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# Specific Carbohydrate Formulation in Late Lactation Diets for Primiparous Sows to Enhance Early Antral Follicle Development, Oocyte Quality and Post-Weaning Fertility

## Final Report
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**Executive Summary**

Suboptimal reproductive performance of primiparous sows is a long-time recognised problem which is linked to the metabolic status of these sows during lactation. The compromised reproductive performance of first litter sows is often referred to as "second litter syndrome", with affected herds (or sows) showing second litter sizes that are hardly greater, or even smaller (in severe cases), than first litters. The syndrome is also manifested in other ways such as a longer weaning-to-oestrus intervals and lower pregnancy rates after the first litter, but litter size is the most prominent, the most financially impacting, and in literature the most common parameter that is referred to.

Effects of feeding level on metabolic changes during lactation have been investigated in previous studies. Energy intake during lactation, in particular, has been shown to affect post-weaning reproductive performance, with a number of studies showing the impact of feed restriction during lactation on either ovulation rate, embryo survival, or both (Kirkwood et al., 1990; Zak et al., 1998; van den Brand et al., 2000c; De Rensis et al., 2005; Vinsky et al., 2006). However, feed intake in primiparous lactating sows is generally insufficient to meet nutrient requirements for milk production, with excessive mobilisation of body reserves as a consequence (Dourmad et al., 1994; van den Brand et al., 2000a).

Since feed intake capacity limits energy intake in lactating primiparous sows, the dietary energy source may provide a means to manipulate post-weaning reproductive performance. Increasing energy density of diets by varying the fat content, however, did not result in any improvement of sow body condition during lactation and post-weaning reproductive performance in studies by Heo et al (2008) and Quiniou et al (2008), and actually aggravated the catabolic condition because of increased milk fat output (van den Brand et al., 2000b). In contrast, increasing the carbohydrate content of the diet throughout lactation (van den Brand et al., 2000a,c), improved the energy balance and increased gonadotrophin secretion, and insulin and glucose profiles. Insulin has been proposed as a hormone that acts as an intermediary between nutrition and reproduction (Lucy, 2008), both through effects on gonadotrophins (FSH and LH) but also through direct effects on the ovary. Endogenous insulin levels can be influenced by feeding level (Koketsu et al., 1996), but also by dietary energy source (Kemp et al., 1995) during lactation.

In a recent trial at SARDI, primiparous sows (n=23) were fed a supplement of specifically formulated carbohydrates during the last week of lactation, aiming to increase glucose and insulin secretion (Chen et al., 2012b). The supplement was rich in sugars (sucrose and dextrose) and readily available starch (extruded wheat starch). The supplement increased insulin and glucose profiles compared to controls, and sows fed the carbohydrate supplement had a shorter weaning to ovulation interval (5.7 d vs. 6.2 d; P=0.09), and a greater second litter size (total born 12.4 ± 0.5 vs. 10.7 ± 0.6; P=0.04).

The objective of the present project was to provide a fully formulated diet with the same properties as the supplements in the previous project, to stimulate insulin and glucose secretion in late lactation, and increase reproductive performance after the first lactation. The proviso made by the review panel for this proposal was that the study was to be done under "controlled commercial conditions".

The main part of this trial was performed at Australian Pork Farm Group's piggery at Shea-Oak Log, South Australia. Nulliparous sows (n=228) entered the study at farrowing. Feed intake was monitored and sows and litters were weighed at the start of lactation and at weaning. At 8 days prior to weaning, litters with 10 or more piglets were allocated to receive either a control lactation diet (CON, n=60) or a sugar rich (CHO, n=59) diet (Table 1) until weaning.

In a smaller experiment performed at SARDI's Roseworthy Research piggery, glucose and insulin profiles for the two diets were tested in cannulated sows (8 per diet).

The CHO diet increased the secretion of glucose and insulin compared to the control diet. Intake of the two diets was 5.5 and 5.9 kg/d for CON and CHO, respectively, which was greater than in the previous trial at Roseworthy (5.0 kg on average). Consequently sows lost on average only 7.7 kg and 5.8 kg body weight during lactation in the two treatments. Obviously these conditions would not compromise reproductive performance after the first lactation and this was obvious from the pregnancy rates (88 % and 90 %, for CON and CHO), and second litter size (12.6 and 12.0 total born). It seems that this population of primiparous sows did not have a problem that needed fixing. However, the CHO diet did reduce weaning-to-oestrus by half a day.

