performance in nursery pigs. Earlier studies have demonstrated that body weight is significantly associated with circulating levels of IL-6, IL-8, IL-18, and TNF- α in humans [39,40], and differential expression of IL-1, IL-6, and TNF- α in horses [41]. The lack of association between body weight and hepatic cytokine expression observed in this study is likely due to previous findings which indicate that excess adipose tissue is the primary source of increased levels of circulating cytokines [42-44]. Monitoring adipose-associated cytokines may be useful for evaluating obesity in other species. Overall, cytokine expression in the liver is unsatisfactory for monitoring growth parameters in nursery pigs, although adipose or serum levels of these cytokines may still prove to be useful biomarkers to evaluate growth performance.

One of the limitations of this study was the lack of dietary information for the swine herds. However, diet is

uniform on each farm and farm-level clustering was accounted for in each regression model during the statistical analysis. Furthermore, it may be argued that diet acts as an intervening variable which can introduce considerable variation into the data but would not need to be controlled for the results to be valid.

Measuring the hepatic expression of IGF-1, IGFBP-3, and GHR can be useful biomarkers for evaluating the growth performance of nursery pigs. It is likely that serological levels of IGF-1, IGFBP-3, and GHR are representative of the changes in liver expression levels, however further research is needed to confirm this assumption. The advantages of evaluating gene expression is that it allows for the determination of growth-associated biological activity in specific tissues and it demonstrates that transcriptional diversity is related to overall growth performance. Hence, the results of this study should direct future research to explore whether any polymorphisms in growth factor genes (*IGF1*, *IGFBP3*, *GHR*), or accessory genes such as proteases, are associated with transcriptional diversity and indicative of growth performance in pigs. Identifying polymorphisms related to growth factor expression may improve the selection of genetic lineages for enhanced production performance. Quantifying IGF-1, IGFBP-3, and GHR may also be beneficial in research and experimental settings where they may be used, in addition to conventional methods, to assess interventions, treatments, or clinical pathologies in relation to growth performance of nursery pigs. Future research should focus on whether RNA and serum levels of IGF-1, IGFBP-3, and GHR are indicative of growth performance at other phases of swine production and whether biomarker expression during the nursery phase can predict performance in later phases of production.

Conclusion

Overall, IGFBP-3 and GHR expression in the liver was significantly associated with growth performance across multiple swine production systems despite variation in operating procedures. IGF-1 expression had a tendency to be associated with the measured growth parameters but the large variation reduced its statistical significance in this study. Nevertheless, IGF-1 is likely a useful biomarker of growth performance for comparing pigs from the same herd. IGFBP-3 and GHR appear to be suitable biomarkers for evaluating growth performance in nursery pigs, either in a controlled experimental setting or in population-based studies involving multiple swine herds with varying management practices. Hepatic expression of CRP, Hp, SAA, IFN- α , IFN- γ , TNF- α , IL-1 β , IL-6, IL-10, and IL-18 was not associated with the growth parameters in this study and appear to be inadequate for monitoring growth in pigs.

Competing interests

The authors of this research declare that they have no competing interests.

Authors' contributions

MJS: Field and post-mortem sample collection; RNA extraction, quantification, and qualification; coordinated RT-qPCR; data analysis; writing manuscript. RF: Recruitment of swine producers; coordinated field work; interpretation of data analysis; critical review. CFMDL: Study design and oversight; coordinated RT-qPCR; interpretation of results. MR: post-mortem sample collection; primer design; RNA extraction; DNase treatment; interpretation of results. The final manuscript was read and approved by all authors. AF: Designed study and survey; recruitment of swine producers; pre-mortem examinations and autopsies; data analysis. All authors read and approved the final manuscript.

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Author details

¹Department of Population Medicine, Ontario Veterinary College, University of Guelph, 50 Stone Road, N1G 2 W1 Guelph, Ontario, Canada. ²Department of Animal and Poultry Science, University of Guelph, 50 Stone Road, N1G 2 W1 Guelph, Ontario, Canada.

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