Pig Development
Department

Workshop Proceedings

Importance of nutrition and environment on birth weight, muscle growth, health and survival of the neonate

Animal and Grassland Research and Innovation Centre,
Moorepark, Fermoy Co. Cork, Ireland.
Thursday 4th - Friday 5th May, 2017

Teagasc
Agriculture and Food Development Authority

Workshop
Proceedings 2017

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Organising Committee: Peadar Lawlor (Teagasc, Ireland),
Niels Oksbjerg (Aarhus University, Denmark), Giuseppe Bee (Agroscope, Switzerland),
Gillian Gardiner (Waterford Institute of Technology, Ireland),
Keelin O'Driscoll (Teagasc, Ireland), Linda Giblin (Teagasc Ireland)

Programme Agenda

Thursday May 4th, 2017

08.00	Leave Cork
09.00	Start

Prenatal nutrition and environment (1) Chair: Peadar Lawlor			Abstract No.
09.10	The importance of early incubation temperature for embryonic development, post-hatch growth and ability to walk in chicks – a new research collaboration between industry and SEGS and Aarhus University	M. Therkildsen	1
09.30	Effect of L-arginine and L-carnitine in gestating sow diets on sow output and piglet growth	K. O'Driscoll	2
09.50	Effect of dietary crude fibre level and L-carnitine inclusion during gestation on gilt and piglet performance	H. Rooney	3
10.10	Effect of maternal arginine supplementation on offspring performance in a commercial swine production environment	E. Hines	4

10.30-10.50	**BREAK**

Prenatal nutrition and environment (2) Chair: Niels Oksbjerg			Abstract No.
10.50	Dietary L-arginine supply during early gestation promotes myofiber hyperplasia	G. Bee	5
11.10	Supplementation of L-arginine during gestation and lactation of sows affect muscle traits of offspring related with postnatal growth dependent on birth weight and sex: From conceptus to consumption	N. Oksbjerg	6
11.30	Effect of salmon oil and vitamin D3 on piglet vitality at birth and viability to weaning	A. Lavery	7
11.50	Effects of dietary seaweed extract supplementation on the reproductive performance of sows and suckling pig performance	A.M. Walsh	8

Future Funding Opportunities			
12.10	Opportunities for European funding in this area (COST, H2020, ERA Nets etc.)	R. Kelly/ O. Ni Choncubhair	

12.30-13.30	**LUNCH**

13.30-14.00	Discussions on future funding/activities

Postnatal nutrition and environment Chair: Giuseppe Bee			Abstract No.
14.00	Effect of management strategies for rearing supernumerary piglets on piglet survival and growth rates	O. Schmitt	9
14.20	Effects of energy supplementation to low-birth weight neonatal piglets on their survival, growth and blood glucose level.	O. Schmitt	10
14.40	Voluntary locomotor activity promotes myogenic growth potential in domestic pigs	C. Kalbe	11
15.00	1NMR characterisation of colostrum in pure breed sows and its influence on piglets' performance	P. Trevisi	12
15.20	The effect of different dietary bee pollen consumption on growth intensity and blood parameters of rats: A laboratory study	B. Gálik	13

15.40-16.00	**BREAK**

Intestinal microbiota (1) Chair: Keelin O'Driscoll			Abstract No.
16.00	Porcine feed efficiency (FE)-associated intestinal microbiota and physiological traits: finding consistent cross-locational biomarkers for residual feed intake (RFI)	U. McCormack	14
16.20	Faecal microbiota transplantation in pregnant sows alters the growth and intestinal bacterial profile of pigs	G. Gardiner	15
16.40	Altering the chicken microbiome in ovo – the concept and future perspective	K. Stadnicka	16

17.00	**END**

17.15	Bus to Cork
19.30	Dinner - Montenotte Hotel, Middle Glanmire Road, Cork City

Friday May 5th 2017

08.00	Leave Cork		
09.00	START		

Genetics/gene expression Chair: Linda Giblin			Abstract No.
09.00	Acute and delayed transcriptional responses following livestock-environment interactions	M. Oster	17
09.20	The influence of maternal backfat and gestation feed level on offspring adipose and muscle biomarkers	L. Giblin	18
09.40	Changes in metabolic gene expression during C2C12 myogenic differentiation	M. Brearley	19
10.00	Phenotypic and genetic relationships between litter birthweight characteristics, indicators of intrauterine growth restriction and piglet survival	S. Matheson	20.

10.20-10.40	**BREAK**

10.40-11.30	Review: Maternal nutrition effects on muscle development and body composition of lambs	J. Brameld	21.

Intestinal microbiota (2) Chair: Gillian Gardiner			Abstract No.
11.30	A gnotobiotic piglet model of preterm infants	I. Splichal	22
11.50	Development of an effective *Bacillus* probiotic for use as an alternative to in-feed antibiotics for pigs	G. Gardiner	23
12.10	European network on the factors affecting the gastro-intestinal microbial balance and the impact on the health status of pigs (PiGutNet)	P. Trevisi	24
	Fish		
12.30	Growth and Flesh Quality: From study of muscle tissues to flesh quality determinisms in fish	JC. Gabillard	25

12.50 - 13.00	Future Funding Opportunities/Activities Future meetings?

13.00	**LUNCH**

14.00	Bus to Cork
	Tour of New Pig Research Facility
	Tour of Laboratories
16.00	Bus to Cork

1. The importance of early incubation temperature for embryonic development, post-hatch growth and ability to walk in chicks – a new research collaboration between the industry, SEGES and Aarhus University

M. Therkildsen[1], J.S. Petersen[2] and N. Oksbjerg[1]

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Meat from broilers is highly appreciated by the consumers, and specifically the demand for the breast fillet is increasing. This demand has been met by genetic selection of lines characterized by fast post-hatch growth and greater yield of the breast fillet but not leg muscles. This focus in the selection has caused an imbalance in the body of the chickens, leading to very large pectoralis muscles but decreased leg strength, which cause impaired animal welfare because of decreased ability to walk. To address this animal welfare and productivity problem the broiler industry in Denmark is collaborating with the advisory service (SEGES) and researchers at the university in a project, which manipulate with the temperature during incubation of chicks. The early incubation temperature has been shown to affect the number of muscle fibres in the leg muscle of chick embryos (Hammond *et al.* 2007), and this may support a more balanced development post-hatch between the breast and leg muscles, supporting the chickens ability to walk. An increased incubation temperature from 37.5 to 38.5°C from day 4 to 7 increase the embryonic movements and this may explain the increased number of muscle fibres and ratio of nuclei per fibre at day 18 of the incubation period (Hammond *et al.* 2007).

Methods

Three incubators (Linco Incubator, Ikast, Denmark) were used in the experiment, each with room for 72 fertilized chicken eggs (Ross 308). The eggs were numbered, weighed and placed randomly in the three incubators all adjusted to 37.5°C and the same humidity. From day 4 to 7, the temperature was raised with one degree Celcius (38.5°C) in one incubator and lowered with one degree Celcius (36.5°C) in another incubator. The temperature in the last incubator was not changed (37.5°C). From day 8 the temperature in all incubators were held at (37.5°C) until hatching. At day 7 in the embryonic period the embryonic movements in 5 eggs from each incubator were studied in 3 min. by illumination by a light source. At hatching, d 15 and 32 post hatching the weight of the chickens were recorded and at d 30 the chickens ability to walk were scored according to the gait score method (Bristol method, 1992 - score 0 resembling unhindered walk, score 5 resembling chickens unable to walk). The chickens were killed at day 34, and the sex and the weight of the pectoralis muscle (breast) and the gastrocnemius muscle (leg) were recorded.

Results

The incubation temperature affected the embryo activity at d 7 with low, medium and high activity in eggs exposed to 36.5, 37.5 and 38.5°C, respectively. In addition, the percentage of hatching (88, 97 and 100%) and the percentage of males (53, 36 and 37%) also differed between the incubation temperatures (36.5, 37.5 and 38.5°C, respectively). The response to the different incubations temperatures are shown in table 1. There were a negative effect of the low temperature on the male chick weight at hatching, whereas in the female chicks the low incubation temperature led to a heavier body weight at day 15 post hatching. The different incubation temperatures did not change the weight of the muscles nor the gait score of the chicks.

Table 1: Effect of incubation temperature from d 4-7 on body weight and muscle weight of female and male chickens d 0, 15 and 32 and gait score d 30.

	Female				**Male**			
Temp from d 4-7	36.5	37.5	38.5	p-value	36.5	37.5	38.5	p-value
Wgt d 0, g	44.4	45.3	46.4	0.20	45.8	47.2	47.2	0.01
Wgt d 15, g	622	606	596	0.06	685	655	665	0.21
Wgt d 32, g	2172	2164	2144	0.86	2585	2570	2542	0.81
Pectoralis, g	212	210	211	0.85	257	258	254	0.92
Gastrocnemius, g	11.2	11.1	11.1	0.93	13.0	13.9	13.1	0.41
Gait score	0.83	1.01	0.91	0.83	2.11	1.92	2.07	0.87

Conclusion

The manipulation with the early (4-7 d) incubation temperature did not show a direct effect on body and muscle weight, although the temperature seemed to affect activity in the egg. However, there seemed to be an effect of incubation temperature on sex, a low temperature leading to more male chicks, which is favorable for the industry. This the experiment will be followed by a new design to further study possible effects of incubation temperature on body conditions and sex.

[1.] Hammond, C. L., Simbi, B. H., & Stickland, N. C. 2007. In ovo temperature manipulation influences embryonic motility and growth of limb tissues in the chick (Gallus gallus). The Journal of Experimental Biology 210, 2667-2675.

2. Effect of L-arginine and L-carnitine in gestating sow diets on sow output and piglet growth.

K. O'Driscoll[1], K. Reid[1,2], E. Magowan[3], J.V. O'Doherty[2] and P.G. Lawlor[1]

[1] AGRIC, Teagasc, Moorepark, Co. Cork, Ireland; [2]Agri-Food and Biosciences Institute, Hillsborough, N. Ireland; [3] University College Dublin, Ireland

In an attempt to increase sow output, dam-line genetic improvement has resulted in considerable increases in litter size over the past 10 years. However, large litters can result in problems with regard to health and growth of the piglets, with one of the major issues for piglets being low birth weight. A large number of embryos results in increased uterine crowding, leading to more competition for space and nutrients prior to birth. This study evaluated the effect of supplementing highly prolific multiparous sows with L-Arginine and/or L-Carnitine on sow and piglet performance in a commercial setting. L-arginine has been shown to improve piglet birth weight, and number of piglets born, in sows which are of average prolificacy (1). It is involved in placental angiogenesis, embryogenesis, and placental and foetal growth. Carnitine is hypothesised to be involved in muscle fibre development and is involved in regulating transport of fatty acids across the mitochondrial membrane. Similar to L-arginine, it has been shown to have beneficial effects on piglet birth weight, as well as reducing the number of non-viable piglets (2), in sows of average to low prolificacy. We hypothesised that both L-arginine and L-carnitine supplementation to highly prolific sows during gestation would increase mean birth weight and total born, and that when both were added, the effect would be additive.

Methods

Sows (n=429) were used in the study, which was carried out using 16 replicates over time. Within replicate, sows were blocked on their breed, parity, back fat depth, and previous numbers born alive. At day 28 of gestation they were randomly assigned within block to one of 4 dietary treatments; 1) control (CON), which consisted of the standard sow diet provided on the farm: 2) control plus 25g/day of L-arginine per day (ARG): 3) control plus 0.125g/day of L-carnitine (CAR): and 4) control plus 25g/day of L-arginine plus 0.125g/day of L-carnitine (ARGCAR). Sows remained on their treatment until they farrowed. The total number of piglets born (TB) and born-alive (BA) were recorded. Piglets from a subset of 216 litters were weighed at birth (n = 3309) and at weaning (n=1836). Piglets were ranked into 5 categories on the basis of birth weight, to investigate whether the supplements were beneficial for piglets which were particularly weak or heavy at birth. Data were analysed using the mixed models procedure in SAS (v9.4) using a two by two factorial arrangement.

