LOW DOSE DIETARY STRATEGIES IN LATE GESTATION TO ENHANCE BORN ALIVE AND PIGLET SURVIVAL AND PERFORMANCE

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By

Dr Jessica Craig

c/o Rivalea (Australia) Pty Ltd
Redlands Road
Corowa NSW, 2646

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Australasian
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APRIL

Executive Summary

The current project investigated three novel feed additives associated with (or having a similar action to) arginine and/or leucine, on litter characteristics at birth when fed from day 90 of gestation until farrowing. These novel materials were ß-hydroxy ß-methyl butyrate (HMB), N-carbamylglutamate (NCG), and calcium nitrate ($Ca[NO_3]_2$; CAN), fed at low doses in the late gestation diet. These additives were selected for this project based on a review of the literature on late gestation feed additives aimed at improving the birth weight and pre-weaning survival of piglets. Arginine, HMB, NCG, and CAN have all been reported to improve piglet vitality at birth and possibly stillbirth rate through enhanced placental efficiency and piglet viability (CAN), and/or increased piglet birth weights (arginine, HMB, and NCG), most likely as a result of improved oxidative status and enhanced placental blood flow in late gestation (Flummer et al., 2012b; van den Bosch et al., 2019a,b; Wu et al., 2012). Most previous studies investigating these additives, especially NCG and CAN, have been proof-of-concept using limited numbers of sows (<20 sows per treatment), and to our knowledge there have been no studies published that compare all three additives to L-arginine supplementation.

Therefore, the aims of the current project were:

1. To evaluate the effects of supplementation of 0.5% L-arginine and three novel feed additives ß-hydroxy ß-methyl butyrate (HMB), N-carbamylglutamate (NCG), and calcium nitrate $Ca(NO_3)_2$ (CAN) on litter characteristics at birth when fed from day 90 of gestation;
2. To assess each treatment for piglet vitality, number of still born, number born alive and weaned as well as subsequent reproductive performance of all sows; and,
3. To provide the industry with effective strategies for improving the efficiency of reproduction and progeny performance.

Five hundred and thirty-seven mixed parity multiparous sows (parity 2-7, ave. 3.8 ± 2.3 SD) were allocated across five dietary treatments commencing at day 90 of gestation. These sows were allocated from 4 weeks of matings at the Module 1 facility in Corowa NSW, with each week representing a time replicate and containing five group housed gestation pens, with one dietary treatment fed per pen. The dietary treatments consisted of: a control diet (CON); a 0.5% Arg diet (ARG); a 0.15% HMB diet (HMB); a 0.15% NCG diet (NCG); and a 0.1% $Ca(NO_3)_2$ diet (CAN). Total piglets born (TB) and born alive (BA), number of stillborn piglets, individual and litter birth weight and litter weight at day 7 and at 25 days of lactation, and

litter survival rate during lactation were recorded for each litter, as well as subsequent reproductive performance for each sow.

There was a significant difference in sow P2 at day 90 of gestation between the dietary treatments, before the beginning of supplementation ($P = 0.041$). Sows in the NCG group had the highest P2 backfat at this stage (21.0 ± 0.5 mm), being significantly fatter than HMB (19.7 ± 0.5 mm) or ARG sows (19.0 ± 0.5 mm; $P < 0.05$). However, this was not expected to significantly impact the other variables studied. Both TB and BA were not significantly different between dietary treatments ($P = 0.99$ and $P = 0.88$, respectively). Similarly, there was no difference in total number of stillborn piglets per litter ($P = 0.65$) or the proportion of stillborn piglets ($P = 0.79$) between dietary treatments.

Diet tended to influence litter weight at birth of all pigs ($P = 0.083$) but had no overall effect on the total litter weight of live pigs ($P = 0.17$). However, CAN sows had significantly higher litter weights of all pigs (19.3 ± 0.4 kg; $P = 0.040$) than ARG sows (17.5 ± 0.5 kg). There was no difference ($P > 0.10$) in litter weight at day 7 and 25 of lactation between dietary treatments. There was no difference in litter number post-foster ($P = 0.17$) or at day 7 of lactation ($P = 0.25$) but a significant ($P = 0.041$) difference at day 25, with HMB and CAN litters having less pigs per litter at this stage of lactation than the other treatments. Piglets born alive from CAN sows had the heaviest average weight at birth (overall diet effect $P = 0.061$), which was significantly heavier than that of ARG sows ($P = 0.044$), but similar to that of CON sows ($P = 1.00$). There was no difference ($P = 0.79$) in birth weight coefficient of variation (CV) between dietary treatments. At day 25 of lactation, average weight of piglets born to HMB sows was the heaviest (overall dietary treatment $P = 0.042$), tending to be heavier than that of CON ($P = 0.053$) sows, and piglets born to NCG sow tended to be heavier ($P = 0.086$) than those of ARG sows. Post-foster mortality was lowest in NCG and CAN piglets ($P = 0.028$), and CAN sows had significantly less piglets born <1.1 kg than the other treatments ($P < 0.001$). There was little impact of diet on subsequent reproductive performance of sows. From these results and the subsequent cost benefit analysis, HMB and CAN were determined to be the most cost-effective supplemental feed ingredients.

In conclusion, supplementation of CAN to sows in late gestation may improve weight of piglets at birth, reduce the proportion of piglets born <1.1 kg, and improve their pre-weaning survival chance. Supplementation of HMB may also improve the growth performance of piglets to weaning (25 days of age). Therefore, these two additives show the most promise as alternatives to supplementary l-arginine in gestating sow diets.

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1. Introduction

Increasing litter size in Australia (and globally) is associated with reductions in piglet birth weights, and an increased stillbirth rate. As litter size has increased in hyper-prolific sows overseas, so do concerns of declining piglet birth weight and survival (Foxcroft et al., 2009; Rekiel et al., 2014). Low piglet birth weight also has negative whole of life consequences. A number of specific feed additives for the sow in late gestation have been investigated for their role in improving piglet birth weight and vitality at birth, and hence their impact on piglet survival, but often with mixed results.

Recently there has been an increased interest in the use of L-arginine in sow gestation diets for its positive impacts on performance (Che et al., 2013; Mateo et al., 2007; Mateo et al., 2008), largely due to being a precursor of nitric oxide (NO) and polyamines, which exert an antioxidant effect in the body (Costa et al., 2019; Kim et al., 2007; Tan et al., 2010; Wu et al., 1998). Arginine and the products of its metabolism are involved in cellular growth and production (Ishida et al., 2002; Meininger et al., 2002), ovulation and implantation of embryos (Zeng et al., 2013), regulation of placental blood flow (Bird et al., 2003; Wu et al., 2006; Wu et al., 2010a), and regulation of hormones such as insulin and growth hormone (Alba-Roth et al., 1988; Chew et al., 1984; Davenport et al., 1995; Flynn et al., 2002).

Recent results suggest that at 0.5% L-arginine fed in late gestation can enhance birth weight and piglet survival during farrowing (Nuntapaitoon et al., 2018). Other authors have reported improvements in litter birth weight (Gao et al., 2012; Mateo et al., 2007; Wu et al., 2010a), litter weight gain during lactation and hence weaning weight (Hong et al., 2020; Mateo et al., 2008; Oksbjerg et al., 2019) and lower variation in birth weights due to a reduction in the proportion of low birth weight piglets (Dallanora et al., 2017; Hong et al., 2020; Quesnel et al., 2014) when L-arginine was fed in gestation and/or lactation. Improvements in birth weights and variation in birth weight may be due to an increase in placental weight (Gao et al., 2012; Wu et al., 2018).

Despite the number of advantages of supplemental L-arginine shown in the literature, widespread adoption is limited due to the significant cost of the additive (Hu et al., 2019; Wu et al., 2007) and required supplementation levels are often quite high (Luise et al., 2020). Therefore, there is a need for suitable additives with a similar mode of action to be investigated as alternatives to L-arginine in sow gestation diets. A few such additives that have been investigated recently for their potential to replace L-arginine in sows diets, but that still remain to be fully developed as feed additives, are ß-hydroxy-ß-methyl butyrate (HMB), N-carbamylglutamate (NCG), and calcium nitrate ($Ca(NO_3)_2$; CAN).

