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# Alleviating seasonal infertility and increasing the female: male ratio in litters by manipulating dietary intake of omega 6 and omega 3 fatty acids

**Final Report**
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**Research Organisation**
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## Executive Summary

Two factors which continue to negatively affect the productivity and efficiency of Australian, and global, pig production are the suppression of sow fertility during summer (seasonal infertility) and boar taint. This project was designed to determine whether feeding sows with diets containing different levels of linoleic acid (n-6 PUFA; LA) and α-linolenic acid (n-3 PUFA; LNA) would affect subsequent reproductive performance during summer and winter, and the percentage of female progeny produced in the subsequent litter. Altering dietary intake of LA and LNA has the potential to affect reproductive processes in a number of ways, including: altered synthesis of prostaglandins, which play an important role in uterine contractility and involution, communication between, and development of, the embryo-maternal interface, embryo implantation and pregnancy maintenance, as well as oocyte and embryo metabolism and growth potential. Recently, it has been proposed that minimum intakes of 10 g / day of LNA and 100 g / day of LA are required during lactation to optimise reproductive efficiency of sows (Rosero et al., 2016), and there is growing evidence that high dietary intake of n-6 PUFAs can result in female biased litters in sheep and mice (Fountain et al., 2008; Clayton et al., 2017). Therefore, this study tested the hypothesis that feeding sows a diet containing 0.27% LNA and 1.8 – 2.0% LA during lactation would increase sow reproductive performance, and that feeding sows a diet containing 0.13% LNA and 2.5 – 2.7% LA diet would increase the percentage of female piglets born at the subsequent litter.

A total of 641 Large White x Landrace Sows (PIC Genetics, parities 1-7), were allocated to 1 of 6 dietary treatments (Summer - Low n-6 (SL, n = 104 sows), Summer - Medium n-6 (SM, n = 103 sows); Summer - High n-6 (SH, n = 102 sows); Winter - Low n-6 (WL, n = 119 sows); Winter -Medium n-6 (WM, n = 104 sows), Winter - High n-6 (n = 109 sows) at entry to the farrowing house. Dietary treatments were designed as a 2 x 3 factorial, incorporating the effects of season (summer versus winter) and three lactation diets (Low n-6 versus Medium n-6 versus High n-6 PUFA). The following measurements were collected: sow weight on day 1 of lactation and at weaning; sow subsequent reproductive performance (timing of post-weaning service, pregnancy rates, total born, born alive, mummified fetuses, stillbirths, the number of male and female piglets present in the litter (including alive, and stillborn); and, suckled litter size and litter weight on day 21 of lactation.

There was no effect of treatment on the weaning to service interval; however, sows lactating during summer were heavier at the start and end of lactation and were re-mated sooner ($P = 0.001$) after weaning than those lactating during winter. The total number of piglets born and the number of piglets born alive and the number of mummifed fetuses delivered at the subsequent farrowing were unaffected by treatment or season. However, the number of piglets born dead at the subsequent farrowing was lower ($P = 0.032$) for the medium compared with low n-6 PUFA treatment group (0.82 ± 0.11 versus 1.27 ± 0.11 piglets). There was a tendency ($P = 0.1$) for lactation diet to affect the number of mummified fetuses, with the medium and high n-6 diets resulting in fewer mummies than the low n – 6 diets. There were no season of lactation or dietary treatment effects on the proportion of females born at the subsequent farrowing. Subsequent gestation lengths were shorter for sows which lactated during summer compared with winter ($P < 0.001$); but were unaffected by dietary treatment ($P = 0.107$). Based on farrowing rates, per 100 sows weaned, and subsequent litter size, it is clear that the medium and high n-6 diets resulted in more piglets being born per 100 sows weaned. However, the medium n-6 diet resulted in 32 fewer stillborn piglets per 100 sows weaned compared with the low n-6 diet and 26 fewer stillborn piglets per 100 sows weaned compared with the high n-6 diet.

Total litter sizes were smaller ($P < 0.047$) when the proportion of females in the litter were high ($\geq$ 0.60) compared with a low ($\leq$ 0.40) or normal (0.41 – 0.59) (12.26 ± 0.44 versus 13.47 ± 0.28 and 13.40 ± 0.23 piglets). The number of live born piglets was similar regardless of the proportion of

females present in the litter; however, there were fewer ($P < 0.001$) still born piglets in litters with a high compared with a normal and low number of females (0.47 ± 0.19 versus 0.98 ± 0.10 and 1.34 ± 0.12 piglets). Interestingly, there was a tendency ($P = 0.082$) for previous weaning to service intervals to differ between sows which produced litters with a high (5.90 ± 0.64), a normal (7.19 ± 0.33) or low (6.23 ± 0.41) proportion of females.

In conclusion, the current data demonstrated that feeding sows a lactation diet containing low levels of n-3 and n-6 PUFA reduced subsequent reproductive performance, regardless of season. Importantly, feeding diets with higher levels of n-6 PUFAs during lactation increased reproductive efficiency (based on number of piglets born per 100 sows weaned). However, the current study also demonstrates for the first time, as far as I am aware, that feeding a lactation diet containing approximately 0.27% LNA and 1.8 – 2.0 % LA reduces stillbirth rates. Therefore, these data indicate that feeding higher levels of n-3 PUFA in conjunction with higher levels of n-6 PUFA improve reproductive efficiency. This finding lends further support to the suggestion (Rosero et al., 2016) that reproductive efficiency is improved when sows receive 10 g / day of LNA and 100 g / day of LA during lactation. It is suggested that higher intakes of LA may promote embryo and uterine function, such that incidences of post-implantation fetal death were reduced (eg fewer mummies), while the combination of increased LNA and LA may have increased the capacity of neonatal piglets to cope with the challenges associated with parturition (eg lower stillbirths). Interestingly, litters with a high female bias had fewer stillbirths, supporting suggestions that female fetuses and neonates may be more robust, and better able to cope with environmental challenges, which increases the imperative to understand how to increase the female bias of swine litter.

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# 1. Background to Research

Two factors which continue to negatively affect the productivity and efficiency of Australian, and global, pig production are the suppression of sow fertility during summer (seasonal infertility) and boar taint.

Australian, and global, pork industries have spent a considerable amount of money and time trying to alleviate seasonal infertility and understand the physiological causes of summer / heat induced suppression of sow fertility. However, whilst understanding has progressed, a ‘cure’ for seasonal infertility remains elusive. This project was, therefore, developed to determine whether increasing the ratio of n-6 to n-3 polyunsaturated fatty acids in sow lactation diets would improve the reproductive performance of sows which lactate and are mated during summer, and reduce the proportion of male pigs born in the subsequent litter.