Results

There was no interactive effect between CAR and ARG on any variable measured. Arginine reduced TB and BA compared to non-ARG sows (Table 1). However piglets from CAR sows were 60g heavier at birth than those from non-CAR supplemented sows (Table 1). There was no treatment effect on average daily gain from birth to weaning, or on weaning weight (P>0.05). However, there tended to be an interaction between CAR and rank for both (P = 0.06, P = 0.1, respectively), with piglets that were in the lightest rank at birth gaining more during lactation from CAR sows, whereas piglets from heavier ranks gained less.

Table 1. Effect of L-arginine and L-carnitine (CAR) supplementation during gestation on litter size, birth weight, and weaning weight

	Arg				**Car**			
	Y	**N**	**sem**	**P-value**	**Y**	**N**	**sem**	**P-value**
Total born (No.)	15.03	15.70	0.22	0.03	15.31	15.41	0.22	0.75
Born alive (No.)	14.09	14.72	0.21	0.02	14.26	14.52	0.21	0.37
Birth weight (kg)	1.28	1.29	0.02	0.54	1.31	1.25	0.02	0.03
ADG to weaning (g/d)	242	245	3	0.20	241	246	3	0.18
Weaning weight (Kg)	7.97	8.03	0.08	0.61	7.93	8.07	0.08	0.19

Conclusions

In conclusion, in contrast to previous work Arg tended to reduce no. born but had no effect on birth weight, possibly due to the level of prolificacy of the sows used, however, CAR was beneficial in increasing piglet birth-weight, and appeared to improve weaning weight for the lightest piglets born.

[1.] Wu, X. et al.(2012). Animal reproduction science 132:187-192;

[2.] Birkenfield, C. et al. (2006). Animal Feed Science and Technology 129: 23-38

3. Effect of dietary crude fibre level and L-carnitine inclusion during gestation on gilt and piglet performance

H.B. Rooney*[1,2], K. O'Driscoll[1], J.V. O'Doherty[2] and P.G Lawlor[1]

[1] *AGRIC, Teagasc, Moorepark, Co. Cork, Ireland;* [2]*University College Dublin, Ireland*

Over the past decade, selection for increased sow prolificacy has resulted in a reduction in mean piglet birth-weight and increased piglet mortality. Therefore strategies to increase piglet birth-weight and robustness at birth are of increasing importance. In general, gilts tend to give birth to lighter piglets than sows (1). Previous research at our group found that supplementing gestating sows with L-carnitine increased piglet birth-weight; however, there is little information available regarding L-carnitine supplementation in gilt diets. Piglets from gilt litters are lighter at birth and also have a lower pre-weaning growth rate than piglets from multiparous sows (2). During lactation, high feed intake is important to maximise milk production and subsequent reproductive performance. In general, gilts have lower lactation feed intakes than multiparous sows, which is in part due to their lower gut capacity. Increasing the level of fibrous ingredients in the gestation diet should increase gut capacity at farrowing thereby facilitating increased feed intake during lactation. The aim of this experiment was to determine the effect of increasing fibre level and L-carnitine inclusion in gilt gestation diets on gilt and piglet performance. We hypothesised that L-carnitine supplementation to gilts during gestation woould increase mean birth weight, and that increased fibre, provided by 40% sugarbeet pulp, in the gestating gilt diet would increase gilt lactation feed intake, thereby increasing piglet survival and growth to weaning.

Methods

Eighty four pregnant gilts (Landrace * Large White) were used in the study, which was conducted in 4 batches (~ 28 gilts each) over time. At d38 of gestation, gilts were blocked within batch, on live-weight and back fat depth before being randomly assigned to one of four dietary treatments; (1) Control fibre [crude fibre (CF) = 4.5%], (2) High fibre (CF= 9.8%; HF), (3) Control plus L-carnitine (0.125g/day; CAR) and (4) HF plus L-carnitine (0.125g/day; HFCAR), up to farrowing. Gilts were weighed and back-fat depth was recorded at d38, d90, d108 and at weaning (~d26 of lactation). Saliva samples were collected every 3 weeks from gilts between d90 and d108 of gestation. The number of piglets born alive and born dead per litter was recorded. Individual piglet weight was recorded at birth, 24h after birth, d6, d13 and weaning. A blood sample was taken from the ear vein of piglets at 24h post-partum and glucose concentration (an indication of survival to weaning) was determined. Data were analysed using the mixed models procedure in SAS (v9.4) for a two by two factorial arrangement.

Results

Birth-weight of piglets was not affected by treatment. However, sex did have an effect (P<0.05), with males (1.22kg) being born heavier than females (1.18kg). The total number born and the number born alive were similar for all treatments (P>0.05). Cortisol levels were numerically higher for Fibre (Y; 0.53 u/DI) when compared with Fibre (N; 0.46 u/DI), however, the difference was non-significant P>0.05). Glucose levels were not significantly affected by treatment (P>0.05). Although sex did have an effect (P=0.008), with males (5.03 mg/dL) having higher glucose levels than females (4.80 mg/dL). Glucose level was affected by birth-weight (P<0.001).

Table 1. Effect of L-carnitine (CAR) supplementation and increased fibre levels during gestation on litter size, birthweight, glucose and cortisol level

	CAR				Fibre			
	Y	**N**	**sem**	**P-value**	**Y**	**N**	**sem**	**P-value**
Total born (No.)	14.8	14.9	0.37	0.87	14.5	15.2	0.37	0.23
Born alive (No.)	14.0	13.9	0.42	0.82	13.5	14.4	0.42	0.12
%Born alive	93.1	93.2			96.0	92.9		
Birthweight (kg)	1.19	1.21	0.030	0.61	1.19	1.21	0.030	0.65
Glucose (mg/dL)	4.80	5.03	0.167	0.28	5.10	4.73	0.168	0.10
Cortisol (u/DI)					0.53	0.46	0.045	0.18

Conclusion

These data do not support our original hypothesis. The absence of a response to L-carnitine may be because this experiment was conducted with gilts as opposed to sows.

[1.] MAHAN, D (1993). Journal of Animal Science 71: 1991-1995;
[2.] CARNEY-HINKLE, E. et al. (2013). Journal of animal science 91: 2885-2893

4. Effect of maternal arginine supplementation on offspring performance in a commercial swine production environment

E.A. Hines[*1,2]; M. Romoser[1]; A.F. Keating[1]; L.H. Baumgard[1]; J. Niemi[1]; N.H. Williams[2]; B. Haberl[2]; B. Kerr[1]; K. J. Touchette[3] and J.W. Ross[1]

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Feeding the growing pig is undoubtedly one of the most intensive financial inputs for the swine industry. As much as 70% of operating costs in commercial pig production can be accounted for in feed alone, with protein making up approximately 30% of diet costs. During gestation, amino acid requirements of the developing fetus change with increasing gestational age (Wu *et al.*, 1999; NRC, 2012) and arginine (ARG) appears to be an important source of nitrogen in fetal pigs at all stages of development. Supplementing ARG is also necessary for neonatal growth and conditionally necessary for sows during gestation (NRC, 2012). However, in commercial production settings, it is unclear what level dietary ARG should be supplied or what stages of gestation may most benefit from additional ARG. Furthermore, the benefit of gestational supplementation of ARG on offspring performance is ill-defined. Project objectives were to evaluate supplementation of L-ARG (at 1% inclusion, as fed) at different stages of gestation and determine its influence on offspring performance in a commercial swine production system.

Methods

Commercially reared pubertal gilts (n = 548) were divided into four diet by stage of gestation treatments: Control (n = 143; 0% supplemental ARG); Early (n = 138; 1% supplemental ARG 15-45d of gestation); Full (n = 139; 1% supplemental ARG 15d of gestation until farrowing); and Late (n = 128; 1% supplemental ARG 85d of gestation until farrowing). The Control diet served as the base for all dietary treatments, with an average base ARG level at 0.65% analyzed total, as fed. Individual piglets were weighed at birth and weaning (17.6 d of life). After weaning, individual pig weights on a subset of offspring were collected at targeted average weights of 30, 60, and 100 kg to evaluate growth performance, and carcass data were collected on a subset of pigs at harvest. Statistical analyses (SAS 9.0, Cary, NC) were performed on gilt and offspring performance. Gilts and offspring participating in any cross foster events during lactation were removed for pre- and post-weaning performance parameters. All offspring growth performance data were analyzed using birth weight and weaning weight age as covariates.

Results

Supplementation of ARG at any point in gestation did not affect total born (14.28 ± 0.21; P = 0.96), number born alive (13.04 ± 0.22; P = 0.37), or number of pigs weaned (11.51 ± 0.49; P = 0.83). Birth weight of viable pigs was also not affected (P = 0.20), with piglets weighing 1.34, 1.37, 1.38, and 1.34, ± 0.02 kg for offspring from Control, Early, Full, and Late treatments, respectively. Individual wean weights tended to be increased (P = 0.09) in offspring from gilts supplemented with ARG at 5.16, 5.24, 5.32 ± 0.10 kg for Early, Full, and Late treatments, respectively, as compared to offspring from the Control maternal diet (5.10 kg ± 0.10 kg). This observation was similar in pre-wean total weight gain (3.72, 3.78, 3.86, and 3.94 ± 0.10 kg; P = 0.09) and pre-wean average daily gain (ADG; 0.220, 0.223, 0.228, 0.232 ± 0.006 kg/d; P = 0.08) for offspring from Control, Early, Full, and Late maternal dietary treatments, respectively. Maternal ARG supplementation did not affect (P = 0.76) postnatal growth performance from birth to approximately 100 kg of weight, as illustrated by life time ADG of offspring being 0.65, 0.65, 0.67, 0.64 ± 0.02 kg/d for Control, Early, Full, and Late dietary treatments, respectively. Hot carcass weight (84.1 ± 1.4 kg; P = 0.92) and fat to lean depth ratio (0.28 ± 0.01; P = 0.74) were also similar in offspring from different maternal dietary treatments.

Conclusion

This data analysis suggest that maternal supplementation of additional ARG, at 1% inclusion, tended to improve pre-wean piglet performance in a commercial swine facility. Further investigation into supplementing ARG is needed to understand the appropriate level for optimal performance of fetal development and postnatal growth in commercial production systems. This project was funded in part by the Iowa Pork Producers Association and Ajinomoto Heartland, Inc.

[1.] National Research Council. 2012. Nutrient Requirements of Swine: Eleventh Revised Edition. The National Academies Press, Washington, DC.
[2.] Wu, G., T. L. Ott, D. a Knabe, and F. W. Bazer. 1999. Amino acid composition of the fetal pig.
[3.] J. Nutr. 129:1031–8.

5. Dietary L-arginine supply during early gestation promotes myofiber hyperplasia

J. G. Madsen[1,2], C. Pardo[1,2], M. Kreuzer[2] and G. Bee[1*]

[1]*Agroscope Posieux, Posieux, Switzerland;* [2]*ETH Zurich, Institute of Agricultural Sciences, Zurich, Switzerland.*

Intra-uterine crowding (IUC) observed in hyperprolific sow impairs myofiber hyperplasia and overall fetal growth. Arginine supplementation (ARG) in gestation diets has been shown to positively affect litter and muscle development. The study objective was to assess whether the effect of ARG on offspring characteristics, with special emphasis on myofiber hyperplasia, differs under IUC conditions from these responses, because in that situation growth retardation is particularly prevalent due to reduced fetal nutrient supply. Unilateral oviduct ligation (OL) was used as a model for an uncrowded and hyperprolificacy (IN) as a model for a crowded intra-uterine environment.

Methods

Five OL and five IN sows were fed a diet supplemented daily with either 43 g l-alanine (Ctrl) or 25 g l-arginine from d 14 to 28 of gestation in a cross-over design (two periods: 5th and 6th parity). At farrowing, two male and two female offspring, respectively, with a low and intermediate birth weight were selected. After euthanization, the Semitendinosus muscle was removed and weighed, and the light and dark portions were prepared for myofiber histochemistry using ATPase staining and the entire Semitendinosus muscle for gene expression analysis of myogenesis-related genes using RT-qPCR. In addition, various organs were removed and weighed. Data were analyzed using the MIXED model in SYSTAT.