ß-hydroxy-ß-methyl butyrate (HMB) is a metabolite of leucine and appears to be a potent stimulator of protein synthesis, which has been reported to enhance piglet birth weight (Krakowski et al., 2002; Tatara et al., 2007; Wan et al., 2016) and colostrum yield when supplemented to sows in late gestation (Flummer et al., 2012b). In humans, HMB is classed as an ergogenic acid and is used widely by sportspeople to increase muscle mass, regenerate muscle cells, and for a range of other health benefits (reviewed by Wilson et al., 2008). Naturally occurring HMB is found in small amounts in some foods such as catfish, alfalfa, grapefruit, cauliflower, asparagus, corn and avocado (Muszyński et al., 2016; Szcześniak et al., 2015). Along with leucine and its other metabolite α-ketoisocaproate (KIC), HMB (both through the diet and exogenously produced) activates mTOR signalling pathways, playing a role in cell metabolism, growth and proliferation (Cieslak et al., 2018; Duan et al., 2016).

N-carbamylglutamate (NCG) is a precursor of arginine and has been reported to markedly enhance milk production in lactating goats (Wang et al., 2019) and endogenous synthesis of arginine in piglets (Frank et al., 2007). It has also been reported to improve piglet survival when added to late gestation sow diets (Wu et al., 2012) and to increase piglet birth weights, sow milk production, and piglet growth rates (Feng et al., 2018; Liu et al., 2012). Supplementation of NCG in pigs may also positively impact pork quality (Ye et al., 2017). A common feed additive used in China, NCG has been shown to have numerous health and reproductive benefits, improving vascular function, influencing hormone status, antioxidant effects on several tissues, and promoting organ development (reviewed by Hu et al., 2019).

There are few papers that have investigated the impacts of $Ca(NO_3)_2$ supplementation in late gestation, but results may indicate that this additive could also be used to improve piglet vitality at birth (van den Bosch et al., 2019a,b). These authors investigated this feed additive as, like HMB, it has been used extensively in human health to improve exercise performance (Jones, 2014; Omar et al., 2016) and nitrate supplementation is often used in ruminants to mitigate methane production and improve performance (Sun et al., 2017). In these seminal pig studies, piglet vitality increased linearly with $Ca(NO_3)_2$ inclusion rate, which appears to be an impact of increased NO levels playing a role in vasculogenesis and/or angiogenesis within the placenta. Supplementation of CAN appears to be a more cost-effective means of increasing NO levels and piglet vitality than supplementing L-arginine.

Overall, given the results of previous studies, these three novel feed additives would seem ideal for enhancing foetal growth in late gestation, as alternatives for L-arginine supplementation in sows. This project aimed to investigate the effects of supplementation of 0.5% L-arginine, 0.15% HMB, 0.15% NCG and 0.1% CAN on sow and piglet performance. It was hypothesised that supplementation of these additives would increase piglet vitality at birth and therefore their overall performance to weaning, increasing the number and/or proportion of live piglets born and weaned. If successful at improving piglet performance (subject to APVMA approval and/or registration), these additives could easily be fed to sows in late gestation to improve overall heard productivity, and hence improve the global competitiveness of the Australian industry.

2. Methodology

All experimental procedures were approved by the Rivalea (Australia) Animal Care and Ethics Committee under protocol number 19R020C, in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council, 2013).

2.1 Experimental Design

The on-farm data collection for the experiment was conducted at Rivalea's Research and Innovation (R&I)/Module 1 facility from February to May 2020, with subsequent reproduction performance data collected from April to October 2020. A total of n = 537 sows were included in the experiment. The design was a five-treatment comparison with dietary treatments allocated based on sow parity (multiparous sows, parities 2-7), with an even spread of parities amongst dietary treatments (Appendix 1; Table S1). Allocations were also balanced for sow body weight. Sows were grouped into electronic sow feeder (ESF) pens after mating over four time replicates (mating weeks) and were housed with non-trial sows that returned to oestrus or were removed before day 90 of gestation.

Sows were fed one of five diets from day 90 of gestation until farrowing:

1. CON - A basal (control) diet (common gestation diet with no supplemental arginine added; n = 108);
2. ARG - The basal diet + 0.5% added arginine (n = 101);
3. HMB - The basal diet + 0.15% added ß-hydroxy ß-methyl butyrate (n = 107);
4. NCG - The basal diet + 0.15% N-carbamylglutamate (n = 113); or,
5. CAN – The basal diet + 0.1% $Ca(NO_3)_2$ (n = 108).

Sows entered the farrowing house at day 108 (± 1; SD) of gestation and continued on their experimental diets until the day of farrowing. Sows were then fed a common commercial lactation diet until weaning (27 ± 2 days of lactation). Daily sow feed intakes were recorded from day 90 of gestation until weaning.

Average total born (TB) and born alive (BA) per litter from previous parities were calculated for each sow from previous farm records. Sow body weight and P2 backfat thickness was recorded for each sow at day 90 of gestation, at entry to the farrowing house, and at weaning. At farrowing, date, TB, BA, number of stillborn piglets (SB) and number of mummified piglets were recorded for each sow. Piglets were individually weighed at birth (both piglets BA and SB), from which litter weight at birth and birth weight coefficient of variation (CV) were calculated. Fostering was kept to a minimum as per commercial practices, and all fosters were recorded. Number of piglets per litter after fostering was calculated and litters were weighed again on days 7 and 25 of lactation. All piglet and sow mortalities and removals were recorded throughout the experimental period. Subsequent reproduction data was collected from farm records for each sow, including wean to remate interval (WRI), farrowing outcome, gestation length, TB, BA and number weaned in the subsequent litter.

2.2 Diets and Feeding

Sows were allocated to their experimental diet on day 90 of gestation and fed these diets from then until the day of farrowing. Day 90 of gestation to farrowing was chosen as the dietary supplementation period, as this is the period that corresponds with the highest rate of growth and development of piglets *in utero* (Hułas-Stasiak et al., 2019). The composition of the experimental diets is shown in Table 1. Diets were fed on a pen basis in their gestation pens, each fitted with a single electronic sow feeder (ESF). The feed hoppers were filled with the corresponding diet at least twice daily, and sows were restrict-fed throughout gestation according to the commercial feed curves for gestating sows. Briefly, sows were given an allowance of 2.6 kg/day for the first 32 days of gestation, 2.0 kg per day in mid gestation (from the 33rd to the 89th day of gestation), and 2.3 kg/day in later gestation (from the 90th day of gestation onwards).

Table 1: Composition of the experimental diets.

Ingredient (%)	Diet CON	 ARG	 HMB	 NCG	 CAN
Wheat	40.9	40.9	40.9	40.9	40.9
Barley	40.0	40.0	40.0	40.0	40.0
Millmix	6.7	6.7	6.7	6.7	6.7
Canola meal (38% CP)	6.7	6.7	6.7	6.7	6.7
Water	1.5	1.5	1.5	1.5	1.5
Semi-refined fish oil	0.20	0.20	0.20	0.20	0.20
Tallow	1.0	1.0	1.0	1.0	1.0
Liquid betaine	0.40	0.40	0.40	0.40	0.40
Phytase	0.01	0.01	0.01	0.01	0.01
Salt	0.33	0.33	0.33	0.33	0.33
Limestone	1.0	1.0	1.0	1.0	1.0
Dicalcium phosphate	0.67	0.67	0.67	0.67	0.67
Arginine[1]		0.50			
HMB[2]			0.15		
NCG[3]				0.15	
$Ca(NO_3)_2$[4]					0.10
Lysine	0.23	0.23	0.23	0.23	0.23
Threonine	0.02	0.02	0.02	0.02	0.02
Vitamin and mineral blends	0.31	0.31	0.31	0.31	0.31
Calculated composition					
DE (MJ/kg)	13.3	13.3	13.3	13.3	13.3
Protein (%)	13.1	13.5	13.1	13.1	13.1
Fat (%)	2.8	2.8	2.8	2.8	2.8
Fibre (%)	4.5	4.5	4.5	4.5	4.5
Avail. lysine (%)	0.57	0.57	0.57	0.57	0.57

[1] L-arginine powder (ARG; Hebei Huayang Biological Technology Co. Ltd., Hengshui City, China).