In conclusion, the sugar supplemented diet did have expected effects on insulin and glucose secretion. The reproductive performance of the sows used in the trial, however, was too good to show positive effects of the CHO diet. The CHO diet is designed to fix problems related to a second sow litter syndrome. The feed intake during lactation was too high and the body weight loss too small in this study to expect any such problem. We recommend in future testing these diets in an experimental situation where feed intake can be controlled or where there is a clear and predictable second litter syndrome, associated with insufficient intake and excessive body weight loss during lactation.

## Backgrounds to Research

Suboptimal reproductive performance of primiparous sows is a long-time recognised problem which is linked to the metabolic status of these sows during lactation. The compromised reproductive performance of first litter sows is often referred to as "second litter syndrome", with affected herds (or sows) showing second litter sizes that are hardly greater, or even smaller (in severe cases), than first litters. The syndrome is also manifested in other ways such as a longer weaning-to oestrus interval and lower pregnancy rates after the first litter, but litter size is the most prominent, the most financially impacting, and in literature the most common parameter that is referred to.

A second litter syndrome can be defined at a herd level but also at sow level. Estimations of affected herds range wildly in literature from 12% to 50%, whereas sow level estimates are more consistent at around 50%. Note that even in herds that do not have a second litter problem at the herd level, still 45-55% of the sows suffer from the second litter syndrome (Hoving, 2012) but this is masked by other sows performing better. The syndrome can also be masked by a higher percent of sows returning to service and (having had time to recover metabolically) performing better from the repeat insemination.

Feed intake in primiparous lactating sows is generally insufficient to meet nutrient requirements for milk production, with excessive mobilisation of body reserves as a consequence (Dourmad et al., 1994; van den Brand et al., 2000a). Effects of feeding level on metabolic changes during lactation have been investigated in previous studies. Energy intake during lactation, in particular, has been shown to affect post-weaning reproductive performance, with a number of studies showing the impact of feed restriction during lactation on either ovulation rate, embryo survival, or both (Kirkwood et al., 1990; Zak et al., 1998; van den Brand et al., 2000c; De Rensis et al., 2005; Vinsky et al., 2006).

Post weaning nutritional strategies can improve reproductive performance, however, the post weaning window of final follicle development is rather short (4-5 days). Baidoo et al. (1992) demonstrated that even if sows are generously fed after weaning, previous restriction during lactation still impacts on embryo survival. There is evidence for lactational catabolism having "imprinted" effects on inherent follicle and oocyte quality at weaning, compromising the number of follicles that develop after weaning (Quesnel et al., 1998) as well as the developmental potential of the contained oocytes (Zak et al., 1997).

Similarly, evidence from cyclic gilt models shows that feed restriction during the period of early antral follicle development (luteal phase preceding follicular phase), has carry-over effects and reduces the number of antral follicles (Hazeleger et al., 2005) and eventually ovulation rate and number of embryos (Chen et al., 2012a). In a recent study from our group, a high feed level during the luteal phase preceding the follicular phase and oestrus at which gilts were mated (Chen et al., 2012a), improved early antral follicle development, ovulation rate (+1.7 ovulations; $P<0.05$), embryo survival (+7%; $P<0.10$), and the number of embryos (+2.2 embryos at day 9 of pregnancy; $P<0.05$).

These results suggest that follicle development can be manipulated nutritionally during the early (pre) antral stages, improving follicle and oocyte quality. In primiparous sows, this stage of follicle development would coincide with late lactation, prior to the post-weaning follicular phase.

**Objectives of the Research Project**

- To provide extra carbohydrates to lactating primiparous sows, to stimulate insulin and glucose secretion, rather than increasing energy intake per se. To achieve this, starch and sugars will be added to the diet to replace fats. Conventional diets for lactating sows generally have an increased fat density to increase the energy intake, but we believe that this may actually aggravate the negative energy balance and reduce insulin and glucose levels at the same time.
- Stimulate follicle development during the last week of lactation, to shorten the wean-to-oestrus interval and increase the number of ovulations.
- Increase pregnancy rate after the first lactation and size of the second litter (total born, born alive).