Results

No effect of either IUC or dietary treatment was found in litter characteristics. Offspring of ARG sows displayed a greater muscle area in Semitendinosus muscle ($P < 0.01$) as a result of the greater myofiber hyperplasia ($P < 0.01$). The increase was more distinct in the light portion ($P < 0.05$) than in the dark portion of the Semitendinosus muscle ($P = 0.131$). Offspring of OL sows were heavier at birth ($P < 0.01$), had a heavier Semitendinosus muscle ($P < 0.05$), liver ($P < 0.01$) and kidney ($P < 0.05$), but when expressed relative to birth weight, these differences were absent. In addition, IUC had an effect ($P < 0.05$) on the expression of one of the myogenesis-related genes investigated.

Conclusion

Independent from the extent of IUC, ARG improved birth weight, muscle and organ weights and myofiber hyperplasia in offspring.

6. Supplementation of L-Arginine during gestation and lactation of sows affect muscle traits of offspring related with postnatal growth dependent on birth weight and sex: From conceptus to consumption.

[1]Oksbjerg, N., [2]Krogh, U., [1]Askov Jensen, J., Møller, H.S., [3]Ramaekers, P., [1]Krøyer Rasmussen, M. and [2]Theil, P.K.

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It is well known that piglets born small (low birth weight, (LWB) have a higher mortality rate and lower performance traits compared to littermates with a high birth weight (HBW). This is most likely due to poor nutrition during foetal development, resulting in formation of a lower outcome of the myogenesis, i.e.,the number of muscle fibres, which is proportional to daily gain, feed conversion rate and meat percentage. This call for means to prevent development of LBW. Nutrients for foetal growth across the placenta follow Fick's principles and is the product between the blood flow and the arteria-venous differences. The functional amino acid, L-Arginine, besides being used for protein syntheses also is a precuser for nitric oxide production, which is a potent vasodilator. We thus, hypothesize that L-Arginine supplementation to pregnant sows increases foetal nutrients up-take, resulting in more muscle fibres and consequently enhance postnatal performance. The present study aims to study the influence of L-Arginine supplementation to pregnant sows on the performance, muscle histology, total DNA and some meat quality in offspring following supplementation of L-arginine (ARG).

Methods

In the present study, offspring (Danish Duroc x Danish Landrace x Danish Large White) from 13 multiparous sows was used. The sows were fed 25 g of L-arginine (Nutreco) daily as a topdressing (n=7) or iso-nitrogenous amounts of alanine (CON) (n=6) from d 30 of gestation to d 28 of lactation (TREAT). Selected offspring were low birth weight piglets (LBW) and high birth weight (HBW) piglets of each sexes (castrated male pigs and female pigs). Daily gain was measured and at slaughter at day 140, the cross-sectional area of semitendinosus (CSAST) was estimated. Muscles (semitendinosus and longissimus dorsi) were sampled for enzyme analyses, histochemical traits and meat quality traits. Statistical analyses were performed by means of the SAS version 9.3 (SAS Institute lnc, Cary, NC, USA) using the MIXED procedure according to a 2x2x2 factorial design. The model includes the main effects and their interactions and as random factor sow within treatment was included.

Results

Treating the sows with ARG increased post weaning daily gain (890 vs. 834; $P<0.05$) and CSAST (44.8 vs. 42.7 cm2). At slaughter, the size of the response to ARG was larger in LBW female pigs (5.2 cm2) compared to HBW male pigs (2.9 cm2). Some muscle traits reflecting muscle growth such as the number of S-fibres, was lower in LBW female pigs born to control sows compared with LBW female pigs born to ARG sows. The muscle total DNA also was lower in LBW female pigs but restored following treatment of the sows with ARG. These muscle traits were unaffected in HBW male pigs born to either CON or ARG sows. Supplementation of ARG reduced pro-glycogen, and macro-glycogen, while OH-acyl- CoA-dehydrogenase (HAD) and citrate synthase (CS) were unchanged. On the other hand, lactate dehydrogenase (LDH) was increased after ARG treatment. Offspring born to sows treated with ARG affected meat tenderness (shear force) dependent on sex.

Conclusion

Treatment of the sow with ARG increase offspring performance most pronounced in small females pigs most likely due to increased number of S-fibres and increased muscle DNA suggesting that both myogenesis and satellite cell proliferation are involved.

7. Effect of salmon oil and vitamin D_3 on piglet vitality at birth and viability to weaning

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Additional fats are often added to increase energy density in pig diets but there are currently no recommended inclusion rates of n-3 fatty acids in sow diets. Docosahexanoic acid (DHA) is a n-3 fatty acid commonly found in fish oils that has been shown to increase piglet vitality at birth (Rooke J., *et al*, 2001), improve the growth of nursing piglets and increase both sow and piglet n-3 fatty acid serum concentration in immune cells. Vitamin D is important for the promotion of calcium homeostasis, bone health and both innate and adaptive immunity, with vitamin D_3, Cholecalciferol, commonly added to pig diets. An inclusion rate of 800IU/kg is recommended for gestating and lactating sows, although levels up to 2000IU/kg are frequently added to sow diets on Irish farms. Indeed increasing maternal supplementation of vitamin D_3 has been shown to increase milk, sow and piglet vitamin D_3 levels (Flohr J.R., *et al*, 2014), reduce stillbirths and prolong immune responses. The aim of this study was to investigate the use of salmon oil as well as the inclusion rate of vitamin D_3 in sow gestation diets as a solution to improve piglet vitality at birth and viability to weaning.

Methods

A total of 120 PIC F1 cross (large white x landrace) multiparous sows were selected for use on the experiment. Sows were blocked and allocated to treatment by parity, body condition score and weight on day 28 of gestation. Sows were offered 2.5kg/day of control diet (diet 1) from service until day 29 of gestation. Experimental diets were fed from day 30 of gestation until farrowing (Table 1). Sows were offered their respective experimental diet at 2.5kg/day until day 79 of gestation and then 3.0kg/day from day 80 until parturition. Diets were cereal soya based (DE 12.9 MJ/kg, CP 14 %, Fibre 7.67% and Lysine 0.7%). All sows were weighed, back-fat scanned, body condition and locomotion scored on day 28, 107 and at weaning. Twenty sows per treatment were selected for blood sampling on day 28, 107 and weaning. Sows were not induced but rather allowed to farrow naturally.

Table 1. Oil and Vitamin D_3 content in sow gestation diets

Diet	n	Oil source	% Oil inclusion	Vitamin D_3 IU/kg
1	30	Soya	2.5	2000
2	30	Soya	2.5	800
3	30	Salmon	2.5	2000
4	30	Salmon	2.5	800

Piglets from attended births underwent detailed vitality scoring (n=80 litters). Information was collected regarding birth order, birth interval and farrowing duration. Immediately after birth the vitality of each piglet was visually scored using a categorical scale (0-3). The piglets were then weighed and an umbilical blood sample was used to determine blood lactate level (The Edge Blood Lactate Monitor, Arctic Medial, U.K). Each piglet was then marked noting their birth order and placed back with the sow. Each litter was observed to note time to first suckle, allowing for an estimation of colostrum intake. At 24 hours old, all vitality scored piglets were weighed and crown to rump and abdominal circumference measured. Rectal temperature was taken and each piglet was given a visual intra-uterine growth retardation (IUGR) score. Sows whose farrowing was unattended (n=40) provided both colostrum and milk samples while the piglets were weighed and one piglet of mean birth weight was euthanized to provide blood, brain, liver and hind leg samples. Total numbers of piglets born, born alive, born dead and mummified were recorded for each sow. Cross fostering took place within treatment groups only when required to standardise litters to 14. Within 24 hours of birth all piglets had their teeth clipped, tails docked, an iron injection and were tagged with an individual ID number. Any piglets that died before weaning were weighed and cause of death noted. All piglets in the experiment were weighed at birth, day 14 and at weaning (approx. 28 days old).

[1.] *Rooke, JA, Sinclair, AG, and Edwards, SA (2001) Feeding tuna oil to the sow at different times during pregnancy had difference effects on piglet long-chain polyunsaturated fatty acid composition at birth and subsequent growth. British Journal of Nutrition. 86: 21-30.*

[2.] *Flohr, JR, Tokach, MD, Dritz, SS, DeRouchey, JM, Goodband, RD, Nelssen, JL, and Bergstrom, JR (2014) An evaluation of the effects of added vitamin D in maternal diets on sow and pig performance. Journal of Animal Science. 92: 594-603.*

8. Effects of dietary seaweed extract supplementation on the reproductive performance of sows and suckling pig performance

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Recent research has demonstrated that maternal dietary supplementation of LactoShield® (seaweed extract) had beneficial effects on the growth performance, immune status and gastrointestinal health of weanling pigs (Leonard *et al.*, 2011). The primary objective of the present study was to evaluate the effect of maternal dietary LactoShield® inclusion on the reproductive performance of sows and the piglet during the suckling period.

Methods

The farm used for this study was a commercial Truly Irish pig farm (Parknageragh Pig Breeders). In all, 138 crossbred pregnant sows (Large White x Landrace genetic lines) were randomly assigned, accounting for parity and anticipated farrowing date, to 1 of 2 dietary treatments (n = 69 sows/treatment); (1) basal lactation diet and (2) basal lactation and LactoShield® from day (d) 109 of gestation until weaning (d 28). Upon farrowing, the numbers of pigs born alive, stillborn and mummified were recorded. All pigs born alive were weighed and individually identified with numbered ear tags. At weaning, each litter was selected and all pigs within each litter were individually weighed. Data were analyzed using the GLM procedure (SAS Inst. Inc., Cary, NC) as a completely randomized design.

Results

The gestation length, number of pigs born alive, stillborn number, mummified number, average birth weight, total litter birth weight at birth, minimum birth weight and maximum birth weight within litters were not influenced by sow dietary treatment ($P>0.05$). However, maternal supplementation of LactoShield® resulted in a significant reduction in the number of stillborn pigs (0.43 vs. 0.68; $P<0.05$). The total litter weight at weaning was 5 % higher (94.55 vs. 90.22 kg) with the pigs from the LactoShield® supplemented sows when compared with the control group; however, this effect was not significant ($P>0.05$). Of particular interest, feeding LactoShield® during late gestation and lactation resulted in a significant reduction in the coefficient of variation (-10 %) of weaning weights within litters ($P<0.05$). Consequently, supplementation of maternal LactoShield® resulted in a significant increase in the minimum weaning weight within litters when compared with the control group (5.86 vs. 5.47 kg; $P<0.05$). In addition, the percentage of pigs weaned per sow on weaning day was significantly higher with LactoShield® supplemented sows when compared with the unsupplemented sows ($P<0.01$). There was a tendency for the LactoShield® sows to have a higher body condition score at weaning when compared with unsupplemented sows ($P=0.065$). As a result, maternal supplementation of LactoShield® reduced sow body condition loss when compared with unsupplemented diets (0.42 vs. 0.56; $P<0.05$). The weaning-to-service interval did not differ between the two experimental groups ($P>0.05$), but a tendency for a higher proportion of sows displaying oestrus by day 7 was observed in the LactoShield® treated sows, compared with those offered unsupplemented diets ($P=0.066$).

Conclusion

The results of the present study show that LactoShield® supplementation reduced the number of stillborn pigs. Maternal dietary treatment decreased within-litter variation in piglet weaning weight and increased the weaning weight of the smaller pig within litters. Furthermore, LactoShield® sows had reduced body condition loss during lactation, which positively influenced subsequent reproduction (more sows in oestrus by day 7 post-weaning).

[1.] Leonard, S.G., Sweeney, T., Bahar, B., Lynch, B.P. & O' Doherty, J. V. 2011. Effects of dietary seaweed extract supplementation in sows and post-weaned pigs on performance, intestinal morphology, intestinal microflora and immune status. British Journal of Nutrition, 106, 688-699.