[2] β-hydroxy β-methyl butyrate (HMB) as a calcium salt (Ca-HMB; Awell Ingredients Co. Ltd., Hefei, China).

[3] N-Carbamylglutamate (NCG; Beijing Animore Sci & Tech Co. Ltd., Beijing, China).

[4] Calcium nitrate ($Ca[NO_3]_2$; Ixom, East Melbourne, Vic, Australia).

When sows entered the farrowing house, they were fed 3 kg of lactation feed per day up until the morning before farrowing, and *ad libitum* access to lactation feed thereafter, from the morning after farrowing until weaning. Lactation feed intakes were calculated including the feeding in the morning that the sows farrowed and were included in the data for all sows that remained on trial through the entirety of their lactation. Average daily feed intake (ADFI) was calculated for day 90 until entry to the farrowing house, entry to the farrowing house until farrowing, and farrowing to weaning (lactation ADFI).

2.3 Sow and Piglet Management

Sows and piglets were housed under commercial conditions at the R&I/Module 1 facility. Each gestation pen was fitted with an electronic sow feeder (ESF) and sows were housed in groups of 40. Feeders were checked daily for blockages and sows that had not entered the feeder in >1 day. Sows remained in their static groups and home pens for the whole length of gestation until farrowing house entry. Sows were removed from the pen as they were detected as being not in pig, returning to oestrus, or of ill health.

Sows farrowed over three separate farrowing sheds with a similar floor plan and crate design; conventional farrowing crates, each fitted with a drinker for the sow and the piglets, a heat lamp, and a creep mat. Fostering was conducted within 12 to 24 hours of farrowing and minimal fostering was conducted, within treatment where possible. Piglets were tailed at 3 days of age using a cauterising iron and were given an injection of 2 mL of supplemental Fe (Uniferon® 200; Abbey Animal Health Pty Ltd, Glendale NSW). Piglets were weaned when they were approximately x days of age.

2.4 Statistical Analysis

Continuous variables were analysed as a linear mixed model using the MIXED procedure of SPSS statistical software (version 25; IBM, Armonk NY, USA). Diet was fitted as a fixed factor, with replicate (mating week) fitted as a blocking factor. Random effects and covariates were tested and added into the model where appropriate and those that were included in the models herein (where they made a significant contribution to the model) are indicated in the Outcomes section. Sows were grouped into parity groupings based on the parity of the sow on day 90 of gestation, with the groups being parities 1 and 2 (1st group; n = 198), 3 and 4 (2nd group; n = 142), 5 and 6 (3rd group; n = 126), and 7 and above (4th group; n = 68). To examine effects of sow parity and its interaction with dietary treatment, parity group was tested as a fixed factor, and parity group and the interaction term between diet and parity were included when parity group made a significant contribution ($P < 0.05$) to the model. Average daily gain (ADG) from day 0 until day 25 of lactation was calculated for each litter where no fostering was conducted. This parameter was not able to be calculated for other litters, as fostered piglets were not individually weighed and hence a post-foster litter weight was not able to be obtained.

A subsequent analysis was carried out with total born group (TB_GRP), with sows grouped as either having a TB of ≤ 12 (n = 147), 13-14 (n = 108), 15-16 (n = 139) or ≥ 17 (n = 115; see Appendix 1, Table S2). Data were further analysed within the TB ≥ 17 group only with the simple model (diet as a fixed factor, blocked by replicate). These analyses were conducted to see whether diet effects could be seen within a certain group of sows with a certain litter size (particularly the higher litter size sows) as the work of other authors has shown that uterine blood flow to the placenta decreases with increases in litter size (Père et al., 2000) and hence these litters have higher variations in birth weight of piglets (De Vos et al., 2014) and sows having larger litters ('high prolific sows') may see the main benefit from these feed additives (Hong et al., 2020; Nuntapaitoon et al., 2018). Average total born (TB), born alive (BA), and average total stillborn (SB) piglets in previous litters was calculated for each sow to account for her prior performance. Models for TB, BA and SB in the experimental litter were analysed with and without these prior averages as covariates to adjust for the individual sows' prior performance in the herd.

Results presented herein are from the diet x parity group factor analysis unless stated otherwise, as results were similar between the three analyses. Pairwise comparisons were made between treatment means (and the interaction with parity group) where appropriate using Fisher's least significant difference (LSD) method with a Bonferroni adjustment for multiple comparisons. Seven pairwise comparisons were made in total, testing between CON and the other four dietary treatments, as well as ARG and the remaining three dietary treatment levels.

Mortality, sow removals, remating rate, and farrowing rate were analysed as binomial variables (dead or alive, removed or retained, mated or not, successful or unsuccessful) using chi-square (X^2) analysis.

3. Outcomes

3.1 Sow Performance in Late Gestation

Average prior total born (TB) and born alive (BA) in previous parities was not significantly different between sows in the 5 dietary treatment groups (*P* ≥ 0.10; Table 2). Of the sows that were selected for the experiment, 8.8% had been re-mated after a failed mating and a return to oestrus. As a preliminary analysis, the proportion of sows in each dietary group that was a re-mated sow was analysed by chi-square and it was found that there was no significant difference between dietary treatment groups ($X^2 = 1.60$; $P = 0.81$; data not shown).

Sows were allocated to dietary treatment balancing for parity and sow body weight at day 90 of gestation. Hence, sow body weight at day 90 was not significantly different between treatments ($P = 0.57$; Table 2). However, sow P2 backfat at day 90 of gestation was significantly different ($P = 0.041$) between sows allocated to different dietary treatments before the experimental conditions had been applied (Table 2). Sows allocated to the NCG group were significantly ($P < 0.05$) fatter than those allocated to the CON and ARG groups (Table 2). Therefore, this was used as a covariate for P2 backfat measured at day 108 and weaning. From what we know from previous studies in this area, sow P2 backfat at day 90 would not be expected to significantly impact other parameters measured in this study such as birth measures or lactation performance. Indeed, sow P2 backfat modelled as a covariate did not improve the statistical models for average piglet or litter weights at birth or in lactation and did not show a linear relationship with any of these factors and was therefore not included in these statistical models. However, changes in backfat thickness in sows may be related to colostrum production (Decaluwe et al., 2013) and could therefore impact piglet survival. Number of piglets born alive and hence average birth weight may also be impacted by sow P2 backfat in late gestation (Lavery et al., 2019), but again in the current study these parameters were not linearly related to or significantly influenced by P2 at day 90 of gestation. Similarly for lactation average daily feed intake (ADFI), which Lavery et al. (2019) found may be influenced by sow P2 backfat thickness throughout gestation, which may warrant further investigation in future studies.

Average daily feed intake (ADFI) between day 90 of gestation and entry to the farrowing house was similar for all dietary treatments ($P = 0.62$; data not shown).

This was unsurprising given that sows were given a restricted daily ration via automatic feeders. Any small differences between treatments were likely due to the treatment being confounded with pen, and hence the feeder operation etc. Wu et al. (2013) suggested that a ratio of 3:1 digestible arginine to digestible lysine be used in sow diets to avoid arginine/lysine antagonism, and ratios higher than this may impact sow feed intake. The ratio of these two amino acids in our ARG diet was calculated to be approximately 2.05:1 and hence we wouldn't expect this to impact ADFI of sows (Moreira et al., 2018). Similarly, HMB supplementation did not impact gestation feed intake in previous studies on spiny mice (Muszyński et al., 2016) and sows (Hu et al., 2020) and CAN did not impact pre-farrowing feed intake of sows in the study by van den Bosch et al. (2019a). However, impacts of NCG supplementation on sow feed intake in late gestation have not been well studied.

There was no difference ($P = 0.72$) in sow body weight at entry to the farrowing house (day 108) between dietary treatments (Table 2). Sow P2 backfat at day 108 was highest in NCG sows ($P = 0.043$), being significantly different to that of CON, ARG and CAN sows, and tending to be higher than that of HMB sows ($P = 0.093$; Table 2). Sow P2 backfat at day 108 was corrected for P2 at day 90 as a covariate, to adjust for differences at the start of the experiment that would have been independent of treatment effects. After this adjustment there was no significant difference ($P = 0.77$) in sow P2 backfat between dietary treatments (CON, 20.4 ± 0.2 mm; ARG, 20.4 ± 0.2 mm; HMB, 20.1 ± 0.2 mm; NCG, 20.4 ± 0.2; and CAN, 20.2 ± 0.2 mm).