## 2. Objectives of the Research Project

This project set out to determine whether altering the quantity and ratio of n-3 and n-6 polyunsaturated fatty acids included in sow lactation diets during summer and winter would affect subsequent reproductive performance (weaning to oestrus interval, farrowing rate and subsequent litter size) and the proportion of female progeny produced in the subsequent litter.

## 3. Introductory Technical Information

Feeding lactating sows diets containing 3.3% linoleic acid (n-6 PUFA; LA) and 0.45% α-linolenic acid (n-3 PUFA; LNA) improved reproductive performance (i.e. total born and farrowing rate increased by 0.8 piglets and 10%, respectively (Rosero et al, 2016). Previous studies from the same authors demonstrated improved subsequent reproductive performance when supplemental fat was included in lactating diets of sows exposed to high ambient temperature (Rosero et al., 2012), presumably reflecting the reduced heat increment associated with fat digestion and a possible increase in energy intake. Further, in a recent review, Rosero et al. (2016) concluded that subsequent reproductive performance is optimised when sows have a minimum dietary intake of 10 g / day of LNA and 125 g / day of LA acid during lactation.

Both linoleic acid and α-linolenic acid are essential precursors for the biosynthesis of prostaglandins. Within the body, LA is converted to Dihomo-gamma-linolenic acid (DGLA), which is converted to either 1-series prostaglandins (PG) ($PGE_1$ and $PGF_{1\alpha}$ etc) or arachidonic acid (AA), which is in turn converted to 2-series PGs (PGF2α and $PGE_2$). LNA is converted to eicosapentaenoic acid (EPA), which is then converted to 3-series PGs ($PGE_3$, $PGF_{3\alpha}$ etc). Importantly, conversion of LA and LNA to AA and EPA, respectively, is catalysed by the same enzymes, which means that the ratio of these n-6 and n-3 fatty acids in the diet determines the quantity of series 2 and series 3 PG which are produced. Based on this, it can be assumed that high levels of LA promote production of 1-series PGs and the 2 series PG (eg PGF2α and $PGE_2$), while high levels of LNA promote production of the series three PG. Furthermore, high levels of EPA depresses metabolism of n-6 PUFAs and, thus, synthesis of the 2 series PGs. There is substantial evidence that altering dietary consumption of PUFA changes both the type and quantity of PG produced by production animals (ruminants and pigs) (reviewed by Wathes et al., 2007). For example, diets which promote synthesis and release of PGF2α, a known luteolytic factor, would be expected to be detrimental to pregnancy maintenance especially during the period of elongation and implantation when conceptus stimulated inhibition of PGF2α is vital to prevent luteolysis. Therefore diets with a high n-6 PUFA content would be expected to promote luteolysis and reduce pregnancy rates. In contrast diets rich in n-3 PUFA suppress PGF2α from bovine endometrial cells, and would be expected to favour establishment and maintenance of pregnancy (Halle et al., 2015). However, it does not appear to be as simple as this, *in vitro* studies using bovine tissue suggest supplementary LA shifts production of 2-series PG in favour of $PGE_2$, which promotes intra-uterine vascular permeability and placental development, and is a potent luteotrophic factors, and is, therefore, favourable for pregnancy maintenance (Chartrand et al., 2003; Wathes et al., 2007). Furthermore, recent studies in pigs (reviewed by Waclowik et al., 2017) suggest that PGF2α stimulates interactions between the conceptus and maternal environment and promotes angiogenesis of the uterine endometrium, which may positively affect implantation and pregnancy maintenance. Therefore, while increasing dietary n-3 PUFA intake has positive effects on reproductive function, and Smits et al. (2011) reported increased litter size in response to supplementary n-3 PUFA during lactation, increased intake of n-3 PUFA intake alone may lead to deficiencies in AA synthesis and thus inadequate production of 2 series PGs. There are also other periods during the reproductive cycle when elevations in PGF2α may be beneficial. Elevated PGF2α during the post-partum period are likely to promote regression of the CL of pregnancy, facilitating the onset of lactogenesis and reducing progesterone (P4) levels in milk (P4 is known to reduce activity in neonates), and also promote uterine involution and repair and thus uterine health at the first post-partum mating. Interestingly, the immune status and growth rate of the piglets was higher when their dam was fed a diet with n-6: n-3 ratios of 9:1 compared with 3:1 and 13:1 (Yao et al., 2012). Based on the current evidence, it is clear that further work is required to determine the impact of altering dietary intake of n-3 and n-6 PUFAs on sow reproductive performance.

Recent studies in sheep and mice demonstrated that feeding diets with high levels of n-6 PUFAs prior to mating significantly increased the proportion of female progeny produced. In the absence of any change in fertility or the number of progeny produced, the proportion of females born increased from 44 to 54% (Clayton et al., 2015) and from 44% to 58% (Gulliver et al., 2013) in sheep, and from 47%

to 60% in mice (Fountain et al., 2008). Interestingly, previous work in mice indicated that feeding diets rich in saturated fats decreased the proportion of female progeny produced. Specifically, the proportion of female progeny produced was decreased from 61% to 33% when mature female mice were fed iso-calorific diets with energy provided by carbohydrates as opposed to saturated fats (Rosenfield et al., 2003). The mechanisms whereby mammalian females can influence the sex of their progeny remain to be fully elucidated. However, maternal factors pre-conception may result in the ovulation of oocytes which are fertilised preferentially by sperm bearing an X as opposed to a Y chromosome, whilst alterations in the development rate of male and female embryos prior to and during implantation may also be mechanisms whereby mammalian females could influence the sex of their progeny (Rosenfield and Roberts, 2004; Grant and Irwin, 2009; Grant and Chamley, 2010). However, despite the significant benefits associated with increasing the number of female pigs produced by each sow annually, there has been almost no work conducted in pigs in this area. Based on the available literature, it was, therefore, hypothesised that feeding lactating sows diets containing higher levels of both linoleic acid and α-linolenic acid during summer would improve subsequent reproductive performance and the proportion of female progeny produced.

# 4. Research Methodology

This study was conducted in accordance with the guidelines set out in 'Code of Practice for the Care and Use of Animals for Scientific Purposes' (Canberra 2004) and with the approval of The University of Adelaide Animal Ethics Committee. All animal work was carried out at SunPork South, South Australia.