The objective of this project was to provide a fully formulated diet with the same properties as the supplements in the previous project, to stimulate insulin and glucose secretion in late lactation. The proviso made by the review panel for this proposal was that the study was to be performed under "controlled commercial" conditions.

**Introductory Technical Information**

Since feed intake capacity limits energy intake in lactating primiparous sows, the dietary energy source may provide a means to manipulate post-weaning reproductive performance, through LH secretion and insulin production (Koketsu et al., 1996; van den Brand et al., 2001). Insulin has been proposed as a hormone that acts as an intermediary between nutrition and reproduction (Lucy, 2008), both through effects on gonadotrophins (FSH and LH) but also through direct effects on the ovary. Endogenous insulin levels can be influenced by feeding level (Koketsu et al., 1996), but also by dietary energy source (Kemp et al., 1995) during lactation.

Increasing energy density of diets by varying the fat content, however, did not result in any improvement of sow body condition during lactation and post-weaning reproductive performance in studies by Heo et al (2008) and Quiniou et al (2008), and actually aggravated the catabolic condition because of increased milk fat output (van den Brand et al., 2000b). In contrast, increasing the carbohydrate content of the diet throughout lactation (van den Brand et al., 2000a,c), improved the energy balance and increased gonadotrophic secretion, and insulin and glucose profiles.

In a recent pilot trial at SARDI, primiparous sows (n=23) were fed a supplement of specifically formulated carbohydrates during the last week of lactation, aiming to increase glucose and insulin secretion (Chen et al., 2012b). The supplement was rich in sugars (sucrose and dextrose) and readily available starch (extruded wheat starch). The supplement (1 kg), was top-dressed and replaced 1 kg of the standard diet, with total feed intake equal to controls (n=24). The supplement increased insulin and glucose profiles compared to controls, and sows fed the carbohydrate supplement had a shorter weaning to ovulation interval (5.7 d vs. 6.2 d; P=0.09), and a greater litter size (total born 12.4 ± 0.5 vs. 10.7 ± 0.6; P=0.04).

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## Research Methodology

### *Experiment 1 Main Commercial Trial*

This project consisted of a controlled commercial trial to test the effects of a specific carbohydrate (CHO) formulation on pregnancy rate and litter size. The study was performed at APFG's production unit at Shea-Oak Log in South Australia. This unit was assumed to have a mild second litter syndrome based on historical data. The trial was run over 3 batches, with a total of 228 first litter sows (gilts) farrowing.

Nulliparous sows entered the farrowing shed, farrowed and had their litter sizes standardised as per normal procedure. Sows and piglets were weighed by research staff at the start of lactation after litters had been standardised (day 1-4 of lactation), and at weaning. Sows were hand-fed using calibrated dippers provided as part of the project. The feed allowance (number of dippers fed) was recorded on a sheet provided by the researchers and attached to each pen, to estimate the feed intake. Feed allowance was increased gradually from farrowing to match the increasing feed intake.

From start of lactation until 8 days before weaning sows were fed the normal APFG lactation diet. After that until the end of the lactation sows were fed either a control diet or the CHO diet, both of which were provided in bags. Again feed allowance was recorded on a daily basis as above. Allocation to treatments was based on suckled litter size. Only litters with 10 or more piglets were included, resulting in 60 litters per treatment being allocated at 8 days pre-weaning. Crates were colour marked to identify the type of treatment. The diets were food-dyed using the same colours (red and green) matched to the crate to facilitate identification of treatment and prevent feeding the wrong diet. Staff were not aware of what colour represented which treatment.

At weaning sows were treated as per normal procedure for heat checks, mating, preg-checks etc. Mating day, cull sows, pregnancy diagnosis, and second litter size (TB and BA) were recorded.