Change in sow body weight from day 90 of gestation until farrowing house entry was significantly impacted by dietary treatment ($P = 0.007$). Sows on the HMB treatment lost significantly less weight ($P < 0.05$) than sows in the other treatment groups (Table 2). A similar result was found in the study by Hu et al. (2020), where HMB supplemented sows lost 5.3 kg less weight than sows fed a control diet from 10 days pre-farrowing until day 2 of lactation. However, in that study, the overall diet effect was not significant ($P = 0.23$) and this may have been a result of the low sample size ($n = 4$ sows per treatment). Animals supplemented with HMB exhibit higher levels of insulin resistance (Duan et al., 2018b; Wan et al., 2016; Yonamine et al., 2014), which may have altered the glucose metabolism of these sows and contributed to this difference in body weight change. Further studies in sows are required to confirm these mechanisms, as the change in P2 backfat from day 90 of gestation until farrowing house entry was not significantly affected by diet (*P* = 0.77; Table 2) in the current study. Gestation length was similar between dietary treatments (116 days; *P* = 0.98). The diet x parity group interaction was not significant (*P* ≥ 0.10) for any parameters in late gestation (data not shown).

Table 2: Results from the linear mixed models analysis of sow performance in late gestation when fed one of five diets from day 90 of gestation until farrowing, comparing a control (CON) diet, with four experimental diets supplemented with either 0.5% arginine (ARG), 0.15% ß-hydroxy-ß-methyl butyrate (HMB), 0.15% N-carbamylglutamate (NCG), or 0.1% calcium nitrate, $Ca(NO_3)_2$ (CAN). Results are presented as least square mean ± SE.

	Diet					Diet
Parameter[1]	CON	ARG	HMB	NCG	CAN	*P*-value
n	108	101	107	113	108	-
Parity	3.8	3.8	3.6	3.6	3.7	0.97
Prior BA (n)	12.0 ± 0.2	12.1 ± 0.2	11.9 ± 0.2	11.9 ± 0.2	12.0 ± 0.2	0.97
Prior TB (n)	13.2 ± 0.2	13.3 ± 0.2	13.1 ± 0.2	13.2 ± 0.2	13.4 ± 0.2	0.95
Prior SB% (%)	6.2 ± 0.5	6.1 ± 0.6	6.8 ± 0.6	7.2 ± 0.5	7.5 ± 0.5	0.25
Sow BWT (kg)						
Day 90	270.9 ± 2.1	267.5 ± 2.3	271.5 ± 2.3	272.7 ± 2.2	270.2 ± 2.2	0.57
Day 108[2]	284.5 ± 2.1	282.8 ± 2.4	283.2 ± 2.4	287.0 ± 2.4	283.4 ± 2.2	0.72
Δ Day 90-108	13.5 ± 0.5^{a}	13.6 ± 0.5^{a}	11.2 ± 0.5^{b}	13.6 ± 0.5^{a}	13.0 ± 0.5^{a}	0.007
Sow P2 (mm)						
Day 90	19.7 ± 0.5^{a}	19.0 ± 0.5^{a}	20.2 ± 0.5^{ab}	21.0 ± 0.5^{b}	19.9 ± 0.5^{ab}	0.041
Day 108[2]	20.0 ± 0.5^{a}	19.6 ± 0.5^{a}	20.5 ± 0.5^{ab}	21.8 ± 0.5^{b}	20.1 ± 0.5^{a}	0.043
Δ Day 90-108	0.5 ± 0.2	0.5 ± 0.2	0.2 ± 0.2	0.5 ± 0.2	0.3 ± 0.2	0.77

^{a,b,c} Different superscripts within rows denotes a significant difference ($P < 0.05$) between dietary treatment means; pairwise comparisons were made using the least significant difference (LSD) method.

[1] BA = born alive; BWT = body weight; P2 = backfat thickness at the P2 site, measured by ultrasound. SB% = stillbirth rate; TB = total born.

[2] Day 108 corresponds to the day of gestation that sows entered the farrowing house.

3.2 Sow Performance in Lactation

The number of days from farrowing house entry until farrowing was similar for all dietary treatments ($P = 0.20$) averaging 9 (± 2 SD) days. Pre-farrowing ADFI from farrowing house entry was not different between sows on each dietary treatment ($P = 0.53$; Table 3). Similarly, sow ADFI in lactation was not impacted by diet ($P = 0.68$; Table 3). Although it is commonly thought that the bitter taste of L-arginine may impact feed intake in pigs (Pérez Laspiur et al., 2001), supplemental L-arginine did not impact ADFI of sows when fed at similar levels in more recent studies (Holanda et al., 2019; Hong et al., 2020; Mateo et al., 2008; Zhu et al., 2017) and its inclusion isn't expected to influence feed intake when at levels of <2% of the diet (Wu et al., 2013). Supplementation of HMB has previously been shown to reduce lactation feed intake (Wan et al., 2017), thought again to be related to insulin resistance in HMB-supplemented sows. These results were not replicated in the current study; however, HMB was only supplemented in lactation in the study of Wan et al. (2017) which may be why they observed a reduction in lactation ADFI. Lactation feed intake was numerically highest in NCG-supplemented sows in the current study, which is in agreement with Feng et al. (2019) who saw an improvement in lactation ADFI when NCG was supplemented in the last month of gestation and throughout lactation together with vitamin C, under summer heat stress conditions. Dietary NCG supplementation has also recently been shown to increase feed intake in chickens (Ma et al., 2020); however, the mechanisms for the impact of NCG on feed intake remain unclear and must be further explored. Recently, van den Bosch et al. (2019a) observed that lactation feed intake was not impacted by CAN supplementation, similar to the findings of the current study.

Sow body weight at weaning was not significantly impacted by diet (*P* = 0.92; Table 3). Lactation body weight loss of sows (from entry to the farrowing house until weaning) was not significantly different (*P* = 0.27) between dietary treatments (Table 3). Sow P2 backfat at weaning tended to be different between dietary treatments (*P* = 0.079; Table 3); however, when adjusting for sow P2 backfat at day 90 of gestation, this effect was no longer significant (CON, 19.2 ± 0.3 mm; ARG, 19.8 ± 0.3 mm; HMB, 19.6 ± 0.3 mm; NCG, 19.4 ± 0.3 mm; and CAN, 19.6 ± 0.5 mm; *P* = 0.59). Change in P2 backfat throughout lactation was not different (*P* = 0.27) between dietary treatments (Table 3). There were no significant interactions (*P* ≥ 0.10) between diet and sow parity group for any of the sow performance parameters during lactation (data not shown).

In the current study, although not significant, ARG sows had the lowest lactation body weight and P2 backfat loss of all dietary groups. This is in accordance with the findings of some authors (Nuntapaitoon et al., 2018; Pérez Laspiur et al., 2001) who have found that supplementation of L-arginine to sows significantly reduced their body weight loss in lactation, but in contrast to those of others (Hong et al., 2020; Krogh et al., 2017; Mateo et al., 2008; Moreira et al., 2018; Zhu et al., 2017) who have found no difference. It is unclear why there is conjecture between studies, but this may be due to low sample sizes or differences in supplementation period (mid, late gestation and/or lactation), which should be further studied. For the remaining additives in our study, the literature on the subject of changes in body weight or P2 backfat of sows in lactation is either lacking or shows conflicting results between studies. For example, Flummer et al. (2012b) found that supplementation of HMB to sows resulted in a higher P2 backfat loss in lactation, while Cieslak et al. (2018) found a reduction in P2 backfat loss, and Hu et al. (2020) found no change. Our results certainly seem to be in agreement with the more recent results of Hu et al. (2020), but further work is required in this area, especially since there was a discrepancy in P2 backfat between our sows at the start of the experiment. Similarly, studies that have reported lactation performance of sows supplemented with NCG rarely measure or report changes in body condition of sows during lactation (Cai et al., 2018; Feng et al., 2018; Liu et al., 2012). The recent study by van den Bosch et al. (2019a) reported, similar to our results, that CAN did not influence sow body weight or backfat loss in lactation. However, as far as we are aware, this is the only report of such a finding in the recent literature.