9. Effect of management strategies for rearing supernumerary piglets on piglet survival and growth rates

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Sows that give birth to more than 14 piglets do not have enough teats to rear them all. Consequently, management strategies facilitating the rearing of supernumerary piglets are needed to optimise weight gain of all piglets born. One strategy involves the use of nurse sows that have their own litter weaned or removed to another sow so they can rear supernumerary piglets from large litters. The nurse sows' own piglets, which are under 21 days-old and therefore too young to wean, are fostered onto another nurse sow whose piglets are weaned. Another strategy involves the use of milk replacer to artificially rear piglets. Such piglets are moved to a specialised enclosure in a separate room where they are fed milk replacer until weaning. In both strategies, it is critical to ensure the growth of fostered piglets. Two experiments were conducted on commercial farms to assess the effects of these two management strategies on piglet growth. Data on weight and average daily gain (ADG) were analysed using GLM, accounting for the random effect of sows and rep and the repeated effect of day, age was used as a covariate for post-weaning data.

Methods and Results (1)

In the first experiment supernumerary piglets were obtained from sows that had large litters (12 piglets were left with her), either all born onto the sow (R, n=9 litters) or with up to 15% of piglets exchanged to homogenize litter weight (i.e. cross-fostering, CF, n=10 litters). At 1 day-old, supernumerary piglets were fostered onto nurse sows that were either 7 (N2-A, n=9 litters) or 21 (N1, n=10 litters) days into lactation. Nurse sows' piglets were either reared by another nurse sow (i.e. 7 day-old piglets, N2-B, n=9 litters) or early weaned (data not collected). Piglets were weighed on the day of fostering (D0), 24 h after (D1), every week (D2, D9, D16, D23) until weaning (W), and then a week after weaning (W7) and at transfer to second stage weaner accommodation (i.e. approx. 8 weeks-old, S2). Pre-weaning mortality of piglets was not affected by treatment (N1: 8.3±2.5%; N2-A: 4.7±2.1%; N2-B: 7.4±2.5%; R: 6.0±2.2%; RC: 13.6±3.2%, P>0.05). Treatment did not affect weaning weight of piglets (R: 9.85±0.46, CF: 10.19±0.45 kg, N1: 10.08±0.45 kg, N2-A: 10.17±0.47 kg, N2-B: 9.43±0.46 kg; P>0.05), or their ADG between fostering and weaning (D0-W; R: 0.221±0.010 kg, CF: 0.234±0.009 kg, N1: 0.214±0.009 kg, N2-A: 0.222±0.010 kg; P>0.05). However, piglets reared by N1 were heavier than siblings reared by their dam at S2 (N1: 13.44±0.48 kg vs. R: 11.11±0.49 kg; P<0.005), probably due to a higher ADG during the week following weaning (W-W7; N1: 0.234±0.032 kg vs. R: 0.124±0.032 kg).

Methods and Results (2)

The second experiment assessed the consequences of artificial rearing (AR, n=10 litters) on piglet growth. AR piglets were transferred to a specialized rearing enclosure at 7 days-old and fed milk replacer until weaning. Sow reared (SR, n=10 litters) piglets remained with their dam until weaning. Piglets were weighed on the day of transfer (D0), 24 h after (D1), every week (D8, D15, D22) until weaning (C = 29.2±0.4 d, RD = 26.3±0.4 d; P<0.01), and at slaughter (C = 164.3±0.8 d, RD = 167.9±0.9 d; P<0.01). Pre-weaning mortality of piglets was not affected by treatment since only 1 piglet died in each treatment. AR and SR piglets had similar weights on D0 (2.77±0.07 kg vs. 2.80±0.07 kg; P>0.05) but by D1 (3.13±0.07 kg vs. 3.32±0.07 kg) and until weaning (6.53±0.07 kg vs. 7.97±0.07 kg) AR piglets were lighter than SR. Overall, the pre-weaning growth rate of AR piglets was lower than SR (0.24±0.005 kg vs. 0.26±0.005 kg, P<0.05). However, SR and AR pigs had a similar ADG post-weaning (0.75±0.025 vs. 0.75±0.024 kg, P>0.05) and from D0 to slaughter (0.68±0.013 kg vs. 0.69±0.015 kg, P>0.05), thus they had similar slaughter weights (109.17±1.13 kg vs. 109.14±1.22 kg, P>0.05).

Conclusion

The transfer of 1 day-old piglets onto a nurse sow did not affect their growth. Piglets reared by a nurse sow in late lactation (N1; 3-7 weeks post-farrowing) coped better with weaning. Fostering piglets at 7 days-old onto a nurse sow did not affected their growth. On the other hand, 7 day-old AR piglets experienced a growth check following their transfer to the enclosure and were unable to catch-up with sow-reared piglets before weaning. However, their growth rates improved during the post-weaning period.

10. Effects of energy supplementation to low-birth weight neonatal piglets on their survival, growth and blood glucose level.

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Birth is a great challenge for piglets and a major cause of mortality in the first days post-partum. At this time, the energy requirements of the newborn piglet is high as they must maintain their body temperature and compete with littermates for teat acquisition, in order to ensure adequate colostrum and milk intake. Low birth-weight piglets (i.e. generally under 1.00 kg) have lower energy reserves and a greater relative surface area at birth than their heavier siblings, and consequently, are more at risk of dying in the first days post-partum. Providing an exogenous source of energy to these low birth-weight piglets may help their survival.

Methods

In the present pilot study, piglets (n=104) born under 1.10 kg were within litter (randomly) given a single 2 ml oral dose of either Energyn® (E, n=34 piglets), coconut oil (C, n=35 piglets) or distilled water (W, n=35 piglets), approximately 3 h post-partum. Supplementation of piglets was by inserting a syringe into their mouth and gently squirting the content, ensuring that all was ingested. To keep the fluidity and temperature of each product constant, the three supplementation products were heated to ~35°C (on a heat pad) for at least one hour before use. In so far as possible, the three treatments were applied to individual piglets within each selected litter (n=27 parity 1 to 3 sows,). Piglets were weighed at day 0 (D0; time of supplementation) and at 24 h (D1), 7 days (D7) and 14 days (D14) after supplementation. Average daily gain (ADG) was calculated for individual pigs at each interval between weighings. A small blood sample was obtained from each piglet by pricking an ear vein at 24 h after supplementation in order to measure piglets' blood glucose concentration using an Accu-chec® glucose monitor.

Analysis of pig weight and growth was performed using General Linear Models (GLM), accounting for the repeated effect of piglet and the random effect of sow. Birth weight was added in the model as a covariate. Blood glucose concentration was also analysed using GLM. The model accounted for the random effect of sow.

Results

There was no treatment difference for survival, piglet weight, growth or blood glucose concentration of piglets (P>0.05). There was also no gender effect on any of the parameters (P>0.05).

Table 1. Mean (±S.E.) piglet weight, average daily gain and blood glucose concentration of piglets that received a single dose (2 ml) of coconut oil, Energyn or water at 3 h post-partum.

	Day	Treatment		
		Coconut	Energyn	Water
Weight (kg)	D0	0.92 (±0.1)	0.86 (±0.1)	0.97 (±0.09)
	D1	0.94 (±0.1)	0.91 (±0.1)	1.00 (±0.1)
	D7	1.92 (±0.1)	1.82 (±0.1)	2.07(±0.09)
	D14	3.42 (±0.1)	3.35 (±0.1)	3.72(±0.09)
Average daily gain (kg)	D0-D7	0.14 (±0.01)	0.13 (±0.01)	0.15 (±0.01)
	D0-D14	0.18 (±0.02)	0.17 (±0.02)	0.19 (±0.02)
	D7-D14	0.21 (±0.02)	0.22 (±0.02)	0.23 (±0.02)
Glucose (mmol/ml)	D1	8.9 (±0.06)	9.1 (±0.06)	8.9 (±0.05)

Conclusion

In this pilot study, energy supplementation of piglets did not influence survival, growth, or blood glucose content. This work has informed further studies which will consider the growth of piglets until weaning, the vitality of piglets at birth and their cognitive abilities after weaning. In addition, to allow more robust conclusions regarding the effect of energy supplementation on blood glucose concentration, baseline glucose data will in future be collected at birth.

11. Voluntary locomotor activity promotes myogenic growth potential in domestic pigs

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Domestic pigs are commonly reared under limited space allowance. However, locomotion is a prerequisite for the development and adequate function of the skeletal muscle. Detailed information about distances covered by pigs under conventional rearing conditions is scarce. The objective of our pilot study was to determine the individual voluntary locomotor activity of domestic pigs kept in standard rearing conditions. Moreover, we investigated if the extent of voluntary locomotor activities affects microstructural and biochemical properties of skeletal muscle and modifies mRNA expression of genes involved in myogenesis and skeletal muscle metabolism.

Methods

In each of two replicates 24 pigs (German Landrace) were used. At the age of 28 days the piglets were weaned and then feed and water were provided ad libitum. The space allowance was adapted to standard requirements defined by the EU. To determine individual locomotion behaviour the focus animals ($n = 6$ per replicate) were observed via video camera at five time points (47/48, 61/62, 82/83, 110/111 and 138/139 days of age). The digitized videos were analyzed for individual distances walked by the observed focus animals ($n = 12$) within 24h using the VideoMotionTracker® according to Brendle and Hoy (2011). Furthermore, the five values per animal were summed up (mean total distance) and assigned to three categories (long distance (LD) > 3000m, medium distance (MD) between 2500 and 3000m and short distance (SD) < 2500m) containing 4 animals each. At 144 days of age all animals were slaughtered after electrostunning and histological/histochemical, biochemical and mRNA analyses in M. semitendinosus (ST) of the focus animals were done as described by Rehfeldt *et al.* (2012).

All data on muscle structure, biochemistry and gene expression were analyzed with distance category as fixed factor and weight at slaughter as co-variable using the mixed procedure of ANOVA including multiple Tukey-Kramer post hoc tests (SAS). Moreover, all traits were correlated with the mean total distance by the Spearman rank correlation.

Results

The daily distance walked was affected by the observed time point ($P < 0.001$) showing a clear reduction of the distances with proceeding age of the pigs. Moreover, the mean distance walked by the pigs within 24 h was affected by the distance category ($P < 0.01$) with differences between SD and LD animals ($P < 0.01$) and between SD and MD animals ($P < 0.05$). The weight of the animals was affected by the observed time point ($P < 0.001$) but not by the distance category ($P = 0.74$).

The ST weight and circumference were not affected by distance category ($P > 0.05$). In addition, basic microstructural (total fibre number, fibre size, fibre type distribution, capillarity, and intramuscular fat deposition) and biochemical (DNA, RNA, protein and specific activities of creatine kinase, lactate dehydrogenase and isocitrate dehydrogenase) properties of ST did not differ among the distance categories ($P > 0.05$). The mRNA expressions of the growth factors MSTN, IGF2 and EGF (P £ 0.03) and the myogenic regulatory factors MRF4 and MYOD (P £ 0.09) were affected by distance category, whereas none of the genes associated with muscle structure or metabolism (e.g., PRKAA2, SORBS1) was affected ($P > 0.05$). With increasing total distance walked EGF (rs = -0.627, $P = 0.03$), MRF4 (rs = -0.553, $P = 0.06$), and MSTN (rs = -0.783, $P = 0.003$) mRNA were decreased, whereas the IGF2 (rs = 0.713, $P = 0.009$) mRNA was increased. In addition, the ratio between IGF2 and MSTN mRNA expression was higher with increasing walking distance (rs = 0.804, $P = 0.002$).

Conclusion

Our data support that pigs reared under limited space allowance due to conventional rearing conditions used the opportunity to move to a different extent as represented by the long, medium and small distance categories. But the effects of walking distance were obviously too small to lead to adaptions in the skeletal muscle phenotype probably because the voluntary locomotor activity in our study can be seen as a limited possibility to move around and not like a physical activity in the true sense of the words. Nevertheless, the impact of different walking distances seems to promote the myogenic growth potential in terms of different mRNA expression of myogenic transcription and growth factors.