**Table 1: Composition of the control diet and the diet with added sugars and starch (CHO diet).**

| Item | Control, % | CHO diet, % |
|---|---|---|
| Wheat | 26.7 | 28.8 |
| Barley | 23.4 | 15.5 |
| Peas | 5.0 | 5.0 |
| Lupins | 15.0 | 15.0 |
| Biscuit | | 1.0 |
| Extruded wheat | | 3.5 |
| Dextrose | | 2.2 |
| Sugar | | 5.5 |
| Mill Mix | 7.0 | |
| NP Meatmeal | 3.0 | 3.3 |
| Fish meal | 2.5 | 2.5 |
| Canola (expeller) | 5.0 | 5.0 |
| Soya (solvent) | 5.9 | 8.8 |
| Salmate | 0.4 | 0.4 |
| Tallow | 1.0 | 1.0 |
| SPRAY Tallow | 2.5 | |
| Limestone | 1.3 | 1.23 |
| Salt | 0.3 | 0.3 |
| HCL-Lysine | 0.3 | 0.3 |
| Methionine | 0.2 | 0.2 |
| Biofix | 0.2 | 0.2 |
| Oxicap E2 | 0.02 | 0.02 |
| Red Ponceau 4R | 0.1 | |
| | | 0.1 |
| Choline LQ75 | 0.05 | 0.05 |
| Pig Breeder Premix | 0.25 | 0.25 |
| Vitamin E | 0.1 | 0.1 |
| DE, MJ ME per kg | 14.2 | 14.3 |
| Protein, % | 19.5 | 19.8 |
| Fat, % | 6.9 | 4.3 |
| Fibre, % | 5.7 | 5.2 |
| Starch, % | 30.6 | 28.7 |
| Lysine, % | 1.2 | 1.2 |

***Experiment 1A Glucose and Insulin Profiles***

This experiment was conducted at Roseworthy piggery to test the glucose and insulin profiles for the control and the CHO diets. Fifteen primiparous sows were fed ad lib through the first three weeks of lactation and then one week before weaning allocated to receive either the control diet or the CHO diet, which were the same diets as fed in the main trial at APFG. The sows were fed these diets during the last 8 days prior to weaning at 4 weeks. The average intake was 5.9 kg for the control diet (n=7) and 5.6 kg for the CHO diet (n=8).

One day before weaning the sows received an ear vein catheter to allow serial blood samples to be taken. Sows were fixed by a snout rope, and then a 1x1.5-mm PVC catheter (Microtube Extrusions, NSW, Australia) was inserted 50 cm into a lateral or intermediate auricular vein. The exterior part of the catheter was secured in a pouch at the back of the neck and taped by a Tensoplast-Vet tape (BSN Medical, Australia). Prior to the test sows were fasted and for the test they were fed 2.0 kg of either diet. Blood samples (13 in total) were taken at -24, -12, 0, 12, 24, 36, 48, 60, 72, 90, 132, 168, and 204 min relative to feeding. For details see Chen et al. (2012b). For glucose and insulin assays only those sows were included that finished the 2 kg provided within 30 min.

Glucose was analysed by colorimetric automated analysis on a Hitachi 912 automated centrifugal analyser with a commercial kit (Glucose HK assay kit; Roche Diagnostics, NSW, Australia). Insulin was assayed in duplicate in 100 µl plasma using a specific porcine RIA (pI-12K; Millipore, Billerica, USA). The intra- and interassay coefficients of variation were <15%. The minimum detection limit was 2 IU/ml.

**Discussion of Results**

Body weight loss during lactation was 3 to 4 % on average, which is less than generally reported for first litter sows. The (estimated) feed intake during the last 8 days of lactation was slightly higher on the CHO diet (Table 2). Nevertheless, piglet growth rate and weaning weights were slightly higher for control sows (when corrected for piglet weights at the start of lactation), probably because control sows mobilised 2 kg more of their body reserves on average (not significant). The increased mobilisation of body reserves, predominantly fat, was expected because a higher fat content in the diet (such as in the control diet) tends to increase body fat mobilisation which is directly secreted into the milk.