For the duration of the study, n = 61 sows died or were removed from the experiment for a number of reasons, including abortion, culled from the herd after weaning for health or management reasons, agalactia, poor condition, downer, feet and leg issues, or prolapse. When analysed with chi-square, there was no significant difference ($X^2 = 0.63$; $P = 0.96$) in the proportion of sows removed between dietary treatments (10.9% of CON sows, 12.4% of ARG sows, 11.1% of HMB sows, 9.6% of NCG sows, and 9.6% of CAN sows). The numerically lower removal rate in NCG and CAN sows may be an indicator of improved health status through amelioration of oxidative stress by increased NO production (Cao et al., 2016; Omar et al., 2016; van den Bosch et al., 2019a; van den Bosch et al., 2019b; Wu et al., 2010b; Zhang et al., 2016), although this was not the case for the L-arginine sows, which also may have been expected to have increased NO production (Dasgupta et al., 2006; Lin et al., 2008). Impacts on sow health of these additives need to be further elucidated.

Table 3: Sow farrowing and litter characteristics results from the linear mixed models analysis of sows fed one of five diets from day 90 of gestation until farrowing, comparing a control (CON) diet, with four experimental diets supplemented with either 0.5% arginine (ARG), 0.15% ß-hydroxy-ß-methyl butyrate (HMB), 0.15% N-carbamylglutamate (NCG), or 0.1% calcium nitrate, $Ca(NO_3)_2$ (CAN). Results are presented as least square mean ± SE.

	Diet					Diet
***Parameter*[1]**	**CON**	**ARG**	**HMB**	**NCG**	**CAN**	***P*-value**
n	107	92	102	103	105	-
Gestation length (d)	116.3 ± 0.1	116.3 ± 0.2	116.3 ± 0.2	116.2 ± 0.2	116.3 ± 0.2	0.98
BA (n)	12.2 ± 0.3	11.7 ± 0.4	12.2 ± 0.4	12.0 ± 0.3	12.1 ± 0.3	0.88
SB (n)	1.2 ± 0.2	1.5 ± 0.2	1.2 ± 0.2	1.5 ± 0.2	1.3 ± 0.2	0.65
MUM (n)	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.67
TB (n)	13.7 ± 0.3	13.6 ± 0.4	13.8 ± 0.4	13.8 ± 0.4	13.7 ± 0.3	0.99
SB% (%)	7.8 ± 1.0	9.4 ± 1.1	9.1 ± 1.1	10.7 ± 1.1	9.6 ± 1.0	0.41
Sex ratio (% females)	47.1 ± 1.6	48.0 ± 1.7	49.7 ± 1.7	45.7 ± 1.7	49.0 ± 1.6	0.47
Sow BWT (kg)						
Weaning[2]	271.8 ± 2.7	273.3 ± 2.9	274.0 ± 2.9	274.4 ± 2.9	271.2 ± 2.8	0.92
Δ Day 108-wean	-11.6 ± 1.5	-9.5 ± 1.6	-10.2 ± 1.6	-13.4 ± 1.6	-13.4 ± 1.5	0.26
Sow P2 (mm)						
Weaning	18.7 ± 0.5	19.1 ± 0.5	19.6 ± 0.5	20.6 ± 0.5	19.6 ± 0.5	0.079
Δ Day 108-wean	-1.1 ± 0.3	-0.6 ± 0.3	-1.0 ± 0.3	-1.5 ± 0.3	-0.8 ± 0.3	0.27
ADFI (kg/d)						
Pre-farrow[3]	3.12 ± 0.03	3.17 ± 0.04	3.10 ± 0.04	3.15 ± 0.04	3.10 ± 0.03	0.53
Lactation	7.8 ± 0.1	7.8 ± 0.1	7.8 ± 0.1	7.9 ± 0.1	7.7 ± 0.1	0.68

[a,b,c] Different superscripts within rows denotes a significant difference ($P < 0.05$) between dietary treatment means; pairwise comparisons were made using the least significant difference (LSD) method.

[1] ADFI = average daily feed intake; BA = born alive; BWT = body weight; MUM = number of mummified foetuses; P2 = backfat thickness at the P2 site, measured by ultrasound; SB = number of stillborn piglets; SB% = stillbirth rate as a proportion of total piglets born (TB).

[2] Sows were weaned at 27.2 ± 0.1 days of lactation.

[3] Pre-farrowing ADFI presented is from entry to the farrowing house (day 108) until day of farrowing.

3.3 Litter Performance

3.3.1 At Parturition

Total number of piglets born (TB) was similar between dietary treatments (*P* = 0.99; Table 3), even after correcting for the sows' average TB (*P* = 0.93; data not shown). Similarly, BA was not significantly different (*P* = 0.88) between dietary treatments (Table 3), even when correcting for the sows' average BA (*P* = 0.73; data not shown). However, BA was numerically lowest for ARG sows (11.7 vs. 12.0-12.2 for other dietary treatments). There was no significant difference in either total number of stillborn piglets per litter (*P* = 0.65) nor the proportion of stillborn piglets per litter (*P* = 0.41) between dietary treatments (Table 3). There was also no significant difference (*P* = 0.47) in sex ratio between the dietary treatments (Table 3).

These results are largely in disagreement with those of previous authors, who have found that ARG (Nuntapaitoon et al., 2018), NCG (Feng et al., 2018; Liu et al., 2012; Wu et al., 2012; Zhang et al., 2014; Zhu et al., 2015) and potentially HMB (Wan et al., 2016) reduced stillbirth rate and enhanced the proportion of piglets born alive. However, Bass et al. (2011) found no impact of L-arginine supplementation on the number of piglets born alive, and more recently Luise et al. (2020) also reported no impact of L-arginine supplementation on stillbirth rate, in accordance with our findings. It is suggested that these advantages of ARG may only be possible when it is fed for a longer period during gestation (Feng et al 2019; Zhang et al 2014). It is also possible that the most substantial benefit of feeding these additives on farrowing performance and reducing stillbirth rate may be in highly prolific sows with large litter sizes (i.e. may have more potential for use in countries such as Denmark; Hong et al., 2020; Nuntapaitoon et al., 2018). This may also be the case for HMB, with Hułas-Stasiak et al. (2019) finding no difference in number of stillborn piglets when HMB was fed for a similar period to the that of the current study. The study by van den Bosch et al. (2019a) also reported no difference in stillbirths when CAN was supplemented in their study.

Sow diet tended (*P* = 0.083) to influence the litter weights of all pigs at birth (including stillborn and piglets that died before fostering, of which litter number was similar between diets, *P* = 0.91; Table 4). Sows supplemented with CAN had the highest litter weight and ARG sows the lowest, both significantly different from each other (*P* = 0.040). All other treatment differences in birth litter weight were not significant (*P* ≥ 0.10). A similar trend for litter weight of BA piglets was seen (Table 4), except diet was no longer significant (*P* = 0.17). Due to the delay between farrowing and piglets being weighed, some pigs reported as being BA subsequently died but were still weighed with the litter.

For all pigs, the diet tended to influence average birth weight (*P* = 0.060; Table 4). On average, piglets born to ARG and NCG sows were lightest, and those from CAN sows were heaviest, but all diet means were statistically similar (*P* ≥ 0.10) when pairwise comparisons were made. Similarly, diet tended to influence the average weight of live pigs at birth (*P* = 0.061; Figure 1). On average, live piglets born to ARG sows were lightest, and live piglets born to CAN sows were heaviest (Figure 1), with CAN piglets significantly heavier (*P* = 0.044) than ARG piglets, but all dietary treatments were similar (*P* ≥ 0.10) to that of the CON sows. There was no significant effect of diet (*P* = 0.79) on the coefficient of variation (CV) of birth weights within litters, based on the weight of all pigs at birth, excluding those that were mummified (Table 4).

The proportion of piglets born <1.1 kg (as a proportion of all experimental piglets born) was significantly influenced by diet (X^2 = 46.6; $P < 0.001$). Piglets born from CAN sows had the lowest proportion of piglets born <1.1 kg (17.5%), less than both the CON (22.0%) and the ARG treatments (25.4%; Figure 2). However, the other dietary treatments had a higher percentage of piglets born <1.1 kg than the CON treatment (HMB, 24.9% and NCG, 26.5%).