[1.] *Brendle, J., S. Hoy. 2011. Investigation of distances covered by fattening pigs measured with VideoMotionTracker®. Appl Anim Behav Sci 132:27-32.*

[2.] *Rehfeldt, C., B. Stabenow, R. Pfuhl, J. Block, G. Nürnberg, W. Otten, C.C. Metges, C. Kalbe. 2012. Effects of limited and excess protein intakes of pregnant gilts on carcass quality and cellular properties of skeletal muscle and subcutaneous adipose tissue in fattening pigs. J Anim Sci 90:184-96.*

12. 1NMR characterization of colostrum in pure breed sows and its influences on piglets' performance

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Colostrum is the first secretion of mammary gland produced in late pregnancy and during the hours immediately preceding and post-partum. This secretion represents an essential vehicle of passive immunity, prebiotic compounds and growth factors involved in intestinal development. Its composition has been investigated mainly in human and cow, but very little is known about pig colostrum metabolome and how it varies between pig breeds and different farrowing parity. Thus, the aim of the present research is to provide new information about pig colostrum composition and the associations between some metabolites, the breed and the survival and growth rates of their litters

Methods

Fifty-eight colostrum samples were collected from 58 different farrowing of pure breed sows: 12 Duroc (D), 15 Landrace (L) and 31 Large White (LW). All sows were raised in the same farm. Farrowing was not induced, and the colostrum sampling was carried out during natural parturition, after the birth of the first piglet and before the parturition of the last, across all teats. All samples were immediately frozen at -20 °C and then stored at -80°C until the samples preparation for NMR analysis. For each sow, parity and data related with the reproductive performances were recorded: the number of piglets alive and the litter body weight (LBW) were recorded at birth and at day 3, cleansed from the weight of the piglets dead. The litter weight gain (BWG) was then calculated for the period from birth to day 3. Furthermore, the number of weaners per litter was recorded as well as the occurrence of diarrhea during suckling (1= presence of diarrhea events from piglets' birth until weaning, 0= absence of diarrhea event). The PCA was conducted on the 201 bins matrix to identify the outlier samples, and test the existence variables contributing to samples clustering. The identified metabolites included in the significant bins emerged from the loadings' plot and additional metabolites relevant for their biological function were selected and grouped in a new dataset named C-dataset. The C-dataset was used to conduct an ANOVA. Then, a stepwise regression analysis was used to select, among the metabolites included in the C-dataset and sows' reproductive performances, the variables that had to be included with the breed, the farrowing season and the parity order in the final GLM model for the identification of the metabolites related to piglets' performances.

Results

The PC1 explained the 81% of the total variance and separated the colostrum spectra of D and LW, while PC2 (10% of the variance) discriminated the L colostrum composition from the ones of LW and D sows. The PC2-PC3 plot highlighted the season effect. The parity, breed and season effects on colostrum composition were then confirmed through the ANOVA analysis on the identified metabolites described in the C-dataset. Parity order showed only trends nearing statistical significance for creatinine ($P = 0.061$) and UDP-glucose ($P = 0.061$). Breed and season resulted to be the major factors affecting the assigned compounds. Indeed, the amounts of dimethylamine ($P = 0.030$; $P = 0.0001$), creatine ($P <0.0005$; $P < 0.0005$), creatine phosphate ($P = 0.003$; $P < 0.0001$), cis-aconitate ($P = 0.030$; $P < 0.0001$), taurine ($P = 0.002$; $P = 0.001$), and UMP ($P = 0.001$; $P = 0.009$) were affected by both breed and the season of the farrowing respectively. Season affects significantly ($P < 0.0001$) the amount of acetate, creatinine and formate, where a higher level was registered for all the metabolites during the cold months. On the other hand, the amounts of o-acetylcholine ($P < 0.0001$), sn-glycerophosphocholine ($P = 0.036$), UDP-n-acetylglucosamine ($P = 0.001$), lactose ($P < 0.0001$), myo-inositol ($P = 0.001$) and UDP-glucose ($P < 0.0001$) were affected only by sow breed. In particular, the colostrum of L samples showed upper signals for UDP-Glucosio, UDP-galactosio and sn-glycerophosphocholine compared to the other two breeds, while LW colostrum was characterized by a major quantity of lactose, taurine, myo-inositol and glycolate. The stepwise regression analysis revealed that, the litter weight at birth and the concentration of acetate significantly entered in the model for BWG ($P < 0.0001$ and $P = 0.003$, respectively); the higher number of alive piglets at birth and the increased concentration of colostrum cis-aconitate resulted to be important variables affecting positively the number of weaned piglets ($P < 0.0001$ and $P = 0.019$, respectively), while dimethylamine ($P = 0.0002$) and taurine ($P = 0.013$) entered as variables in the model for the number of dead piglets per litter at day 3. The outcomes of the stepwise regression analysis were then tested with the GLM. Both the higher average piglets' weight at birth ($P < 0.0001$) and the colostrum acetate concentration ($P = 0.003$) affected positively BWG. The number of dead piglets at day 3 was mainly influenced by the concentration of dimethylamine ($P = 0.001$) and taurine ($P = 0.027$) in colostrum and partially by the litter size at birth ($P < 0.1$).

Conclusion

This research represents a preliminary step towards the knowledge of pig colostrum composition and it is one of the first studies focusing on the associations between different swine colostrum compositions and litter performances using the 1H-NMR technique. Further investigations are needed to extend the identification of the different compounds in swine colostrum and to elucidate their effects on newborns. Furthermore, the possible interaction between sows' feeding and microbiota in the modulation of colostrum metabolome deserves further investigations.

13. The effect of different dietary bee pollen consumption on growth intensity and blood parameters of rats: A laboratory study

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Phytoadditives have a potential in animal nutrition and production (Karaskova *et al.*, 2015). Bee pollen is rich in different nutrients like crude proteins, crude fat and sugars (Galik, 2012), but also in minerals and enzymes (Zuo and Xu, 2003).

Methods

In the experiment, 40 Wistar rats (45 days old) were used in 4 groups (5 male and 5 male per each). Animals were housed individually in plastic cages (Tecniplast, Varese, Italy). In control group, granular complete feed mixture was fed. In experimental groups, feed mixtures were supplemented with different rape seed bee pollen dosage, 3 % (group T1), 5 % (group T2), and 7.5 % (group T3). Bee pollen was obtained from local bee pollen keepers. The experiment lasted 90 days with individual ad libitum feeding and drinking water supplied. Feed mixtures were analyzed for nutrient contents with standard laboratory principles and procedures in Laboratory of Quality and Nutritive Value of Feeds (Department of Animal Nutrition, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Slovak Republic). During the trial, animals were individually observed for body weight and feed consumption. At the end of experiment, animal were killed by decapitation following anaesthesia with ether. Collected blood samples were centrifuged and blood serum was analyzed for biochemical indicators such as triglycerides, cholesterol, glucose, total protein, urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP).

Results

At the beginning of trial, the mean of body weight of male rats were 168.0±10.5 g, and female 129.0±11.0 g. Insignificant differences ($P>0.05$) were detected in average body weight of rats at the end of experiment. A tendency ($P>0.05$) of the highest male body weight at the end of trial was found in T3 group (the highest bee pollen concentration in feed mixture) 388±52.52 g; in comparison to control male group (371±11.40 g). The same effect was typical for female rats; in control group the average body weight at the end of trial was 247±15.65 g, in T3 group 388±52.52 g. In average, a tendency ($P>0.05$) of the highest body weight gain was found in T3 group male rats, however significantly the highest ($P<0.05$) in T3 group for females. Feed conversion ratio was insignificantly ($P>0.05$) different in males (control: 6.11±0.31 kg, T1: 6.14±0.52 g, T2: 6.40±0.04 g, T3: 5.54±0.62 kg). In female rats a tendency ($P>0.05$) of higher feed conversion ratio as an effect of higher bee pollen intake was analyzed. In T3 females group significantly higher ($P<0.05$) feed conversion ratio was found in comparison to control females group (7.77±0.93 kg vs. 6.82±0.59 kg). In male biochemical indicators significant ($P<0.05$) differences were detected in cholesterol concentrations (control vs. T2 group), total protein (control vs. T1 group), ALT and ALP activity (control vs. T1 and T3 respectively). In female biochemical indicators at the end of experiment, concentration of triglycerides (control vs. T1 and T3 groups), cholesterol (control vs. T1 and T3 groups), total protein (control vs. T3 group), urea (control vs. T1 and T3 groups), activity of AST (control vs. T1 group), ALT (control vs. T2 group) and ALP (control vs. T1 and T3 groups) were significantly ($P<0.05$) different.

Conclusion

Bee pollen as a feed additive has a potential in animal nutrition. On the base of trial results, rape seed bee pollen had positive effect on the final body weight (rat males and females) and feed conversion ratio (males). In males bee pollen increased serum cholesterol concentration, in females reduced serum triglycerides and increased cholesterol, as well as total proteins.

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[2.] *Karaskova, K., Suchy, P. and Strakova, E. 2015. Current use of phytogenic feed additives in animal nutrition: a review. Czech J Anim. Sci. 60:521-530.*

[3.] *Zuo, J. and Xu, S. 2003. Study of application of bee pollen as feed additive. Feed Rev. 11:33-35.*

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14. Porcine feed efficiency (FE)-associated intestinal microbiota and physiological traits: finding consistent cross-locational biomarkers for residual feed intake (RFI)

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Feed efficiency is critical in the pig industry, as feed accounts for ~70% of production costs (1). The gut microbiota may influence FE in pigs, considering its role in host metabolism and immunity (2, 3). The aim here was to investigate the intestinal microbiota composition of pigs ranked on residual feed intake (RFI; as a metric for FE) reared at three geographical locations [Republic of Ireland (ROI), Northern Ireland (NI), Austria (AT)], using common genetics and management protocols.

Methods

Four trials, comprising 369 pigs were conducted across three geographic locations; ROI [(two batches; one medium herd health [ROI1; n=80] and one high herd health [ROI2; n=138]); NI (one batch; n=87) and AT (one batch; n=64)]. Pigs were ranked as either high or low RFI between day 42 and 91 post weaning (pw), and a total of 100 pigs (50 high RFI and 50 low RFI) were selected for sampling (ROI1; 20, ROI2; 40, AT; 16, NI; 24). Microbiota analysis using high-throughput 16S rRNA gene sequencing was performed on faeces, collected at day 42 and 105 pw, and in ileal and caecal digesta collected at slaughter, and functionality predicted using PICRUSt software. Other potentially FE-associated physiological parameters (salivary cortisol, haematology, serum biochemistry and intestinal immunology) were measured. Growth performance and physiological parameter data were analysed using a mixed linear model in SAS 9.3 and microbiota analysis was performed in R.

Results

Intestinal microbial diversity varied with geographic location, health status and intestinal site, but not by RFI. For the high health batch of pigs (ROI2), low RFI (more feed efficient) animals had higher species diversity in the ileal and caecal digesta, as measured by the Shannon and Simpson indices (high RFI=0.90, low RFI=0.95 and high RFI=5.38, low RFI=6.78, respectively; $P<0.05$). In addition, some bacterial taxa varied consistently in relative abundance between high and low RFI pigs across two geographical locations and/or across the two ROI batches. *Lentisphaerae* (high RFI: ROI1=0.59%; ROI2=0.007%; low RFI: ROI1=1.44%; ROI2=0.013%), *Mucispirillum* (high RFI: NI=0.004%; AT=0.002%; low RFI: NI=0.008%; AT=0.030%), *Methanobrevibacter* (high RFI: ROI1=0.006%; ROI2=0.003%; low RFI: ROI1=0.014%; ROI2=0.010%), *Ruminoccocaceae* (high RFI: ROI2=9.5%; AT=6.0%; low RFI: ROI2=11.1%; AT=6.65%), RF16 (high RFI: ROI1=0.05%; ROI2=0.12%; low RFI: ROI1=1.13%; ROI2=0.56%), and two unknown bacterial species were more abundant within the faecal/caecal microbiota of low RFI pigs ($P<0.05$). Of the other FE-associated physiological parameters measured, only salivary cortisol differed, tending to be lower in low versus high RFI pigs (1.34 versus 1.76 ng/mL, respectively; $P=0.06$).

Conclusion

The rearing environment and intestinal site greatly impacted the pig gut microbiome, which in turn presents challenges when identifying consistent reliable microbial biomarkers for FE in pigs. However, some FE-associated enterotypes (*Lentisphaerae, Mucispirillum, Methanobrevibacter, Ruminococcaceae* and RF16) were common across trials and related to a potentially 'healthier' and metabolically more capable microbiota. These taxa could therefore potentially be used as probiotics or targeted by dietary means as a strategy for improving FE in pigs in the future, but on a site-specific basis.