Despite the low average body weight loss, 13.5 % and 15.5 % of the sows had a delayed (>10 d) weaning-to-mating interval or remained anoestrous. This may be a reflection of one of the ways second litter syndrome manifests itself, however, some of the delayed matings may be sows that were not picked up at their first post weaning oestrus and mated at their second oestrus. For those sows that were mated within 10 days after weaning, the weaning-to-mating interval was reduced by feeding the CHO diet by half a day, which is considerable in terms of reproductive physiology (Figure 2). This confirms our finding from the previous work at Roseworthy where the CHO supplement reduced the weaning to ovulation interval.

There was no evidence of a second litter syndrome in terms of pregnancy rate (88 %) or litter size (12.6 total born) in the control sows. Therefore, it was obvious that these sows did not experience a second litter syndrome and hence the CHO diet could not be expected to improve pregnancy rate (90 %, n.s.) or litter size (12.0 total born, n.s.), which is shown in Table 2.

A separate analysis was performed on sows with a total energy balance over lactation of lower than -150 MJ ME, representing about 50% of the sows. However, the energy balance in these sows was still only -18 MJ ME/d on average, and there was no difference between treatments in reproductive performance in these sows either (total born 11.4 ± 0.8 vs. 12.0 ± 0.5 for control and CHO, respectively).

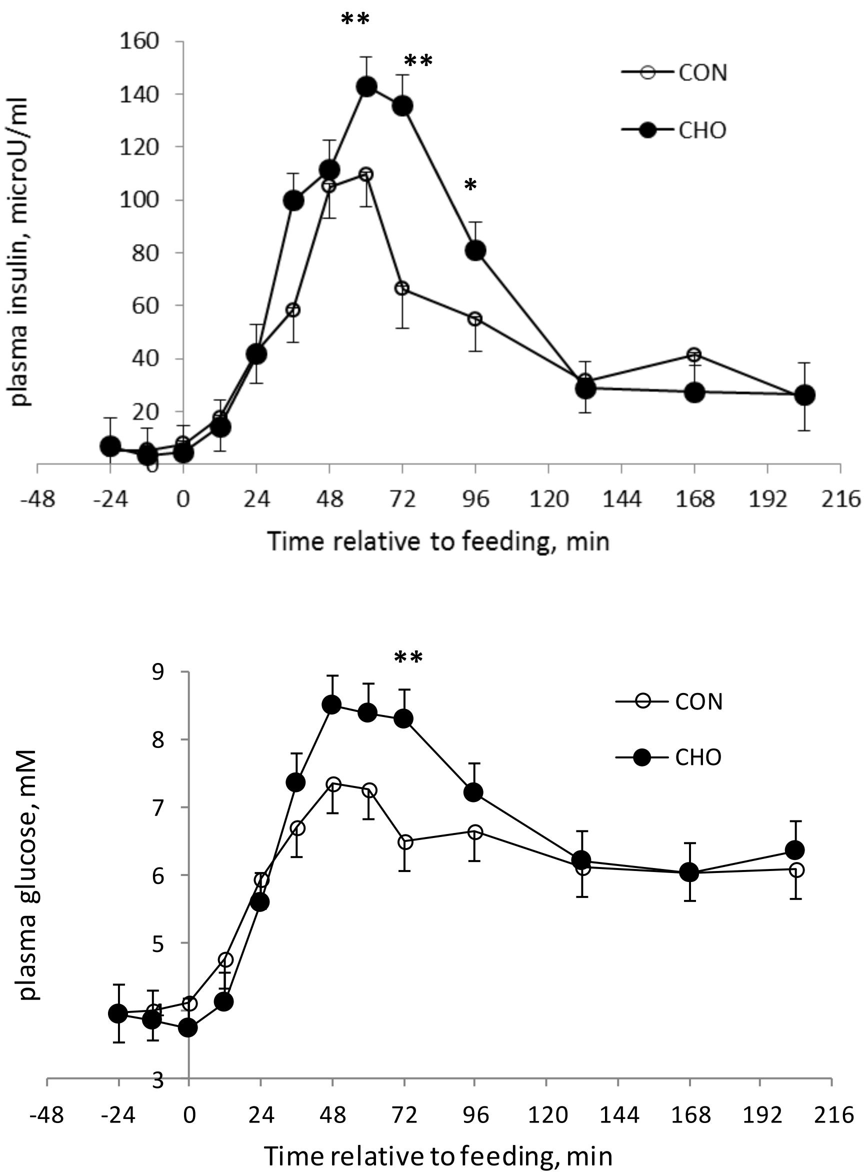


**Figure 1: Insulin and glucose concentration relative to feeding 2.0 kg of either the control (CON) or carbohydrate (CHO) diet after fasting. *P<0.10, **P<0.05**