### *4.1 Animals, Housing and Treatments*

This study used a total of 641 Large White x Landrace Sows (PIC Genetics, parities 1-7), and was designed as a 2 x 3 factorial, incorporating the effects of season (summer versus winter) and three lactation diets (Low n-6 versus Medium n-6 versus High n-6 PUFA). Farrowing dates were the 20$^{th}$ January to 6$^{th}$ March 2017 (summer block) and the 5$^{th}$ of August and 14$^{th}$ September 2017 (winter block). At farrowing shed entry sow were allocated to one of the following 6 treatments (Summer - Low n-6 (SL, n = 104 sows), Summer - Medium n-6 (SM, n = 103 sows); Summer - High n-6 (SH, n = 102 sows); Winter - Low n-6 (WL, n = 119 sows); Winter -Medium n-6 (WM, n = 104 sows), Winter - High n-6 (n = 109 sows). Full diet specifications are detailed in Appendix 1: the low n-6 diet contained 4.1% Tallow, the medium n-6 diet contained 1.75% canola oil and 2.25% soybean oil, and the high n-6 diet contained 4% Sunflower oil. The fat content of the diets and estimated daily intake is presented in Table 1. Sows were weaned on day 25.1 ± 0.13 days post-partum.

### *4.1 Experimental Measures*

Sows were weighed on day 1 of lactation and at weaning, with the following subsequent reproduction parameters recorded: lactation length, weaning to service interval, pregnancy status, gestation length and subsequent litter size (total born, born alive, born dead and mummified fetuses delivered). Blood samples were collected on day 14 of lactation from a subset of sows during summer and winter (number of samples analysed were 28 (low n-6), 40 (medium n-6) and 41 (high n- 6) sows), and analysed for PGF2 metabolites (as a measure of PGF2α levels), plasma urea nitrogen (PUN) and non-esterified fatty acids (NEFAs). A Cobas Integra 400 plus chemistry analyser was used to measure PUN and NEFA levels. Plasma samples were analysed using a commercial Arbor Assays enzyme immunoassay kit for 13,14-dihydro-15-keto-PGF2α (PGFM: K022-H5). Validation was confirmed with a parallelism of pooled animal samples against the standard curve. Samples were analysed neat following the manufacturer's protocol. Inter - and intra-assay variability was 7% and 10%, respectively. The number of piglets alive on day 21 post-partum and at weaning were recorded. Litter weight was measured on day 21 post-partum, and used to calculate mean piglet weight on day 21.

### *4.2 Statistical analysis*

Data were analysed using Genstat (version 15; VSN International Ltd., Hemel Hempstead, UK). Unless otherwise specified, data are presented as Mean ± SEM, with significance accepted at $P < 0.05$, and tendencies at $P < 0.1$. In the absence of interactive effects of season of lactation and dietary treatment main effects only are presented. The effects of season of lactation and dietary treatment on all measures were analysed using an ANOVA (unbalanced treatment design), with parity included in the model as a co-variate. Day 21 litter size was added as a co-variate when determining the effects of season of lactation and dietary treatment on piglet weight on day 21 of lactation. The number of piglets born dead at the subsequent litter was not normally distributed and, therefore, a general linear mixed model (negative binomial) was used, with total litter size included, to determine the effects season of lactation and dietary treatment on this measurement (non-transformed means and SEM presented). The effects of season of lactation and dietary treatment on the proportion of sows returning to service at set intervals and pregnancy rates were analysed using a chi-squared. Sows were divided into groups based on their parity (lactation number at time of treatment), the three groups were: young (parity 1 and 2), medium (3 and 4) and old (5 plus). The effects of these groups and their interaction with dietary treatment, on weight loss and subsequent reproduction were analysed, using an ANOVA (unbalanced treatment design), and on the number of stillbirths using a general linear mixed model (negative binomial), with total litter size included (non transformed means and SEM presented). Sows were also divided into groups based on the proportion of females born at the subsequent farrowing (Low, $\leq$ 0.4; normal, 0.41 – 0.59; high, $\geq$ 0.60). The effects of these groups on total litter size, number of piglets born alive, and number of mummified fetuses delivered were analysed, using an ANOVA (unbalanced treatment design) and on the number of stillbirths using a general linear mixed model (negative binomial), with total litter size included (non transformed means and SEM presented).

*Table 1 Dietary fatty acid levels (%) and estimated daily intake of n-3 and n-6 polyunsaturated fatty acids (based on 6 and 7 kg / day intake) in summer and winter, based on levels in diet (%) and 91.2% dry matter content of diet*

| Dietary treatment | Low n – 6 diet | | Medium n-6 diet | | High n-6 diet | |
|---|---|---|---|---|---|---|
| Season of lactation | Summer | Winter | Summer | Winter | Summer | Winter |
| Fat content, % | 5.80 | 4.97 | 5.58 | 5.34 | 6.60 | 5.89 |
| Saturated fatty acids, % | 2.06 | 1.65 | 1.08 | 1.08 | 1.31 | 1.09 |
| Monounsaturated fatty acids, % | 1.63 | 1.45 | 1.59 | 1.53 | 1.67 | 1.44 |
| **n-6 polyunsaturated fatty acids (PUFA), %** | **1.18** | **1.10** | **1.99** | **1.85** | **2.70** | **2.55** |
| **n-3 polyunsaturated fatty acids (PUFA), %** | **0.13** | **0.12** | **0.27** | **0.26** | **0.14** | **0.13** |
| Trans fatty acids, % | 0.13 | 0.09 | 0.02 | 0.02 | 0.03 | 0.02 |
| Conjugated linoleic acids, % | 0.01 | 0.85 | 0.00 | 0.66 | 0.00 | 1.87 |
| **Ratio of n-6: n-3 PUFA** | **8.99** | **9.23** | **7.23** | **7.19** | **19.11** | **20.34** |
| n-6 PUFA intake, g / kg feed | 11.8 | 11.0 | 19.9 | 18.5 | 27.0 | 25.5 |
| n-3 PUFA intake, g / kg feed | 1.30 | 1.20 | 2.70 | 2.60 | 1.40 | 1.30 |
| **Estimated PUFA intake** | | | | | | |
| When daily feed intake is 6 kg / day | | | | | | |
| n-6 PUFA, g / day | 70.8 | 66.0 | 119.4 | 111.0 | 162.0 | 153.0 |
| n-3 PUFA, g / day | 7.8 | 7.2 | 16.2 | 15.6 | 8.4 | 7.8 |
| When daily feed intake is 7 kg / day | | | | | | |
| n-6 PUFA, g / day | 82.6 | 77.0 | 139.3 | 129.5 | 189.0 | 178.5 |
| n-3 PUFA, g / day | 9.1 | 8.4 | 18.9 | 18.2 | 9.8 | 9.1 |

## 5. Results

### *5.1. Sow weight and weight change during lactation and litter characteristics*

There was no difference in sow weight on day 1 of lactation or sow weight change during lactation between the different dietary treatment (Table 2). Litter size on day 21 of lactation was higher (P = 0.034) for the Low compared with High n-6 dietary treatment (10.46 ± 0.12 versus 10.03 ± 0.12 piglets). Individual piglet weight was lower (P = 0.023) on day 21 for the Low compared with High n-6 dietary treatment (5.89 ± 0.06 versus 6.14 ± 0.07 kg); however, day 21 litter weight was unaffected (P = 0.851) by dietary treatment (Table 2).