It was initially hypothesised that these feed additives would be largely successful in improving birth weights, piglet vitality and litter uniformity at birth, as they have been shown to improve placental growth and prevent IUGR (Gao et al., 2012; Wu et al., 2004a; Zhu et al., 2015). The likely mechanism for this of ARG, NCG, and potentially CAN is the production of nitrate and/or nitric oxide (NO) from ARG and NCG metabolism in pregnant mammals, which can promote placental angiogenesis and vasculogenesis, thus enhancing blood flow to the placenta (Bird et al., 2003; Chang et al., 2008). Dallanora et al. (2017) previously found that L-arginine supplementation increased the average birth weight of piglets born and reduced the proportion of piglets of low birth weight, in agreement with previous studies by Mateo et al. (2007, 2008), but which was not seen in the current study. A recent study by Hong et al. (2020) found that while litter birth weight was improved by ARG supplementation, the proportion of BA piglets born light was reduced, owing to an improvement in survival of these lighter piglets in the later part of gestation. Zhang et al. (2014) found an impact of NCG supplementation on average piglet birth weight, and Tatara et al. (2007) reported a similar outcome for HMB in sow diets. However, Flummer et al. (2012a) reported no impact on birth weight of HMB supplementation, as was the case with the current study. Recently, a paper by Cieslak et al. (2018) speculated that the low inclusion rates used in that study (15 mg/kg sow body weight) may have been the cause, which may have also been the case in the current study. In the case of the study by Flummer et al. (2012a), sows would likely have received more HMB per day than they would in the current study, and that experiment was conducted throughout gestation. Further experiments are required to evaluate the impact of these additives on birth weights of piglets, and the best inclusion rates and feeding times that maximise the chance of high piglet vitality at birth.

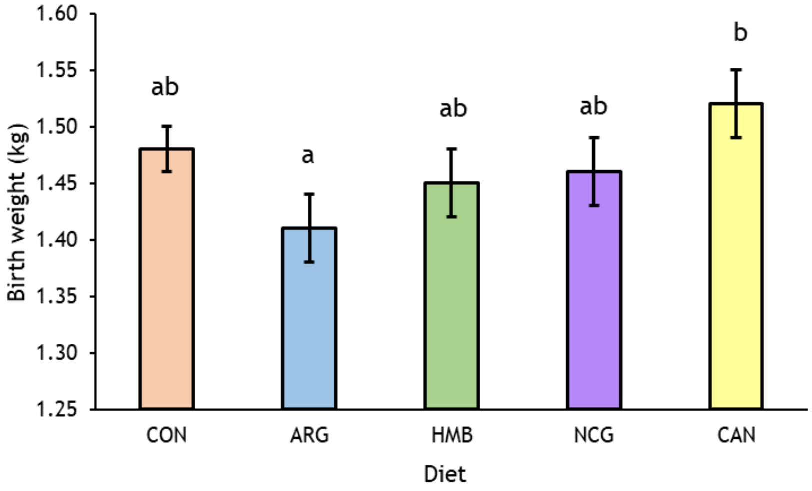

Figure 1: Average piglet weight of live pigs at birth when various supplements were fed to sows in late gestation from day 90 of gestation until farrowing (control, CON; 0.5% supplemented arginine, ARG; 0.15% supplemented ß-hydroxy-ß-methyl butyrate, HMB; 0.15% supplemented N-carbamylglutamate, NCG; and 0.1% supplemented $Ca(NO_3)_2$, CAN). The overall diet effect tended towards significance ($P = 0.061$). Different superscripts denote a significant ($P < 0.05$) pairwise difference between two dietary treatments; pairwise comparisons were made using the least significant difference (LSD) method with a Bonferroni adjustment for multiple comparisons.

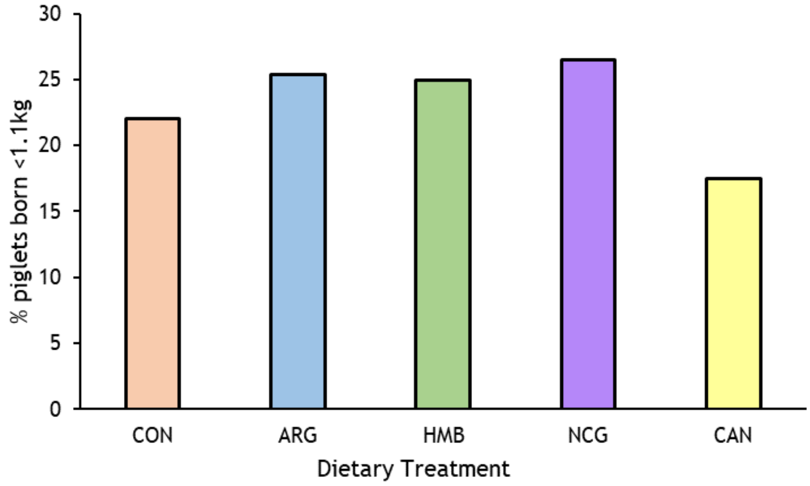

Figure 2: Proportion of total piglets born weighing <1.1 kg when various supplements were fed to sows in late gestation from day 90 of gestation until farrowing (control, CON; 0.5% supplemented arginine, ARG; 0.15% supplemented ß-hydroxy-ß-methyl butyrate, HMB; 0.15% supplemented N-carbamylglutamate, NCG; and 0.1% supplemented $Ca(NO_3)_2$, CAN). Dietary treatment effect was analysed by chi-square ($X^2 = 46.62$; $P < 0.001$).

Table 4: Litter performance results from the linear mixed models analysis of sows fed one of five diets from day 90 of gestation until farrowing, comparing a control (CON) diet, with four experimental diets supplemented with either 0.5% arginine (ARG), 0.15% ß-hydroxy ß-methyl butyrate (HMB), 0.15% N-carbamylglutamate (NCG), or 0.1% calcium nitrate, $Ca(NO_3)_2$ (CAN). Results are presented as least square mean ± SE.

	Diet					Diet
***Parameter*[1]**	**CON**	**ARG**	**HMB**	**NCG**	**CAN**	***P*-value**
Litter number (n)						
All pigs at birth[2]	13.3 ± 0.3	13.0 ± 0.4	13.4 ± 0.4	13.5 ± 0.4	13.4 ± 0.4	0.91
Post-foster (day 0)	11.5 ± 0.2	11.5 ± 0.2	11.1 ± 0.2	11.5 ± 0.2	10.9 ± 0.2	0.17
Lactation day 7	10.2 ± 0.2	10.1 ± 0.2	9.7 ± 0.2	10.1 ± 0.2	9.7 ± 0.2	0.25
Lactation day 25	9.9 ± 0.2	10.1 ± 0.2	9.4 ± 0.2	10.1 ± 0.2	9.7 ± 0.2	0.25
Litter weight (kg)						
All pigs at birth[2]	18.6 ± 0.4^{ab}	17.5 ± 0.5^{a}	18.5 ± 0.5^{ab}	18.1 ± 0.5^{ab}	19.3 ± 0.4^{b}	0.083
Live pigs at birth	16.6 ± 0.4	15.5 ± 0.5	16.2 ± 0.5	16.0 ± 0.5	17.0 ± 0.4	0.17
Lactation day 7	28.5 ± 0.6	28.2 ± 0.7	28.1 ± 0.7	29.1 ± 0.7	28.2 ± 0.6	0.83
Lactation day 25	71.4 ± 1.6	70.4 ± 1.7	70.5 ± 1.7	73.4 ± 1.7	69.6 ± 1.6	0.52
ADG day 0-25 (g/d)	234 ± 8	221 ± 10	252 ± 10	235 ± 9	247 ± 8	0.18
Ave pig BWT (kg)[2]	1.44 ± 0.02	1.38 ± 0.03	1.40 ± 0.03	1.38 ± 0.03	1.47 ± 0.03	0.060
Birth weight CV (%)	22.2 ± 0.7	22.0 ± 0.8	23.2 ± 0.7	22.5 ± 0.7	22.2 ± 0.7	0.79

[a,b,c] Different superscripts within rows denotes a significant difference ($P < 0.05$) between dietary treatment means; pairwise comparisons were made using the least significant difference (LSD) method with a Bonferroni adjustment.