For the glucose and insulin profiles, 2.0 kg was fed from either diet. Based on the formulation details in Table 1, this equated to 310 g barley, 575 g wheat, 70 g extruded wheat, 20 g biscuit meal, 44 g dextrose, and 110 g sucrose as the main carbohydrate sources for the CHO diet. For the CON diet this equated to 460 g barley, and 540 g wheat. In total, therefore, the carbohydrates provided in 2.0 kg of the two diets amounted to 1,129 g and 1,000 g respectively. Therefore, dietary differences were primarily in the content of fast carbohydrates (sugars) rather than the total of carbohydrates.

In the previous trial (Chen et al, 2012c), intakes of various carbohydrate sources were similar to the current trial when based on a similar feed intake. However, the difference in insulin secretion was more pronounced between the control and sugar rich diet. This may be due to the fact that in the previous trial, for the purpose of the glucose and insulin profiles, 1 kg of the lactation diet was supplemented with 0.5 kg of the carbohydrate supplement whereas the controls were fed 1.5 kg of the lactation diet. This resulted in a higher proportion of starch and sugars in the supplemented sows (200 g barley, 300 g wheat, 275 g starch and sugars) compared to the sows receiving lactation diet only (300 g barley, 450 g wheat, no sugars), and also a higher proportion of sugars in the test (1/3) compared to a sow with for example a daily intake of 5 kg (1/5).

Average intake in the previous trial was 5.1 kg during the treatment period, whereas in the current trial feed intake was 5.7 to 5.9 kg in the Roseworthy trial and around 6.0 kg in the APFG trial. This makes it difficult to translate the glucose and insulin profiles in the test to actual secretion during the treatment period. Considering the higher intake in the current trial and the lower proportion of sugars in the actual diet compared to the test mix provided in the previous trial, it may be assumed that the difference in glucose and insulin secretion may be less pronounced in the current trial than in the previous study.

Regardless, the differences between studies in the actual diets provided and the intake of the sows, another major difference between the current study and the study reported by Chen et al (2012c), is the extent of BW loss and the absence of a second litter syndrome in the current study. The small loss of BW and the large second litters are likely related. In a herd with a lower feed intake and greater body weight loss, one would expect this to be reflected in wean-to-oestrus interval, pregnancy rate, or second litter size. This would be a herd that consistently suffered from second litter syndrome, or a herd that has seasonal variation in performance of the primiparous sows due to effects of temperature in summer on lactation feed intake. Clearly, the primiparous sows at APFG that were part of this study did not express such problems, except possibly in terms of wean-to-oestrus interval. In other words, there was no problem to fix.

**Table 2: Sow body weight loss, post weaning oestrus and reproductive performance (second litter), and piglet performance during first lactation for sows fed a diet with specific carbohydrate formulation (CHO) or a standard lactation diet (CON).**

| | CON | CHO |
|---|---|---|
| N* | 60 | 59 |
| BW at start of lactation, kg | 201 ± 2.5 | 197 ± 2.5 |
| BW at wean, kg | 194 ± 2.6 | 191 ± 2.5 |
| BW loss, kg | -7.7 ± 1.4 | -5.8 ± 1.2 |
| Energy balance, MJ ME/d | -11 ± 2[a] | -5 ± 2[b] |
| % anoestrous** | 15.5 % | 13.5 % |
| WMI, d*** | 4.8 ± 0.1[a] | 4.3 ± 0.2[b] |
| ADFI during treatment, kg | 5.5 ± 0.1[a] | 5.9 ± 0.1[b] |
| Pregnant, % | 88 % | 90 % |
| Total born second litter | 12.6 ± 0.4 | 12.0 ± 0.5 |
| Born alive second litter | 11.8 ± 0.5 | 11.5 ± 0.5 |
| LS at allocation | 11.1 ± 0.1 | 11.1 ± 0.1 |
| Lac length | 24.6 ± 0.3 | 24.6 ± 0.3 |
| ADG****, g/d | 224 ± 4[a] | 214 ± 4[b] |
| Piglet BW at wean, kg | 6.51 ± 0.11[a] | 6.21 ± 0.11[b] |

*litters ≥10 piglets. **Sows mated >10 d post weaning or not mated were considered anoestrous, ***WMI only for sows with mating ≤ 10 d post weaning (45 and 49 for the CHO and CON treatments respectively). ****Average daily gain of piglets calculated over the period between first weight and last weight, which excludes the first 1 to 4 d of lactation depending on farrowing date.