Sows which farrowed, and subsequently lactated, during summer were 12.7 kg heavier on day 1 of lactation and 16.3 kg heavier at weaning, than their winter farrowing counterparts (P < 0.001; Table 2). Similarly, weight loss during lactation was lower (P <0.01) during summer compared with winter (Table 2). Litter size on day 21 was lower (P = 0.007) in summer compared with winter (10.07 ± 0.10 versus 10.45 ± 0.10 piglets), as were day 21 litter weights (60.12 ± 0.82 versus 63.16 ± 0.81 kg; P = 0.009); however individual piglet weights were unaffected by season (P = 0.559). There were no interactions between season and treatment and parity group for sow weight or weight loss. However, parity grouping affected (P < 0.01) weight on day 1 and 21 of lactation; parity 1 and 2 sows (224.6 ± 5.46 and 221.1 ± 3.85 kg); parity 3 and 4 sows (24.3 ± 2.40 and 241.5 ± 1.74 kg) and parity 5 plus sows (259.2 ± 7.14 and 251.1 ± 5.15 kg).

*Table 2 Effect of treatment (low versus medium versus high n-6 PUFA levels) and season (summer versus winter) on sow weight and weight loss during lactation, litter weight, litter size and piglet weight on day 21 of lactation (due to the absence of any interactions between n-6 PUFA levels and season, main effects only are presented)*

| | Dietary Treatment | | | Season | |
|---|---|---|---|---|---|
| | Low n-6 | Medium n-6 | High n-6 | Summer | Winter |
| N | 223 | 207 | 211 | 309 | 332 |
| Sow Parity | 3.54 ± 0.10 | 3.43 ± 0.11 | 3.66 ± 0.11 | 3.70 ± 0.09[b] | 3.40 ± 0.08[a] |
| Sow LW | | | | | |
| Day 1, kg | 246.5 ± 1.99 | 244.9 ± 2.06 | 248.0 ± 2.03 | 253.0 ± 1.68[b] | 240.3 ± 1.64[a] |
| Weaning, kg | 238.4 ± 1.93 | 235.9 ± 1.96 | 238.1 ± 1.92 | 245.7 ± 1.58[b] | 229.3 ± 1.58[a] |
| Loss, kg | 8.70 ± 1.18 | 8.06 ± 1.21 | 10.87 ± 1.20 | 7.42 ± 0.96[a] | 11.1 ± 0.98[b] |
| Loss, % | 3.37 ± 0.48 | 3.17 ± 0.49 | 4.01 ± 0.49 | 2.83 ± 0.40[a] | 4.22 ± 0.40[b] |
| Day 21 piglet weight, kg | 5.89 ± 0.06[c] | 5.96 ± 0.07[cd] | 6.14 ± 0.06[d] | 5.98 ± 0.05 | 6.01 ± 0.05 |
| Day 21 litter size | 10.46 ± 0.12[c] | 10.28 ± 0.12[cd] | 10.03 ± 0.12[d] | 10.07 ± 0.10[a] | 10.45 ± 0.10[b] |
| Date 21 litter weight, kg | 62.07 ± 0.96 | 61.28 ± 1.03 | 61.60 ± 1.00 | 60.12 ± 0.82[a] | 63.16 ± 0.81[b] |

[ab] within row indicate differences between season; [cd] within row indicate differences between treatment

## 5.2. *Reproductive performance*

There was no effect of treatment on the weaning to service interval (Table 3); however, sows lactating during summer were re-mated sooner (P = 0.001) after weaning than those lactating during winter (Table 3). The total number of piglets born and the number of piglets born alive and the number of mummifed fetuses delivered at the subsequent farrowing were unaffected by treatment or season (P = 0.665 and 0.182, respectively). However, the number of piglets born dead at the subsequent farrowing was lower (P = 0.032) for the medium compared with low n-6 PUFA treatment group (0.82 ± 0.11 versus 1.27 ± 0.11 piglets; Table 3). There was a tendency (P = 0.1) for lactation diet to affect the number of mummified fetuses, with the medium and high n-6 diets resulting in fewer mummies than the low n – 6 diets (Table 3). There were no season of lactation or dietary treatment effects on the proportion of females born at the subsequent farrowing (Table 3). There was a tendency (P = 0.09) for dietary treatment to affect the proportion of females present in the subsequent litter differently in the two seasons (Table 4). Subsequent gestation lengths were shorter for sows which lactated during winter compared with summer (P < 0.001); but were unaffected by dietary treatment (P = 0.107; Table 3).

*Table 3 Effect of treatment (low versus medium versus high n-6 PUFA levels) and season (summer versus winter) on subsequent reproductive performance (due to the absence of any interactions between n-6 PUFA levels and season, main effects only are presented)*

| | Dietary Treatment | | | Season | |
|---|---|---|---|---|---|
| | Low n-6 | Medium n-6 | High n-6 | Summer | Winter |
| Weaning to service interval, days | 6.69 ± 0.34 | 6.55 ± 0.34 | 6.66 ± 0.34 | 5.89 ± 0.27$^{a}$ | 7.67 ± 0.27$^{b}$ |
| Gestation length | 115.9 ± 0.12 | 115.6 ± 0.12 | 115.6 ± 0.11 | 116.1 ± 0.10$^{b}$ | 115.3 ± 0.09$^{a}$ |
| Subs. Litter size | | | | | |
| Total born | 13.04 ± 0.27 | 13.38 ± 0.28 | 13.12 ± 0.27 | 13.29 ± 0.23 | 13.07 ± 0.22 |
| Born alive | 11.81 ± 0.25 | 12.49 ± 0.26 | 12.06 ± 0.25 | 12.21 ± 0.21 | 12.02 ± 0.21 |
| Mummies | 0.38 ± 0.05* | 0.26 ± 0.05* | 0.23 ± 0.05* | 0.27 ± 0.04 | 0.31 ± 0.04 |
| Born dead | 1.27 ± 0.11$^{d}$ | 0.82 ± 0.11$^{c}$ | 1.09 ± 0.11$^{cd}$ | 1.07 ± 0.09 | 1.06 ± 0.09 |

$^{ab}$within row indicate differences between season (P < 0.05); $^{cd}$ within row indicate differences between treatment (P  0.05); *within row indicates differences between treatment (P = 0.1).