[1] ADG = average daily gain; BWT = birth weight; CV = coefficient of variation.

[2] All pigs born, not including mummified foetuses.

3.3.2 Litter Growth Performance in Lactation

The number of piglets in the litter after fostering was not different (*P* = 0.17) between dietary treatments (Table 4). There was a tendency for a dietary effect (*P* = 0.055) on the total number of piglets fostered on per litter, with CON, ARG, and NCG sows having the most piglets fostered on per litter (data not shown). However, there was no difference (*P* = 0.19) in total number of piglets fostered off per litter (data not shown), and no difference (*P* = 0.17) in total litter number after fostering (Table 4).

Litter weight at day 7 of lactation was not influenced by diet (*P* = 0.93), and neither was litter number at day 7 (*P* = 0.25; Table 4). Similarly, litter weight at day 25 of lactation was also not influenced by diet overall (*P* = 0.52); however, CAN litters tended to be lighter (*P* = 0.094) than NCG litters at this stage (Table 4). This may have been as a result of the litter number at day 25 of lactation, which was significantly influenced by diet (*P* = 0.041; Table 4). Litters from HMB and CAN sows had the lowest number of pigs in the litter at this stage, CAN litters having significantly less (*P* = 0.035) piglets than NCG litters. The diet x parity group interaction was significant (*P* = 0.028) for litter number at day 25, for both the simple and corrected models. From the corrected model, it was observed that most dietary treatment differences in litter number were within the 2nd (parities 3 and 4) and 4th (parities ≥ 7) parity groups. These interaction effects will not be discussed here but pairwise comparisons P-values (with a Bonferroni adjustment for multiple comparisons) are shown in Appendix 1, Table S3.

Average weight of piglets at day 7 of lactation was not significantly influenced by diet (*P* = 0.21; Figure 3A). There tended to be a diet x parity group interaction in average piglet weight at day 7 of lactation (*P* = 0.064). There was no difference between any of the dietary treatments in the younger (1st and 2nd) parity groups (parities 1,2 and 3,4; data not shown). However, in the 3rd (parities 5 and 6) and 4th (≥ parity 7) parity groups, there were some differences in average piglet weight at day 7 (see Appendix 1, Figure S4). Pairwise comparisons were made between dietary treatment groups in the 3rd and 4th parity groups and as such 14 comparisons were made with P-values adjusted using the Bonferroni method. Piglets born to CAN sows were, on average, significantly heavier (*P* < 0.05) than piglets born to sows in the other treatment groups in the 3rd parity group, but not in the 4th parity group. There may have been more variation in the 4th parity group, but these results may suggest that feeding CAN may be more successful for improving piglet pre-weaning growth rates in mid- to late-parity sows. However, there was no longer a significant diet x parity group interaction (*P* = 0.12) in average piglet weight at day 25 of lactation, and this requires further elucidation.

The differences in litter weight and number at day 25 of lactation were reflected in the average weight of piglets at day 25 of lactation, which was significantly influenced by diet (*P* = 0.042; Figure 3B). On average, surviving HMB and CAN piglets were significantly heavier (*P* < 0.05) than ARG piglets, and NCG piglets tended to be heavier (*P* = 0.086) than ARG piglets. On average, HMB piglets were heaviest at day 25 of lactation, and tended to be heavier than CON piglets (*P* = 0.053; Figure 3B). All other treatments were statistically similar (*P* ≥ *0*.10) to that of the CON diet. Litter ADG was not significantly impacted by diet (*P* = 0.18; Table 4), but was numerically highest in HMB and CAN litters.

The lack of improvement in weight gain of piglets over lactation in the ARG group is in agreement with the recent studies of Holanda et al. (2019) and Luise et al. (2020); however, in the former study, ARG was fed in lactation and not late gestation. In other recent studies (Hong et al., 2020; Oksbjerg et al., 2019; Zhu et al., 2017) feeding ARG in gestation (until farrowing or through to weaning, respectively) has been shown to improve pre-weaning ADG of piglets. In the study of Hong et al. (2020), ARG was included as a higher proportion of the diet (1.0 to 1.5%) than in the current study, while Zhu et al. (2017) and Oksbjerg et al. (2019) fed ARG for a longer period of gestation (day 3 or 30 until weaning, respectively) which may have been a reason for these differences. However, further studies are required to determine the desirable rate of inclusion for ARG in late gestation diets. Authors that did see an improvement in piglet weight gain speculated that improvements in mammary gland blood flow (Holanda et al., 2019; Krogh et al., 2017), uptake of ARG into the mammary glands (Trottier et al., 1997; Wu et al., 2018) and higher milk production (Kim et al., 2009; Mateo et al., 2008; O'Quinn et al., 2002) may have been one of the mechanisms for this improvement. However, this remains to be further elucidated, as some authors have found no improvements in milk yield (Krogh et al., 2017; Moreira et al., 2018; Nuntapaitoon et al., 2018). The result in the current study may also be explained by the differences in sow backfat at day 90 of gestation, even though this was accounted for in the statistical analysis, and this must be confirmed in future studies.