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[2.] *Ramayo-Caldas Y, Mach N, Lepage P, Levenez F, Denis C, Lemonnier G, Leplat JJ, Billon Y, Berri M, Dore J, Rogel-Gaillard C, Estelle J. 2016. ISME J doi: 2973-2977.*

[3.] *McCormack UM, Curiao T, Buzoianu SG, Prieto ML, Ryan T, Varley P, Crispie F, Magowan E, Metzler-Zebeli BU, Berry DP, O'Sullivan O, Cotter PD, Gardiner GE, Lawlor PG. 2016. Proceedings of the ECO FCE one day symposium at EAAP 2016:50-53.*

15. Faecal microbiota transplantation in pregnant sows alters the growth and intestinal bacterial profile of offspring

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The intestinal microbiome is considered an important 'organ' for the host, as it plays an important role in nutrient digestion, metabolism and immune development. Its composition has been associated with growth performance traits in pigs (1). Moreover, recent work from our group has established a possible link between the intestinal microbiota and feed efficiency (FE) in pigs (2). Therefore, the aim of the present study was to determine if faecal microbiota transplantation (FMT), from highly feed efficient donor pigs to pregnant sows, could influence FE in offspring.

Methods

Faeces from four donor pigs [with the lowest residual feed intake (RFI) of 409 pigs from a previous study] was processed anaerobically and stored at -80 °C until use as an inoculum for FMT. Pregnant sows (n=22) were blocked by boar used for insemination and body weight, and assigned to two treatments 1) Control (CON) and 2) FMT at day 70 and 100 of gestation (FMT). At farrowing, offspring were assigned to one of three treatments 1) Control, 2) FMT at birth and 3) FMT at birth and days 3, 7, and 28 of life; however, only data relating to the effect of sow treatment are presented here. At weaning, 84 piglets (n=42 per sow treatment) were selected and individually housed up to slaughter at ~155 days of age. Microbiota analysis (Illumina MiSeq 16S rRNA gene sequencing) was performed on faeces collected throughout the lifetime and on intestinal digesta collected at slaughter from 18 pigs/sow treatment. Body weight and feed intake were recorded weekly. Growth performance parameters were analysed using a repeated measures MIXED model and microbial data were analysed using a generalised linear mixed GLIMMIX model in SAS 9.3.

Results

At slaughter, offspring from CON sows were ~8 Kg heavier (121.6 ± 1.09 vs. 113.5 ± 1.16 Kg; $P<0.05$) and their carcass weight tended to be ~4 Kg heavier (92.9 ± 1.55 vs. 88.7 ± 1.72 Kg; $P=0.07$) compared to offspring from FMT sows, but sow treatment had no influence on RFI ($P>0.05$). As a result of FMT in sows, offspring microbial diversity, as measured by the Shannon index, was higher in the faeces at 50 days of age, but lower in the ileum at ~ 155 days of age ($P<0.05$). Early in life, *Sphaerochaeta* (from *Spirochaetes*) were lower in abundance in the faeces of offspring from FMT than CON sows (3.3 vs. 6.4% relative abundance at weaning; 13.8 vs. 22.8 % at 50 days of age). The same was true of *Oribacterium* (from *Firmicutes*) at weaning (0.37 vs. 1.10 % abundance). Conversely, *Asteroleplasma* (from *Tenericutes*) and *Campylobacter* (from *Proteobacteria*) were lower in abundance in offspring from CON sows at weaning and 50 days of age, respectively (0.4 vs. 1.9% and 2.5 vs. 4.2% relative abundance; $P<0.05$). Due to FMT in sows, offspring had a lower abundance of *Actinobacteria* (0.09 vs. 0.33% relative abundance), *Chlamydiae* (0.15 vs. 0.48%), *Butyricimonas* (from *Bacteroidetes*; 0.05 vs. 0.11%) and *Asteroleplasma* (from *Tenericutes*; 0.29 vs. 0.71%) in the ileum, but a higher abundance of *Verrucomicrobia* in the caecum (0.74 vs. 0.34%), and *Fusobacteria* in the colon (0.13 vs. 0.06%), compared to offspring from CON sows ($P<0.05$).

Conclusion

This study investigated the use of FMT as a novel strategy to beneficially influence FE in pigs. Offspring intestinal microbial diversity and composition were impacted by FMT in sows. The latter was evidenced by the fact that offspring from FMT sows had a lower abundance of bacterial members known to play a role in metabolism and health (*Oribacterium*, *Butyricimonas*, *Sphaerochaeta*), and an increased abundance of potentially pathogenic members (*Chlamydia*, *Campylobacter*). These findings may help to explain the depression in offspring body weight observed at slaughter, due to FMT in sows. Overall, although altering intestinal microbial composition through the use of maternal FMT proved unsuccessful in terms of improving FE, this study highlights the influence of the sow microbiota on offspring lifetime growth performance.

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16. Altering the chicken microbiome in ovo - the concept and future perspective.

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The peri-hatch period is critical for a chicken in terms of immune challenges from external environment and energy consumption for growth of the developing organism. Reinforcing the animal's robustness as early as possible has a crucial impact on its lifelong performance, health, and quality of the final product, including health-promoting properties of the meat. Proposed by our group solution to these challenges is in ovo technology.

Proof of the in ovo concept must be considered from 2 perspectives: formulation of a bioactive compound and the timing of its delivery. In ovo technology allows for early and precise delivery of pre/pro/synbiotics and modulation of microbiota of the gastrointestinal tract (GIT), which takes part in regulating immunity and metabolism of the nutrients. Synbiotics, which are synergistic combinations of prebiotics and probiotics, may exert 2 types of effects: 1/ synergistic effect towards the prebiotic - where the probiotic compound efficiently uses the prebiotic substrate, and 2/ synergistic effect towards the host - where the prebiotic and probiotic compounds mutually improve the microbial balance of GIT. Careful pre-selection of the synbiotic and its further in vitro validation, prior to in vivo, trial is crucial to obtain desired effect on the animal.

Why 12. day of egg incubation? Considering the time point of egg incubation and embryo location, we have evidenced [1,2] that a single in ovo injection of a bioactive solution (0.2 mL) into an air chamber must be performed exactly on the day 12 of egg incubation. Upon the injection, a bioactive solution penetrates through the fully vascularized chorioallantoic membrane into the circulatory system of a chicken embryo and reaches the developing GIT. on the day 12, T and B lymphocytes start to proliferate and differentiate and we hypotetise that this is a proper moment to effectively target the immune system.

A single, multi effective treatment in ovo. In general, synbiotics delivered in ovo improved survivability of the hatchlings, increased surface of intestinal tissue and influenced hormonal regulation in GIT, without deterioration in FCE. The specific effects of a prebiotic or a synbiotic depended on the bioactive compound which was delivered in ovo. For example, synbiotic formulation based on raffinose familly oligosaccharides improved some health-promoting meat traits (tromboigenic and artherogenic indices, ratio of polysaturated to saturated fatty acids). It also increased functionality of pancreas and the liver, which was mirrored by activation of metabolic genes in the liver. On the other hand, the formulation based on galactooligosaccharides modulated development of the central and peripheral lymphatic organs and triggered a down-regulatory pattern in the immune-related gene expression in gut associated lymphoid tissue (GALT). It also provided beneficial shift in microbiota composition in GIT.

Prospective investigations. It is hard to point out a single "golden standard" of a synbiotic. To meet expectations of the stakeholders, we recommend to carefully select and validate novel formulations of bioactives. Such selection should depend on the physiological status of the flock and foreseen environmental challenges. To design novel synbiotics, an unlimited number of pre/probiotic combinations may be screened in vitro using relatively cheap bench assays. One of the drivers of further research is to prove the anti-pathogenic properties of a bioactive substance, which can be used as an alternative to veterinary drugs. A probiotic should be able to colonize developing GIT, but also to compete with pathogenic bacteria, or even show "antibiotic-like" features against pathogens and eliminate them from intestine. Even though in ovo technology for delivering prebiotics and synbiotics has been established, there are research questions that require further investigations. For example, one of the questions refers to epigenetics and understanding whether the effect of in ovo treatment with bioactives might have transgenerational effects.. Also, challenge trials are required to validate effectiveness of in ovo technology in different environments. Currently, we are running two ongoing trials in which we analyze impact of prebiotics, probiotics and synbiotics delivered in ovo on chicken responses to antigenic (LPS) and environmental (heat stress) challenge. Our ultimate goal is to put a concept of designer synbiotics into practice and match their composition with the particular needs of the poultry industry,

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17. Acute and delayed transcriptional responses following livestock-environment interactions

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A variety of environmental and maternal factors that act on individuals at early, even prenatal stages have long term consequences on later life. Abiotic factors like housing conditions or nutritional status provoke adaptive responses and a molecular memory termed 'Metabolic Programming'. The mechanism by which environmental and maternal cues are programmed and transmitted involves the epigenetic regulation of specific genes. Knowledge of the molecular mechanisms underlying metabolic programming may offer the possibility to induce different and stable phenotypes. Using pig and chicken models, strategies are developed to better characterise phenotypic and molecular variations due to dietary and environmental challenges.

Methods

We evaluate environmental and dietary impacts on avian embryos and porcine foetuses and adult offspring via transcriptome profiling (RNAseq, microarrays) and bisulfite sequencing (Reduced Representation Bisulfite Sequencing, RRBS) in various tissues. Furthermore, intrinsic responses were deduced via phenotypic records.

Results

In a pig model, nulliparous German landrace sows were fed isoenergetic diets varying in their protein:carbohydrate ratio. Longitudinal expression changes were monitored in offspring in liver and muscle tissue at prenatal and postnatal stages. The in utero exposure to adverse maternal dietary regimen revealed transcriptional alterations and comprised genes related to energy metabolism, cell cycle regulation, organismal and cellular growth, and glucocorticoid receptor signaling. The longitudinal expression changes suggest adaptation processes towards compensatory growth, possibly at the expense of a predisposition for metabolic disturbances.

Another pig model was used to assess effects of maternal supplementation with methylation-related micronutrients during prenatal and postnatal stages on offspring of two breeds (DL, Pi). Male offspring showed increased foetal weight during late pregnancy but decreased live weight postnatally. Phenotype variation was reflected by compensatory transcriptional variation, since males showed increased mRNA abundances of myogenic key drivers (MyoG, MyoD1, Pax7). In contrast, female weights were unaffected by diet, and mRNA abundances corresponded to a phenotype of cellular reorganization via FABP3, FABP4, SPP1 and Insulin-like Growth Factor-signaling. In liver, RNA-Seq revealed that nucleic acid metabolism pathways were influenced by the methyl-donor-rich maternal diet at the foetal stage, while lipid metabolism pathways were affected at the postnatal stage. RRBS revealed differentially methylated CpGs (DMCs) between maternal dietary treatments, developmental stages, and breed suggesting dynamic patterns of DNA methylation. Several DMCs were mapped to the 5´-regulatory regions. Significant inverse correlations between gene expression and DNA methylation level of the upstream CpG cluster (<100 kb) was observed for CBFA2T3 (SSC6), CPEB4 (SSC16) and TUBB4B (SSC1).

The in-ovo development of the poultry is an ideal model to study the impact of exogenous (physical) effects and to analyse mechanisms of gene-environment interactions. Modification of egg incubation temperature has been evidenced in several poultry species to have a wide-range impact on post-hatch development. In order to reveal molecular routes responsive to variable incubation temperature, broiler eggs were incubated at higher and lower temperature (36.8°C, 38.8°C) compared to controls (37.8°C) at embryonic days (ED) 7-10 and 10-13. Higher incubation temperature led to slight but significant differences in body weight, meat quality traits and mitochondrial respiratory capacity, whereas lower incubation temperature only had subtle effects on a few parameters. However, in muscle tissue lower temperature shifted the expression of more genes than higher temperature. Pathways involved were energy and lipid metabolism, cell signaling, muscle development at higher and cellular function and growth, and development of organs and tissues at lower temperature. Interestingly, the transcriptional response to transiently lower incubation temperature, which did not affect the phenotypes, prompted compensatory effects reflecting resilience. In contrast, higher incubation temperature triggers gene expression and has long-term effects on the phenotype. Such mechanisms of phenotypic plasticity contribute to the biodiversity and broaden the basis for managing poultry populations.