The effects of diet within each season on the cumulative proportion of sows mated within 7, 14 and 21 days of weaning and those mated more than 21 days after weaning are presented in Table 5. During summer, there were no effects of diet on the timing of mating relative to weaning. However, in winter the proportion of sows mated within 7 days of weaning was lower (P<0.1) for the medium compared with high n-6 diet, and fewer (P< 0.05) sows were mated within 14 days of weaning in both the low and medium compared with high n-6 diet (Table 5). Regardless of treatment, the proportion of sows mated within 7 and 14 days of weaning was lower (P<0.05) in winter compared with summer (Table 5). Treatment and Season effects on farrowing rates are presented in Table 6. There were no treatment or season effects on farrowing rates, either as a proportion of sows mated or as a proportion of sows which were weaned. Based on farrowing rates, per 100 sows weaned, and subsequent litter size, it is clear that the medium and high n-6 diets result in more piglets being born per 100 sows weaned. However, the medium n-6 diet resulted in 32 fewer stillborn piglets per 100 sows weaned compared with the low n-6 diet and 26 fewer stillborn piglets per 100 sows weaned compared with the high n-6 diet (Table 6).

Total litter sizes were smaller (P < 0.047) when the proportion of females in the litter were high (≥ 0.60) compared with a low (≤ 0.40) or normal (0.41 – 0.59) (12.26 ± 0.44 versus 13.47 ± 0.28 and 13.40 ± 0.23 piglets). The number of live born piglets was similar regardless of the proportion of females present in the litter (Table 7); however, there were fewer (P < 0.001) still born piglets in litters with a high compared with a normal and low number of females (0.47 ± 0.19 versus 0.98 ± 0.10 and 1.34 ± 0.12 piglets). The number of mummified fetuses produced was unaffected by the sex ratio of the litter (P = 0.344). Interestingly, there was a tendency (P = 0.082) for previous weaning to service intervals to differ between sows which produced litters with a high (5.90 ± 0.64), a normal (7.19 ± 0.33) or low (6.23 ± 0.41) proportion of females.

*Table 4 Effect of treatment (low versus medium versus high n-6 PUFA levels) and season (summer versus winter) on the proportion of females present in the subsequent litter*

| Dietary Treatment | Low n-6 | | Medium n - 6 | | High n - 6 | |
|---|---|---|---|---|---|---|
| Season | Summer | Winter | Summer | Winter | Summer | Winter |
| Prop. female piglets/litter[#] | 0.45±0.02* | 0.42±0.02* | 0.41±0.02* | 0.46±0.02* | 0.45±0.02* | 0.44±0.02* |

*P=0.09; [#]calculated as a proportion of total born

*Table 5 Effect of treatment (low versus medium versus high n-6 PUFA levels) and season (summer versus winter) on the cumulative proportion of sows mated after weaning*

| Season | Dietary Treatment | Cumulative proportion of sows re-mated post weaning | | | |
|---|---|---|---|---|---|
| | | < 7 days | 8 – 14 days | 15 – 21 days | Total |
| Summer | Low n-6 | 0.83[b] | 0.87[b] | 0.92 | 0.97 |
| Summer | Medium n-6 | 0.85[b] | 0.89[b] | 0.94 | 1.00 |
| Summer | High n-6 | 0.83[b] | 0.88[b] | 0.95 | 1.00 |
| Winter | Low n-6 | 0.61[a] | 0.71[a] | 0.88 | 0.97 |
| Winter | Medium n-6 | 0.60[a,*] | 0.68[a] | 0.85 | 0.96 |
| Winter | High n-6 | 0.71[a,*] | 0.82[ab] | 0.94 | 0.99 |
| *Combined across treatment* | | | | | |
| Summer | Combined | 0.84[#] | 0.88[#] | 0.94 | 0.99 |
| Winter | Combined | 0.64[#] | 0.74[#] | 0.91 | 0.97 |
| *Combined across season* | | | | | |
| Combined | Low n-6 | 0.71 | 0.78 | 0.90 | 0.97 |
| Combined | Medium n-6 | 0.72 | 0.79 | 0.89 | 0.98 |
| Combined | High n-6 | 0.77 | 0.85 | 0.94 | 0.99 |

*[ab] within column indicates differences between treatments across seasons; P < 0.05*
**Within column and season indicates treatment differences; *P < 0.1*
*# within column indicates differences between seasons; P < 0.05*

### ***5.3. Blood metabolites***

There was no effect of season (summer versus winter) on NEFA (0.068 ± 0.01 and 0.086 ± 0.009 nmol/l; P = 0.276) or PUN (7.06 ± 0.26 and 7.00 ± 0.16 nmol/l; P = 0.969). Dietary treatment had no effect on NEFA: 0.071 ± 0.016 (low n-6), 0.088 ± 0.012 (medium n-6) and 0.081 ± 0.012 (high n-6) (P = 0.641). However, there was a tendency (P = 0.09) for PUN to be affected by treatment; 6.72 ± 0.22 nmol/l (High n-6), 7.02 ± 0.23 nmol/l (medium n-6), and 7.45 ± 0.28 nmol/l (low n-6). NEFA levels were 0.070 ± 0.014, 0.076 ± 0.011 and 0.10 ± 0.015 nmol/l for parity 1 and 2 sows (young), parity 3 and 4 sows (middle) and parity 5 plus (old) sows (P = 0.268). However, old sows had lower PUN than both young and middle aged sows: 6.23 ± 0.26 versus 7.33 ± 0.25 and 7.28 ± 0.19 nmol/l (P < 0.01).

PGFM levels were similar during summer and winter (322.3 ± 59.21 and 258.2 ± 39.01 pg/ml, respectively), and were numerically, but not significantly, higher (P = 0.567) in the high n-6 (302.1 ± 52.68 pg/ml) and medium n-6 (297.5 ± 53.53 pg / ml) fatty acid diets compared with the low n-6 fatty acid diet (222.5 ± 63.82 pg/ml).