$^{a,b}P < 0.05$

**Implications and Recommendations**

- The APFG herd at Shea-Oak Log does not experience the excessive body weight loss sometimes associated with first lactation; nevertheless, there were about 14 % sows with delayed heats.
- This herd clearly does not suffer from second litter syndrome in terms of pregnancy rate after first lactation and size of the second litter, at least in the period studied.
- The CHO diet reduced the wean-to-mate interval by half a day.
- This herd was probably not the right herd to investigate the potential effects of insulin- and glucose-stimulating diets. The diets were probably formulated correctly and caused a difference in insulin and glucose secretion, but the effects on insulin and glucose may be more pronounced when feed intake is restricted intentionally to provide a model for second litter syndrome, or when the diets are tested in a herd with compromised feed intake.
- Similarly, the effects of these diets on reproductive performance can only manifest itself in a herd that has a clear second sow litter syndrome, or in a model where feed intake is restricted to emulate a second litter problem. The diets were designed to fix a problem and effects would only be apparent if tested in a problem situation.
- We recommend to test the diets in an experimental situation where feed intake can be controlled, or in a herd where a clear second litter syndrome can be expected.
- The added carbohydrates in the CHO should replace fat rather than cereals, to drop the fat % more than in the current formulation and increase the total carbohydrates.

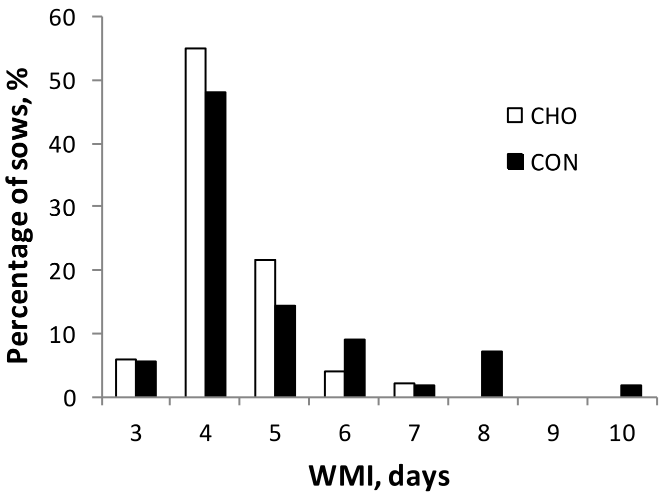


**Figure 2: Weaning to mating interval distribution for those sows that exhibited oestrus within 10 days post weaning.**

## Conclusions

- A diet enriched with sugars and fast carbohydrates does stimulate insulin and glucose secretion more than a standard lactation diet. In sows with a high feed intake (> 5.5 kg/d average) the effects may be less pronounced.
- A diet enriched with fast carbohydrates (sugars and extruded wheat) may not improve reproductive performance in first parity sows (pregnancy rate and litter size) on a farm that has no second litter syndrome, due to the fact that feed intake in first lactation is sufficient to minimise body weight loss and subsequent reproductive performance is good as it is. However, the current trial has shown that even on a farm with no second litter syndrome there are physiological effects of the carbohydrate diet since weaning to oestrus interval was shortened by half a day.
- The carbohydrate enriched diet did show positive effects on second litter size in a previous trial in a herd with considerable body weight loss during lactation. Further work, therefore, should be undertaken in a population that is known for second litter syndrome and where feed intake can be controlled.

## Intellectual Property

N/A

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**Publications Arising**

Full paper for submission to scientific journal is in preparation.
APSA paper will be prepared for APSA 2015.