Conclusion

Our studies show that gestational nutrition with varying protein or methionine content shift gene activity of various metabolic pathways and involved changes of DNA methylation pattern. Considering various diets or conditions, tissues, stages and breeds reveal that there is no common molecular path or gatekeeper mediating transcriptional responses and epigenetic processes. Extensive knowledge of the epigenetic mechanisms and gene regulation offers a new opportunity in animal breeding based on the environment-genome-epigenome interactions.

18. The influence of maternal backfat and gestation feed level on offspring adipose and muscle biomarkers

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Maternal obesity during pregnancy is not uncommon and has far- reaching implications for the mother and the developing offspring. Maternal obesity and level of food intake during pregnancy influence birth weight, growth trajectory and adipose and muscle metabolism. It is now accepted that maternal obesity, in particular, predisposes the offspring to obesity, hypertension and altered glucose regulation resulting in type 2 diabetes. Using a porcine model, the objective of this study was to investigate the influence and interaction of maternal body condition (Thin and Fat) and gestational feed level (Restricted, Control and High feed level) on whole body fat percentage, health biomarkers in blood, adiposity and muscle metabolism in young offspring.

Methods

Seventy eight gilts (Large White x Landrace) were selected (P2 backfat 7-9 mm) at ~154 days of age and restrictively fed (1.8 kg/d) a dry sow diet (6.19 g/kg lysine, 13.0 MJ DE /kg) to reach a backfat depth of 12 mm at 32 weeks of age (Thin gilts). Eighty gilts were also selected (P2 backfat ~ 10-14 mm) at ~154 days of age and provided with ad libitum access to a gilt developer diet (5.85 g/kg lysine, 14.3 MJ DE /kg) to reach a backfat depth of 19 mm at 32 weeks of age (Fat gilts). On day 25 of gestation, gilts from within each body condition group (Fat or Thin) were allocated at random to one of three feeding levels: (a) Restricted (1.8 kg/d), (b) Control (2.5 kg/d) or (c) High feed level (3.5 kg/d), until d 90 of gestation. In total there were six treatment groups; Thin Restricted, Thin Control, Thin High, Fat Restricted, Fat Control and Fat High feed level. Piglets were weighed and tagged at birth. Litter size was standardized at farrowing to ~12 pigs per litter by cross fostering within treatment groups. Within litters, pigs were selected based on their birth weights; low birth weight (LBW; < 1.2 kg), medium birth weight (MBW; ~1.5 kg) and heavy birth weight (HBW; > 1.7 kg). At weaning, all pigs were weighed. One hundred and fifty–five pigs were then sacrificed, blood was collected and body fat percentage measured by a Dual energy x-ray absorptiometry (DXA). Retroperitoneal adipose tissue was harvested from the kidneys The right m.semitendinosus was sectioned and mounted on cork board and snap-frozen for histological examination. The left m.semintendinosus was snap frozen in liquid nitrogen for subsequent mRNA analysis.

Results

At weaning, LBW piglets had greater body fat percentage ($P<0.01$) and tended to have higher serum triglyceride levels ($P=0.07$) that MBW and HBW piglets. LBW piglets had lower mRNA transcript levels of adiponectin in their adipose tissue, than HBW piglets ($P<0.05$). Piglets born to Fat gilts had more mitochondria in muscle, suggesting higher oxidative capacity, than piglets born to Thin gilts ($P<0.05$). Piglets born to Fat gilts tended ($P=0.07$) to have lower mRNA transcript levels of calcineurin in muscle than piglets born to Thin gilts. Birth weight influenced the mRNA transcript levels of a key adipocyte transcriptional regulator, PPARG, with MBW piglets having higher levels expressed in muscle than both LBW and HBW piglets ($P<0.05$).

Conclusion

Piglets with low birth weights had the highest body fat % by weaning. The effect of offspring birth weight overrode the effect of gestational body condition or feed level on markers of adiposity in the offspring. Piglets born to Fat gilts had muscle with a higher oxidative capacity than piglets born to Thin gilts.

19. Changes in metabolic gene expression during C2C12 myogenic differentiation

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Better understanding of molecular mechanisms involved in skeletal muscle growth and metabolism is important to understand how nutrition and other environmental factors might impact upon myogenic development. Through improving our knowledge of early myogenic differentiation in vitro, this might then be translated into growth of neonates and myogenesis in vivo.

The mouse myoblast cell line (C2C12) is often used to model skeletal muscle development. Although the expression of Myogenic Regulatory Factors (MRFs) has been well characterised, less is known about other signalling pathways and metabolic enzymes associated with myogenesis. Here we performed detailed time course experiments to determine changes in mRNA gene expression for various metabolic genes during early differentiation of C2C12 cells. We recently demonstrated (Brown *et al*, 2016) that the expression of many of these genes in skeletal muscle was affected by growth hormone or beta-agonist treatment in growing pigs, but we wanted to investigate their endogenous expression and potential role in myogenic differentiation.

Methods

C2C12 cells were grown in growth media (DMEM+10%FBS) on 6 well plates until 85-90% confluent, when they were switched to differentiation media (DMEM+2%HS; termed 'day 0' of differentiation). Samples (n=4 per timepoint) were taken every 24h from day -2 to 8, then the RNA extracted and used for cDNA synthesis and qPCR analysis. Relative mRNA expression was normalised to total cDNA using Oligreen. Data was analysed by one-way ANOVA using SPSS statistical software, followed by Bonferroni post-hoc tests when appropriate (ANOVA $p<0.05$).

Results

C2C12 cells showed clear morphological changes during differentiation and the expected changes in mRNA expression of the MRFs (Myf5, MyoD and Myogenin). The transition from proliferative myoblasts to differentiated myotubes was associated with a significant decrease in myf5 mRNA ($p<0.001$), a rapid induction and peak in myogenin mRNA at day 2 ($p<0.001$), when myoblasts started to fuse. After this peak in myogenin mRNA, the myosin heavy chain isoforms showed similar patterns of expression to our previous work (Brown, Parr & Brameld, 2012). In addition, there was a significant ($p<0.001$) increase in mRNA expression of both Enolase-3 and Pyruvate carboxylase with differentiation, whereas Isocitrate dehydrogenase-2 mRNA was significantly reduced ($p<0.001$).

The mRNA expression of a number of metabolic enzymes peaked at day 2 of differentiation, which coincided with the peak in myogenin expression. A peak at day 2 was observed for mitochondrial Phosphoenolpyruvate carboxykinase (PCK2; $p<0.001$) and all 3 of the serine synthesis pathway enzymes (PHGDH, PSAT-1 and PSPH; all $p<0.001$), with all 4 genes showing an overall decrease in expression with differentiation.

Conclusion

The peak at day 2 for Pck2 and serine pathway genes appears to indicate a critical point in myogenesis and suggests the importance of energy and amino acid metabolism in myogenic differentiation. Future work will investigate the manipulation of these genes in C2C12 cells and in vivo, and may indicate novel mechanisms for environmental impacts upon muscle development.

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20. Phenotypic and genetic relationships between litter birthweight characteristics, indicators of intrauterine growth restriction and piglet survival

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Selection for hyperprolific sows has resulted in larger litter sizes and a greater variation in birth weight of piglets. This has increased the number of small and/or undersized piglets, which have been exposed to differing degrees of intrauterine growth restriction (IUGR). Selection on individual piglet birthweight is possible, however, gains will not be made if selection for higher birth weight results in greater weight variation and increased levels of IUGR. Therefore, the aim of this study was to look at the phenotypic and genetic relationships between piglet weight, IUGR status assessed from head morphology and survival to processing as a sow trait.

Methods

Piglet births (21,159) were recorded for 52 weeks (between 2015/2016). There were 1,575 individual farrowings from 862 Landrace sows. Sow data included parity, litter size, and week of farrowing. Piglet data gathered at processing (18-24 hours after birth) were: weight (as a proxy for birth weight), sex, head shape, and date and reason for any death. All piglets remained in their birth litters until processing. The degree of intrauterine growth restriction (IUGR) was assessed visually from head morphology and piglets were classified as normal (1), slight IUGR (2) or IUGR (3; steep, dolphin-like forehead and wrinkles perpendicular to the mouth). For each litter, the proportion of IUGR piglets in a litter (IUGRPROP), the within-litter standard deviation in birth weight (sdBWT), and the proportion of piglets surviving to processing (SURVPROP) were calculated.

Results

The fixed effects for the trivariate ASReml model were sow parity and farrowing week. The random factors were the direct additive effect of the sow (ANIMAL) and the permanent environmental effects of the sow (ideANIMAL). The heritabilities for SURVPROP, IUGRPROP, and sdBWT were 0.05±0.035, 0.23±0.06 and 0.13±0.049, respectively. The repeatabilities for each trait were: 0.14±0.038, 0.30±0.036 and 0.18±0.036, respectively. The genetic (g) and phenotypic (p) correlations were: SURVPROP-sdBWT, g= 0.51±0.372, p= -0.11±0.026; IUGRPROP-sdBWT, g= -0.17±0.237, p= 0.27±0.026; SURVPROP-IUGRPROP, g= -0.88±0.273, p= -0.20±0.026.

Conclusion

The positive genetic correlation between the proportion of piglets surviving to processing and the within-litter standard deviation in birth weight is unexpected as it suggests that the proportion of piglets surviving increases as the within-litter variability in birth weight increases. However, it should be noted that the standard errors for the genetic correlations are high and the negative phenotypic correlation suggests that as birth weight variability increases, the proportion surviving decreases. The genetic correlation between proportion of IUGR and birth weight variability also suggests that as birth weight variability increases, the proportion of IUGR decreases, although the phenotypic correlation suggests the opposite, where birth weight variability increases, the proportion of IUGR increases. The heritability for the proportion of piglets surviving to processing is low, however, the genetic and phenotypic correlations with the proportion of IUGR in a litter are negative and moderate-high (although the standard errors for the genetic correlations are high), suggesting that selection for reduced proportion of IUGR will see a corresponding increase in the proportion of piglets surviving to processing. The heritability and repeatability for the proportion of IUGR piglets in a litter is moderate, indicating this may be a good trait to include in breeding programmes.

21. Prenatal factors influencing muscle growth and development

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A review talk, introducing myogenesis and the various factors that have been shown to influence muscle fibre development in cattle, pigs and sheep, with particular emphasis on the numbers of muscle fibres. The factors to be covered include genetics (double muscling), administration of Growth Hormone to pregnant sows and maternal nutrition, with the long term effects of maternal nutrition on muscle and adiposity in sheep being highlighted. Lastly the role of the Insulin-like Growth Factors as a potential mechanism for how many of these prenatal factors might influence prenatal muscle development will be discussed.

22. A gnotobiotic piglet model of preterm infants

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The pig is due to its anatomical and physiological similarities to the human a frequent model in biomedical research. Additionally the epitheliochorial type of its placentation that prevents prenatal transfer of immunoglobulins predetermines the pig as a model in immunology. Surgically-derived colostrum-deprived gnotobiotic piglets with their simple and defined microbiota are suitable models of immunocompromised individuals (preterm infants, elders etc.) to study host × microbiota interactions. At the beginning of the second half of the last century the pioneer attempts to obtain and rear germ-free piglets were done in several laboratories in the USA and later also in a laboratory of gnotobiology in the former Czechoslovakia. Methods of deriving of germ-free piglets were developed and improved from semi-sterile approaches to aseptic surgery techniques. Since the beginning of this century a renaissance of interest in gnotobiotic animal models has been dated. It is mainly due to new methods of study of microbiota – e.g. PCR, high-throughput RNA analysis and next generation sequencing that again attracted attention to study of microbiota composition and its importance for the host.

A lipopolysaccharide (LPS) is a virulence factor of Gram-negative bacteria. Shortening of its chain modifies type of LPS and decreases bacterial virulence. A probiotic *Eschericha coli* Nissle 1917 (EcN) that possesses semirough type of LPS protects children and piglets against diarrhea. *Salmonella* Typhimurium (ST) is enteric pathogen that causes self-limiting gastroenteritis in the human and the pig.