*Table 6 Effect of treatment (low versus medium versus high n-6 PUFA levels) and season (summer versus winter) on farrowing rates (as a % of sows weaned and a % of sows mated), subsequent litter size, and the number of piglets born per 100 sows weaned*

| Season | Dietary Treatment | Farrowing rate | | Subsequent Litter size | | | Piglets born per 100 sows weaned | | |
|---|---|---|---|---|---|---|---|---|---|
| | | % sows Weaned | % sows Mated | Total | Born alive | Born dead | Total | Born alive | Born dead |
| Summer | Low n-6 | 78.6% | 78.6% | 13.31 ± 0.40 | 12.05 ± 0.37 | 1.26 ± 0.16 | 1046.2 | 947.1 | 99.0 |
| Summer | Medium n-6 | 83.5% | 83.5% | 13.25 ± 0.39 | 12.33 ± 0.36 | 0.75 ± 0.15 | 1106.4 | 1029.6 | 62.6 |
| Summer | High n-6 | 86.3% | 86.3% | 13.32 ± 0.39 | 12.26 ± 0.36 | 1.06 ± 0.15 | 1149.5 | 1058.0 | 91.5 |
| Winter | Low n-6 | 81.5% | 84.3% | 12.78 ± 0.37 | 11.58 ± 0.35 | 1.28 ± 0.15 | 1041.6 | 943.8 | 104.3 |
| Winter | Medium n-6 | 84.6% | 88.0% | 13.52 ± 0.40 | 12.64 ± 0.38 | 0.90 ± 0.16 | 1143.8 | 1069.3 | 76.1 |
| Winter | High n-6 | 88.1% | 88.9% | 12.93 ± 0.37 | 11.87 ± 0.34 | 1.13 ± 0.15 | 1139.1 | 1045.7 | 99.6 |
| ***Combined across treatment*** | | | | | | | | | |
| Summer | Combined | 82.8% | 83.6% | 13.29 ± 0.23 | 12.21 ± 0.21 | 1.11 ± 0.09 | 1100.4 | 1011.0 | 91.9 |
| Winter | Combined | 84.6% | 87.0% | 13.07 ± 0.22 | 12.02 ± 0.21 | 1.03 ± 0.09 | 1105.7 | 1016.9 | 87.9 |
| ***Combined across season*** | | | | | | | | | |
| Combined | Low n-6 | 80.2% | 82.4% | 13.04 ± 0.27 | 11.81 ± 0.25 | 1.27 ± 0.11$^{d}$ | 1045.8 | 947.2 | 101.9 |
| Combined | Medium n-6 | 84.1% | 85.7% | 13.39 ± 0.28 | 12.49 ± 0.25 | 0.82 ± 0.11$^{c}$ | 1126.1 | 1050.4 | 69.0 |
| Combined | High n - 6 | 87.2% | 87.6% | 13.12 ± 0.27 | 12.06 ± 0.25 | 1.10 ± 0.11$^{cd}$ | 1144.1 | 1051.6 | 95.0 |

$^{cd}$ within column row indicate differences between treatment; $P < 0.05$

*Table 7 Effect of gender distribution within the litter on the size of the litter (total born, born alive, born dead and mummifies fetuses)*

| Proportion females per litter | Litter size | | | |
|---|---|---|---|---|
| | Total born | Born alive | Born Dead | Mummies |
| Low (< 40%)<br>(n = 156 litters) | 13.47 ± 0.28[a] | 12.13 ± 0.27 | 1.34 ± 0.12[d] | 0.34 ± 0.06 |
| Medium (41 – 60%)<br>(n = 229 litters) | 13.40 ± 0.23[a] | 12.43 ± 0.22 | 0.98 ± 0.98[d] | 0.29 ± 0.05 |
| High (> 60 %)<br>(n = 60 litters) | 12.26 ± 0.44[b] | 11.79 ± 0.42 | 0.47 ± 0.10[c] | 0.19 ± 0.09 |

Different superscripts within column indicate significant differences; [ab] $P < 0.05$; [cd] $P < 0.001$

## 6. Discussion

The current data demonstrate that altering levels of n-6 and n-3 polyunsaturated fatty acids, and the ratio of these two fatty acids, in sow lactation diets can affect day 21 litter size, individual piglet weight at day 21 and post-weaning reproductive performance of the sows. Based on a series of dose response studies, Rosero et al. (2016) concluded that sow reproductive efficiency (based on interval between weaning and farrowing and litter size) was maximised when sows receive at least 10 g / day α-linolenic (n-3 PUFA) acid and a minimum of 100 g / day of α-linoleic acid (n-6 PUFA) daily during lactation. This study, and the diets used, were designed to determine whether this is also true under Australian conditions. Based on an estimated daily intake of 6 or 7 kg / day during lactation, it is estimated that intakes of n-3 PUFA (primarily α-linolenic acid) were less than 10 g / day for both the low and high n-6 diet, with levels exceeding 10 g / day for the medium n-6 diet. Importantly, levels of n-6 PUFA (primarily α-linoleic acid) were < 80 g / day for the low n-6 diet, but exceeded 100 g per day for the medium n-6 diet and high n-6 diet. Based on this, the medium n- 6 diet is the only one which meets the minimum daily n-3 PUFA and n-6 PUFA requirements to maximise subsequent reproductive performance (as described by Rosero et al., 2016). Overall, the current data demonstrated that feeding sows a low n-3 and low n-6 diet during lactation resulted in poorer subsequent reproductive performance compared with the other two diets, and that this was the case regardless of season.

The current data demonstrated that feeding the medium n-6 diet during lactation resulted in fewer still born piglets at the subsequent farrowing compared with the low n-6 diet (0.4 fewer stillbirths / litter). Interestingly, there was also a tendency for the medium and high n-6 PUFA diets to reduce the number of mummified fetuses produced at the subsequent litter. Interestingly, there were no dietary effects on the mean weaning to service interval or farrowing rates. Subsequent reproductive performance figures (proportion sows mated post-weaning, pregnancy rates and subsequent litter size) were used together to create an estimate of reproductive efficiency similar to that used by Rosero et al. (2016). Based on this estimate of reproductive efficiency, it is evident that feeding the medium and high n-6 PUFA diets (which provided more than 100 g / day of α-linoleic acid daily during lactation) increased the number of piglets born per 100 sows weaned. However, feeding the diet which met the minimum requirement of 10 g / day of α-linolenic acid (Rosero et al., 2016; namely the medium n-6 diet) reduced the number of stillborn piglets produced per 100 sows weaned.

The finding that feeding the medium n-6 diet reduced subsequent stillbirth rates, and tended to reduce the number of mummified fetuses delivered is an interesting one. This finding appears to support previous evidence, and suggestions, that increased dietary intake of α-linoleic and α-linolenic acid has the potential to promote uterine recovery post-partum as well as oocyte and embryo development potential (Robinson et al., 2002; Wathes et al., 2007; Lopes et al., 2009; Rosero et al., 2016). The mechanism whereby pre-ovulatory fatty acid intake could affect post-implantation conceptus death (i.e. mummies) and intra-partum deaths (stillbirths) is unclear. However, nutrition during the period when cumulus-oocyte complexes are maturing prior to ovulation are thought to affect not only early embryo development and survival, but also fetal growth (Thompson, 2006). Porcine oocytes have a high lipid content, consisting of both neutral lipids and fatty acids, and these fatty acids play an integral role during nuclear and cytoplasmic maturation of the oocyte. Within the oocyte, lipid composition and fatty acid metabolism are affected by the nutritional intake of the sow (Dunning et al., 2014), with fatty acids acting as a vital energy substrate for the maturing oocyte and developing embryo (Sturney et al., 2009). Maternal intake of n-3 and n-6 PUFAs affected levels in ovine granulosa cells, oocyte and embryos, suggesting that dietary n-3 and n-6 PUFA intake has the capacity to affect ovarian steroidogenesis and embryo development prior to implantation (Wonnacott et al., 2010). It is, therefore, conceivable that altering PUFA intake of sows prior to ovulation may affect oocyte development, early embryo development and potentially fetal development which could in turn account for the differences observed in the incidence of mummified fetuses and stillborns. Certainly, maternal PUFA intake affects pre-natal brain development and has been shown to affect neonatal vitality and viability in a range of species (pigs, sheep and ruminants). However, the capacity of lipid content and fatty acid metabolism of the oocyte to affect neonatal brain development and capacity to cope with intra-partum hypoxia has not previously been reported. However, previous work in sheep (Clayton et al., 2016, 2017a and b) indicates that the fatty acid (n-6: n-3 PUFA ratio) content of diets fed to ewes prior to mating affected the reproductive performance of their progeny, as well as their response to diets containing different levels of n-6 and n-3 PUFAs. This finding appears to support the notion that maternal intake of n-3 and n-6 PUFA prior to ovulation may have long term effects on fetal growth and development. Therefore further work is required to confirm the current finding that the medium n-6 diet reduced stillbirths, and to determine what the mechanisms are. It is also interesting that this reduction in still births occurred despite a numerical increase in total litter size, something which would, normally, be expected to increase stillbirth rates.

In the current study, subsequent reproductive performance was not depressed in sows which lactated during summer compared with winter, which is somewhat unexpected. However, this is consistent with previous studies conducted at these facilities. In contrast to the expectation, but again consistent with previous data, sows which lactated during summer were re-mated earlier post-weaning compared to those which lactated during winter, with subsequent litter size unaffected by season. This outcome can be attributed to the fact that sows were heavier immediately post-farrowing and at weaning during summer compared with winter, and lost less weight during lactation, all of which is consistent with improved subsequent reproduction.

Although not significantly different, the current finding that increasing the n-6 PUFA content of lactating sow diets increased circulating PGFM levels is consistent with previous reports in lactating cattle (eg Juchem et al., 2010; Dirandeh et al., 2013). Linoleic acid and alpha linolenic acid are essential precursors for the biosynthesis of the series 2 prostaglandins ($PGE_2$ and $PGF_{2\alpha}$) and the series 3 prostaglandins ($PGE_3$ and $PGF_{3\alpha}$), respectively. Dietary omega 6 supplementation resulted in more rapid involution of the uterus post-partum, as well as increased PGFM release in response to an oxytocin challenge (Dirandeh et al., 2013). Delayed uterine involution has been associated with extended and difficult parturitions in sows (Bjorkman et al., 2018), and extended parturitions have also been associated with reduced conception rates post-weaning (Peltoniemi et al., 2016). Increased PGF production in response to suckling induced oxytocin release is likely to facilitate more rapid post-partum repair of the uterus, suggesting that supplementing lactating diets with n-6 PUFA may promote uterine repair and thus, potentially, improve subsequent reproductive performance. Increased n-6 PUFA intake has the potential to increase biosynthesis of the luteotrophic factor $PGE_2$, as well as $PGF_{2\alpha,}$ which recent evidence suggests may also promote implantation (Waclowik et al., 2017). Therefore, future studies are required to determine the impact of n-6 PUFA intake alone, and in conjunction with n-3 PUFA intake, on prostaglandin release and post-partum uterine repair.

The secondary objective of this study was to determine whether increasing the n-6 PUFA content of sows diets pre-ovulation would increase the proportion of females born in the litter. However, no dietary effects on the sex distribution of the litter were observed. Previous studies in sheep (Clayton et al., 2016) and Mice (Fountain et al., 2008) demonstrated females which consumed diets with high levels of n – 6 PUFA gave birth to litters containing a higher proportion of female offspring compared to those fed a diet containing high levels of n-3 PUFAs. It is worth noting that the levels of n-6 PUFA contained in the ewe, and mice, studies were substantially higher than those used in the current study. The ewes were fed diets containing between 32% and 36% linoleic acid and the mice were fed diets containing 4.5% n-6 PUFAs. Due to the commercial nature of the current trial these levels were not achieved. However, considering the economic benefits of increasing the proportion of female progeny born it is suggested that intensive trials are conducted to determine whether substantially higher levels of n-6 PUFAs in pre-ovulation and peri-implantation diets can increase the incidences of females born.

The potential benefits of being able to increase the proportion of females present in the litter is also evident from the current finding that in female biased litters fewer piglets die during parturition (i.e. stillbirth rates are lower). Specifically, on average the incidence of stillbirths was only 0.5 of a pig in female biased litters compared to 1 piglet and 1.5 piglets in non-biased and male biased litters. Although total litter size was lower in female biased compared to non-biased and male biased litters, this was accounted for in the statistical model used. Taken together, this reduction in litter size of female biased litters and apparent reduction in the susceptibility of female piglets to succumb to the challenges associated with parturition, suggest that preferential loss of male pigs in response to adverse environmental conditions may be one mechanism whereby sex bias is determined in swine litters.

In conclusion, the current data provide preliminary evidence that feeding diets with low n-3 and low n-6 PUFA content during lactation is detrimental to sow reproductive performance. Although preliminary, the current study also indicates that feeding a diet containing medium levels of n-6 PUFA and high levels of n-3 PUFA may increase subsequent reproductive output, and decrease incidence of still births. There is also evidence that increasing n-6 PUFA intake may increase prostaglandin release, which may promote post-partum uterine repair. Further work is required to determine whether the beneficial effect of this diet is observed in other genotypes, promotes uterine repair following parturition and results in greater viability and vigour of the piglets produced.