Methods

Vitamins A (300,000 IU/ml), D (100,000 IU/ml) and E (50 mg/ml) (ADE-oleosum) were i.m. applied as 0.2 ml per 10 kg of body weight to the pregnant miniature sow on 80th day of gestation (DG). At 105th DG 50 mg of medroxyprogesterone acetate was i.m. administered. A hysterectomy was performed on 112th DG under isoflurane anesthesia. The piglets were transferred into a rearing isolator and their umbilical cords were ligated. They were i.m. treated by 50 mg dextraferranum and 5 mg of phytomenadionum till four hours after the hysterectomy and they were reared in positive-pressure sterile fiberglass isolators with part of a floor heated to 32-35 °C. The piglets were fed to satiety seven times a day via bottle with nipple by an autoclave-sterilized condensed cow's milk-based formula. One-week-old germ-free piglets (GF) or gnotobiotic piglets associated with EcN were infected with *S. Typhimurium* (ST) or its isogenic rfaG mutant for 24 hours. A colonization and possible translocation were evaluated by CFU counting on MacConkey agar. TLR4, MyD88 and TRIF transcriptions in the ileum were evaluated by RT-qPCR.

Results

EcN, ST and its isogenic rfaG mutant colonized the ileum of the gnotobiotic piglets in a comparable ratio. While TLR4 transcription was increased in ST group, the transcriptions in EcN and rfaG groups were comparable with GF piglets. In the group preliminary associated with EcN and later infected with ST was the transcription decreased in comparison with the piglets infected with ST only. MyD88 was decreased but TRIF increased in the groups with ST and EcN while rfaG group was in both cases comparable with GF group.

Conclusion

One of possible unique probiotic properties of *E. coli* Nissle 1917 consists in its semirough type of LPS. The gnotobiotic piglet is the suitable model to study action of probiotics and their possible candidates in a low birth weight preterm infants to evaluate their opportunistic pathogenicity. The gnotobiotic piglets has been highly required animal model of many international collaborative studies.

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23. Development of an effective Bacillus probiotic for use as an alternative to in-feed antibiotics for pigs

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The emergence of antibiotic-resistant bacteria has led to an EU-wide ban on the routine addition of antibiotic growth promoters to pig feed. The use of pharmacological levels of zinc oxide (ZnO) in feed is also currently being phased out by the EU. Effective alternative feed additives which can achieve at least the same level of benefits as in-feed antibiotics/ZnO are therefore required. Probiotics are one such alternative and the objective of this work was to develop an effective probiotic for use in pigs. The marine environment was chosen as a source, given that it represents an untapped supply of potentially novel microorganisms and that antimicrobial production, an important probiotic trait, is common amongst marine microbiota. The focus was on isolation of *Bacillus*, as, being spore-formers, they are generally capable of withstanding intestinal transit, as well as drying and feed pelleting temperatures.

Methods

From an initial screening, 311 bacterial isolates were obtained from seaweed, sand and seawater (1). Of these, 15 isolates, identified as *Bacillus licheniformis* and *Bacillus pumilus*, were selected for their antimicrobial activity. Six were active against *E. coli* and *Salmonella* and for this reason were further characterised in vitro as animal probiotics (2). Resulting from this work, one *B. pumilus* isolate offered most potential, based on its antimicrobial properties, ability to survive simulated intestinal transit and absence of safety concerns. Spores of this *B. pumilus* isolate were then fed to weaned pigs for 22 days to evaluate safety and efficacy in the live animal (3). Forty eight individual pigs (8.7 ± 0.26 kg) weaned at 28 ± 1 d of age were blocked by sex, weight and litter origin and randomly assigned as individual pigs to one of three dietary treatments (n = 16 pigs/treatment): 1. non-medicated diet; 2. medicated diet + apramycin (200 mg/kg) + pharmacological levels of ZnO (2,500 mg Zn/kg) and 3. *B. pumilus* diet (non-medicated diet + 10^{10} spores/day *B. pumilus* WIT 588).

Results

Final body weight and average daily gain tended to be lower (P = 0.07) and feed conversion ratio was poorer ($P<0.05$) for the medicated treatment compared to the *B. pumilus* treatment. Ileal *E. coli* counts were lower for the *B. pumilus* and medicated treatments compared to the non-medicated treatment ($P<0.05$), perhaps as a result of increased ileal propionic acid concentrations ($P<0.001$). However, the medicated treatment reduced faecal ($P<0.001$) and caecal ($P<0.05$) *Lactobacillus* counts and tended to reduce total caecal short chain fatty acid (SCFA) concentrations (P = 0.10). Liver weights were lighter and concentrations of liver enzymes higher ($P<0.05$) in pigs on the medicated treatment compared to those on the non-medicated or *B. pumilus* treatments. Pigs on the *B. pumilus* treatment had lower overall lymphocyte and higher granulocyte percentages ($P<0.001$) and higher numbers of jejunal goblet cells ($P<0.01$) than pigs on either of the other two treatments or the non-medicated treatment, respectively. However, histopathological examination of the small intestine, kidneys and liver revealed no abnormalities.

Conclusion

The *B. pumilus* treatment decreased ileal *E. coli* counts as effectively as in-feed medication. However, it did so without the adverse effects on growth performance, *Lactobacillus* counts and caecal SCFA concentrations and possible liver toxicity experienced with the medicated treatment. Overall, the data obtained to date provide preliminary evidence of the safety and efficacy of this *B. pumilus* strain and indicate its potential as a substitute for in-feed antibiotics/ZnO in pig diets. A follow-on project recently funded by Enterprise Ireland will generate additional data on safety and functionality of the probiotic strain by exploring delivery routes, production stages and product manufacturability not yet explored to date. It will involve *E. coli* challenge studies, dose-response studies and studies on timing, duration and mode of administration (e.g. administration to sows as a route of delivery to offspring) as well as feeding trials at different production stages (sows, neonates, weaners, finishers).

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24. COST Action FA1401 “European network on the factors affecting the gastro-intestinal microbial balance and the impact on the health status of pigs” (PiGutNet)

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The “hoped for” reduction in the use of antibiotics in pig by EU producers has not been materialized as they are still being widely used for the control of enteric infectious diseases. This practice can spread antibiotic resistance in the farm environment and poses a threat to consumer health. Whilst it is widely recognized that a diversified gastro-intestinal tract (GIT) microbiota is essential for optimal health and performance, the underlying factors favoring the development and maintenance of a balanced intestinal microbiota are not fully understood. PiGutNet will establish the first European network focused on this topic, joining specialists in all research areas. It will define both environmental and host genetic factors affecting the GIT microbiota and the complex interactions between microbiota and gut maturation, to maintain a healthy gut throughout life. The network will coordinate databases and unravel innovative tools to define the status of intestinal eubiosis in pigs. The most important outcomes will be genome/metabolome-wide association studies and the provision of a road map to increase pig resistance against GIT infections. This will have an important translational potential, being the foundation for European companies to develop strategies in the areas of feed additives and animal husbandry, resulting in improved animal health and welfare, consumer protection and competitive advantage for the European agriculture.

Dimension and objective of the Action

PiGutNet network was established in 2015 and today includes partners with multidisciplinary expertise from 22 EU countries (57 institutions, 10 companies), and the European Federation of Animal Science (EAAP). Moreover, extra-UE colleagues is involved in the Action. The main objective of the Action is to increase the knowledge about the effect/interaction of environmental and genetic factors on the composition of the microbiota in the gastrointestinal tract of pigs and to improve the risk management associated with antibiotic resistance in pig production. In order to disentangle the factors involved in the gut microbial balance four gaps were detected: i. Molecular microbiology; ii. Environment, host genetics and epigenetic approach; iii. Feeding strategies to manage the risk of dysbiosis; iv. Antibiotic resistance. The 4-years PiGutNet program will be carried out within 5 working groups (WGs) identified according to the 4 main research areas above described, while one WG will be dedicated to the dissemination.

Expected impact

We hope to advance the European research strategy on animal health and welfare in domestic animals by the activities, to create a sustainable network of experts in pig nutrition and to foster transdisciplinary discussions, research and future projects in an important domain. PiGutNet will stimulate and support the European Union’s needs and societal demands and by this increase the competitiveness in the agricultural sector.

Contact and update

The PiGutNet network is open to accept new partners that aim to share information to progress in the field of the gut eubiosis of pigs. In order to have more information on the network (partner involved, Management Committee composition, scientific objectives, activities, etc.), please, visit our webpage http://www.cost.eu/COST_Actions/fa/Actions/FA1401 and www.pigutnet.eu. Moreover, you can contact the Chair and the Vice-Chair of the PiGutNet network, Prof. Paolo Trevisi from the University of Bologna and Prof. Jürgen Zentek from the Freie Universität Berlin.

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25. The INRA team « Growth and Flesh Quality » : From study of muscle tissues to flesh quality determinisms in fish

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In the context of world population expansion, the need for animal proteins, particularly fish proteins, is increasing and will continue to increase worldwide in the future. With the decline of wild stocks and commercial fishing, sustainable aquaculture development is one of the main challenges to support fish consumption and food safety. Muscle constitutes the edible part of the fish and it composes up to 60% of the body weight, highlighting the importance of its development for the competitiveness of aquaculture. Fish of agronomic interest have the extraordinary ability to exhibit a continuous muscle growth associated with the persistent formation of new myofibres (hyperplasia) during the post-larval exponential growth phase. Therefore, the intensity and the duration of the post-larval hyperplasic growth is crucial for the muscle growth potential of fish. In addition to the quantitative aspect of muscle production, some aspects of the flesh quality, such as the texture, are directly dependent on the relative part of muscle tissues. The aim of the "Growth and Flesh Quality" team is to understand the mechanisms of development and muscle tissue growth for improving the production and the quality of flesh. Our research are based on cutting edge technologies (transcriptome, trangenesis, gene knockdown, CrispR/Cas9 gene inactivation) as well as applied technologies for flesh quality analyses (texture, filet yield prediction).

Using an Agilent-based microarray, we recently carried out a time-course analysis of transcript expression during muscle regeneration [1]. This analysis shows a coordinated expression of functionally related genes in trout. Among them, a large set of genes was previously reported to be up-regulated in hyperplastic muscle growth areas, indicating that many features of the transcriptional program underlying muscle hyperplasia are reactivated when new myofibres are transiently produced during fish muscle regeneration.

Given the importance of miRNA in myogenesis, we performed a high throughput analysis and produced a repertoire of trout miRNA (Juanchich *et al.*, 2016). To identify new miRNA potentially involved in satellite cell differentiation, we used a methionine depletion/replenishment protocol to synchronize myogenic cell differentiation in vitro following by a microarray analysis [2]. Our results show that miR-133a and miR-210 had strong expression in white muscle. We also showed that miR-210 expression was upregulated during differentiation of satellite cells, suggesting that miR-210 was involved in the differentiation of satellite cells.

Among the flesh quality parameters, we are particular interested in texture. By comparing the transcriptome of trout muscle with firm or soft texture, we identified several markers correlated with flesh texture. We also confirmed that some of these markers are also relevant in several experimentations.

The control of slaughter stress is of importance with regard to both fish welfare and flesh quality. Muscle characteristics and instrumentally measured quality parameters were determined in rainbow trout lines selected for high-responsiveness (HR) or low-responsiveness (LR) of plasma cortisol to an acute confinement stressor [3]. Our results show that genetic selection for low stress responsiveness does not appear to offer benefits to manage slaughter-stress consequences on flesh quality.

Selection to improve processing yields relies on sib selection, in which live candidates are ranked according to their family breeding value. This approach limits genetic progress, as it only exploits genetic variability between families and not within them. Indirect criteria measured on live candidates could overcome this limitation. Thus, we developed in trout morphometric analyses by external measures combined in a linear regression model, that allow the prediction of phenotypic processing yields [4].

Conclusion

The "Growth and Flesh Quality" team gathers expertises and knowledges in key scientific areas for integrating the impact of nutrition and environment on muscle growth and flesh quality in fish.

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