## 7. Implications & Recommendations

Feeding low n-3 (0.13%) and low n-6 (1.1 – 1.2% PUFA) diets during lactation reduced subsequent reproductive output

Feeding diets containing 0.27% n-3 PUFA and 1.85-1.99% n-6 PUFA during lactation (the medium n-6 diet) tended to reduce incidences of post-implantation fetal death (as evidenced by reduced mummies) and significantly reduced the incidence of stillbirths produced at the subsequent litter

Feeding diets containing 0.13% n-3 PUFA and 2.55 – 2.70% n-6 PUFA during lactation (high n-6 diet) tended to reduce incidences of post-implantation fetal death (as evidenced by reduced mummies) and increase the proportion of sows returning to oestrus within 7 days of weaning during winter

The proportion of females born in the subsequent litter was unaffected by diet; however, due to the commercial nature of the trial we were unable to replicate the high levels of n-6 PUFA found to increase the proportion of females born in sheep and mice

Based on these data, it is suggested that future work is warranted in four areas

- To determine whether the positive effect of the medium n-6 diet prior to ovulation on stillbirth rates is repeated across other genotypes, and results in increased piglet viability at, and immediately after birth and through to weaning

- To determine the effect of the medium and high n-6 diets on ovarian function, oocyte quality, embryo – placental development and uterine function.

- To determine the effect of feeding the medium and high n-6 from parturition to day 30 of pregnancy on prostaglandin release, uterine involution, reproductive outcomes, as well as the physiology of the sow

- To determine the physiological mechanisms responsible for determining sex bias in swine litters

- To conduct smaller scale studies under intensive conditions to determine the effect of exceptionally high n-6 PUFA dietary levels on the sex distribution of swine litter.

## 8. Summary Snapshot

Dietary intake of polyunsaturated fatty acids (PUFAs) prior to ovulation can profoundly influence reproductive function and, therefore, reproductive output of female pigs. This study determined the effect of three dietary intakes of PUFAs during lactation on sow reproduction during summer and winter. The diets were: One (0.13% n-3 PUFA/1.10-1.18% n-6 PUFA); Two (0.27% n-3 PUFA / 1.85 – 1.99% n-6 PUFA); and, Three (0.14% n-3 PUFA / 2.56-2.7% n-6 PUFA). The main outcomes were:

- Feeding diet one resulted in more stillbirths and more mummified fetuses, and resulted in 1046 (total born), 947 liveborn and 102 stillborn piglets per 100 sows weaned

- Feeding diet two reduced the number of stillborn piglets, tended to reduce the number of mummies produced, and resulted in 1126 (total born), 1050 liveborn and 69 stillborn piglets per 100 sows weaned

- Feeding diet three reduced the number of stillborn piglets, tended to reduce the number of mummies produced, and resulted in 1144 (total born), 1052 liveborn and 95 stillborn piglets per 100 sows weaned

Simple catchy title: Sow fatty acid intake affects reproduction

Purpose:

- Is subsequent reproductive performance affected by feeding different levels of n-3 and n-6 PUFAs during lactation

Take home messages –

- Stillbirth rates are higher, and reproductive efficiency lower, when sows are fed a lactation diet containing low levels of n-3 and n- 6 PUFAs
- Stillbirth rates are lowest, and reproductive efficiency high, when sows are fed a lactation diet containing medium levels of n-3 and n-6 PUFAs
- Based on this study, and American data, lactation diets containing medium levels of n-3 and n-6 PUFA promote reproductive efficiency

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## 10. Appendix: Diet specifications and ingredient

| Component | Units | Low n-6 diet | Medium n-6 diet | High n-6 diet |
|---|---|---|---|---|
| Dry Matter | % | 89.95 | 89.9 | 89.9 |
| Protein | % | 20.74 | 20.47 | 20.47 |
| Crude Fibre | % | 4.2 | 4.23 | 4.23 |
| NDF | % | 0.475 | 0.24 | 0.24 |
| DE | MJ / kg | 14.25 | 14.26 | 12.26 |
| Cystine | % | 0.34 | 0.33 | 0.33 |
| Leucine | % | 1.52 | 1.5 | 1.5 |
| Isoleucine | % | 0.73 | 0.72 | 0.72 |
| Lysine | % | 1.23 | 1.23 | 1.23 |
| Methionine | % | 0.36 | 0.36 | 0.36 |
| Threonine | % | 0.82 | 0.82 | 0.82 |
| Trytophan | % | 0.23 | 0.23 | 0.23 |
| Valine | % | 1.04 | 1.03 | 1.03 |
| M + C | % | 0.71 | 0.7 | 0.7 |
| Available Lysine | % | 1 | 1 | 1 |
| Ash | % | 5.29 | 5.33 | 5.33 |
| Calcium | % | 0.95 | 0.98 | 0.98 |
| Phosphorous | % | 0.75 | 0.74 | 0.74 |
| Available Phosphorous | % | 0.56 | 0.56 | 0.56 |
| Sodium | % | 0.18 | 0.19 | 0.19 |
| Salt | % | 0.45 | 0.5 | 0.5 |
| Choline | mg/kg | 2707.02 | 2757.43 | 2697.43 |
| Ingredients | | | | |
| Barley | % | 8 | 10.75 | 10.75 |
| Wheat | % | 35.31 | 36.125 | 36.125 |
| Groats | % | 5 | 2.5 | 2.5 |
| Millrun | % | 12.65 | 12.5 | 12.5 |
| Peas-Field | % | 14 | 13.4 | 13.4 |
| Soybean Meal | % | 9 | 8.6 | 8.6 |
| Blood Meal | % | 2.2 | 2.2 | 2.2 |
| Meat Meal | % | 8 | 8 | 8 |
| Tallow | % | 4.1 | - | - |
| Canola Oil | % | - | 1.75 | - |
| Soybean Oil | % | - | 2.25 | - |
| Sunflower Oil | % | - | - | 4 |
| Limestone Fine | % | 0.1 | 0.19 | 0.19 |
| Salt | % | 0.25 | 0.3 | 0.3 |
| Potassium Chloride | % | 0.2 | 0.2 | 0.2 |
| Betaine (VistaBet) Liq 32% | % | 0.6 | 0.6 | 0.6 |
| DL Methionine | % | 0.08 | 0.08 | 0.08 |
| Lysine HCL | % | 0.12 | 0.15 | 0.15 |
| L-Threonine | % | 0.085 | 0.095 | 0.095 |
| L-Tryptophan | % | 0.015 | 0.02 | 0.02 |
| Natupos E Blend UE 150G Sunpork | % | 0.015 | 0.015 | 0.015 |

| | | | | |
|---|---|---|---|---|
| BioFix MycoFix Plus (Biomin) | % | 0.075 | 0.075 | 0.075 |
| Sunpork Breeder Premix | % | 0.2 | 0.2 | 0.2 |