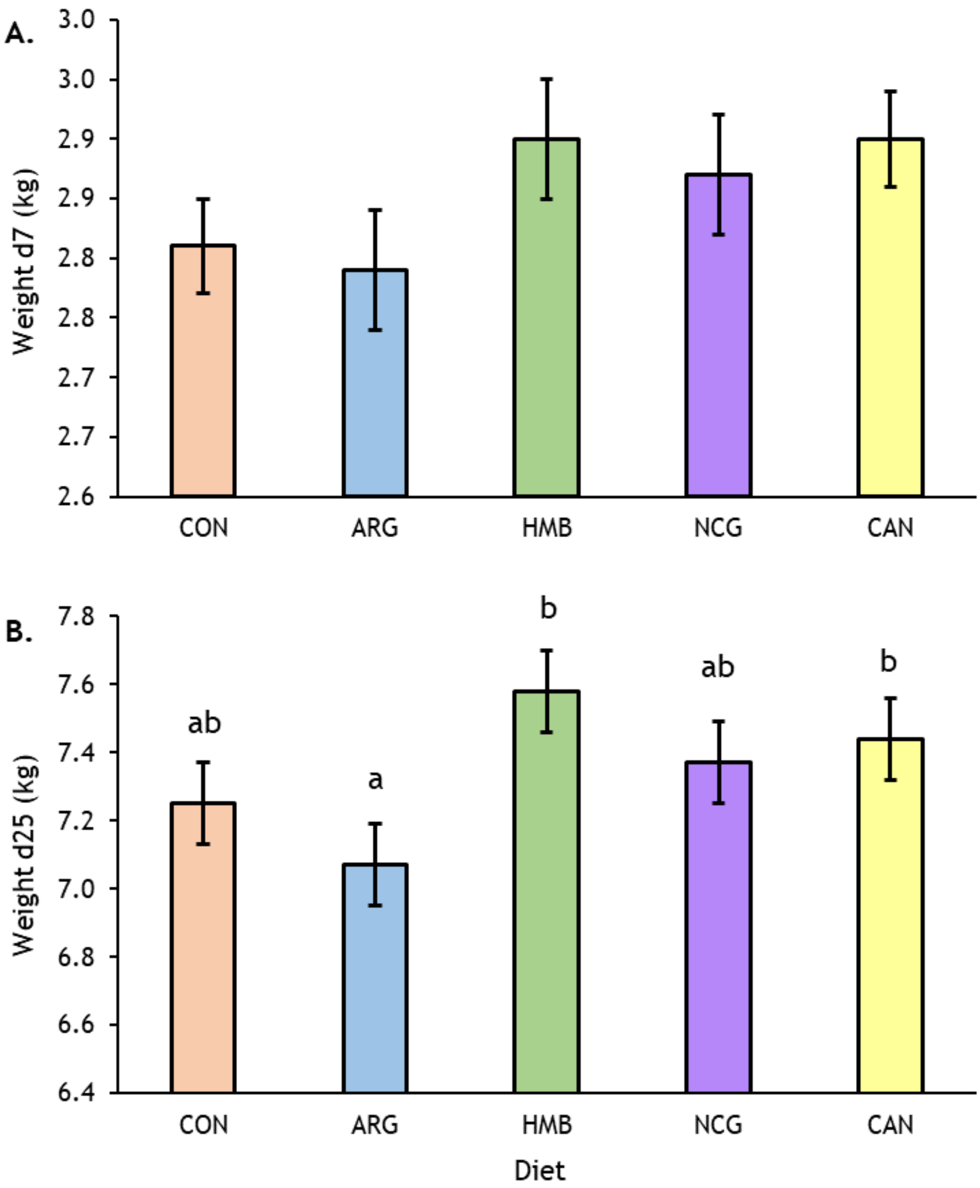

Figure 3: Average piglet weight at days 7 (A) and 25 (B) of lactation when various supplements were fed to sows in late gestation from day 90 of gestation until farrowing (control, CON; 0.5% supplemented arginine, ARG; 0.15% supplemented ß-hydroxy-ß-methyl butyrate, HMB; 0.15% supplemented N-carbamylglutamate, NCG; and 0.1% supplemented $Ca(NO_3)_2$, CAN). Diet effects were $P = 0.21$ and $P = 0.042$ for days 7 and 25, respectively. Different superscripts denote a significant ($P < 0.05$) pairwise difference between two dietary treatments; pairwise comparisons were made using the least significant difference (LSD) method with a Bonferroni adjustment for multiple comparisons.

The apparent improvements in pre-weaning growth of piglets from HMB and CAN sows are in agreement with previous studies that have supplemented these additives to sows, with even NCG supplementation showing a numerical improvement. Supplementation of HMB has been shown previously to improve piglet pre-weaning ADG (Flummer et al., 2012b; Krakowski et al., 2002; Nissen et al., 1994), as has NCG (Feng et al., 2018; Zeng et al., 2015; Zhang et al., 2013). Supplemental NCG is thought to impact piglet growth rates similarly to ARG by improving milk production (Feng et al., 2018), influencing uptake of amino acids by the mammary gland by regulation of insulin (Burnol et al., 1990; Laarveld et al., 1981). It is also likely that NCG supplementation results in improvements in intestinal development and function (Wu et al., 2010b; Zhang et al., 2018) and protein synthesis and muscle fibre development (Frank et al., 2007; Wu et al., 2004b; Zeng et al., 2015).

Unlike ARG and NCG supplementation, HMB does not seem to improve milk production (Flummer et al., 2012b; Hu et al., 2020). More likely, the improvements in piglet growth performance are due to an increased serum IGF-I concentrations in piglets at birth (Tatara et al., 2007), increased levels of fat (Kao et al., 2016; Nissen et al., 1994; Wan et al., 2016) or HMB in the milk, improving skeletal muscle development (Wan et al., 2017) and/or beneficial changes in the intestinal microbiome (Duan et al., 2018a). Supplemental HMB in sow diets has also been shown to improve bone development in piglets (Blicharski et al., 2017).

Improvements in pre-weaning weight gain of piglets in the CAN treatment was surprising, as van den Bosch et al. (2019a) previously found no difference; however, piglet vitality at birth was improved in the follow up to that study (van den Bosch et al., 2019b). Further work is required to determine the impact of CAN supplementation on piglet growth performance in lactation, and to determine possible mechanisms for this improvement.

3.3.3 Piglet Pre-Weaning Survival

There was no difference between diets in terms of total pre-foster deaths per litter (*P* = 0.90; data not shown) or pre-foster mortality as a percentage of the total piglets BA (CON, 5.6 ± 0.9%; ARG, 6.3 ± 1.0%; HMB, 7.6 ± 1.0%; NCG, 5.6 ± 1.0%; and CAN, 6.3 ± 1.0%; *P* = 0.60). There was no significant difference in pre-foster mortality as a proportion of BA (X^2 = 2.42; *P* = 0.66) between dietary treatments (data not shown). Of the subset of litters that had all data collected from farrowing until day 25 of lactation, there was no difference in piglet mortality from birth to fostering (as a proportion of BA; $X^2 = 2.42$; $P = 0.66$) or after fostering to day 7 of lactation ($X^2 = 3.61$; $P = 0.46$). However, there was a significant difference in total litter mortality from post-foster to day 25 between dietary treatments ($X^2 = 10.88$; $P = 0.028$; Figure 4), with all diets showing lower mortality than the CON treatment, except for the HMB treatment.

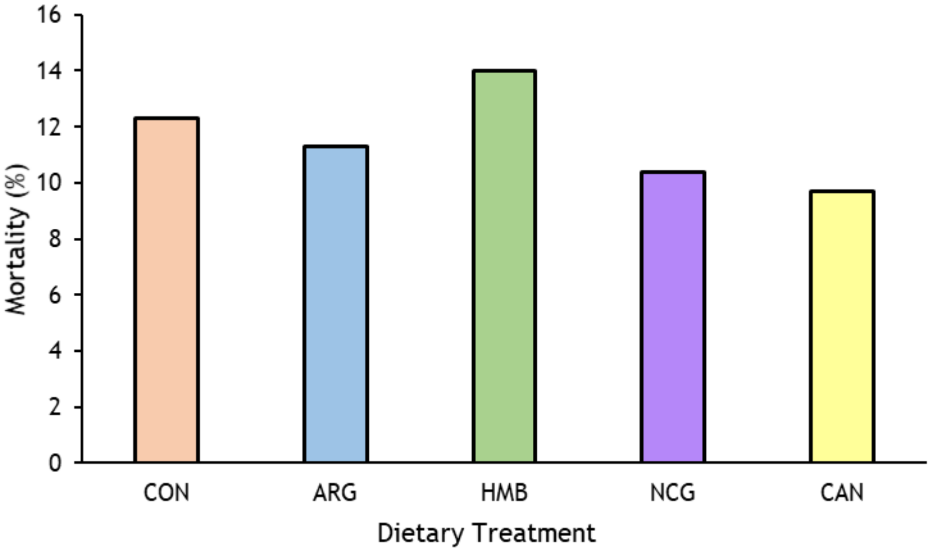

Figure 4: Post-foster mortality from fostering to day 25 of lactation when various supplements were fed to sows in late gestation from day 90 of gestation until farrowing (control, CON; 0.5% supplemented arginine, ARG; 0.15% supplemented β-hydroxy-β-methyl butyrate, HMB; 0.15% supplemented N-carbamylglutamate, NCG; and 0.1% supplemented $Ca(NO_3)_2$, CAN). Dietary treatment effect was analysed by chi-square ($X^2 = 10.88$; $P = 0.028$).

The lack of impact of feeding these additives to sows on early post-natal survival of piglets was surprising, given that previous studies have found an improvement in vitality, placenta weight and efficiency and birth weights (Feng et al., 2018; Gao et al., 2012; van den Bosch et al., 2019a; van den Bosch et al., 2019b; Wan et al., 2016; Wu et al., 2018; Wu et al., 2012). However, differences were seen in overall pre-weaning mortality.

Nuntapaitoon et al. (2018) showed that addition of 0.5% L-arginine to the late gestation sow diet increased the blood oxygen saturation of the piglets at birth, which may explain the lower pre-weaning mortality rate in this group compared to the control group. In this study, L-arginine supplementation also increased the IgG concentration of colostrum which may have further enhanced the piglets' survivability. Piglets in the CAN group had the best rate of survival to day 25 of lactation (close to weaning) in the current study. This in agreement with the results of van den Bosch et al. (2019a,b) who speculated that this was due to an increase in blood flow to the placenta due to the synthesis of NO in these piglets, who they found had increased vitality at birth with a longer placental width.

A similar impact has been previously seen when supplementing NCG to sows in late gestation, with improvements also in IgG concentration of colostrum (when supplemented along with Vitamin C; Feng et al., 2018), gastrointestinal mucosal immunity (Zhang et al., 2013) and intestinal structure (Mo et al., 2018), antioxidative capacity and vasculature of the placenta (Liu et al., 2012; Wei et al., 2020; Wu et al., 2012). Furthermore, CAN and NCG have been shown to improve sow respiration around farrowing (Feng et al., 2018; van den Bosch et al., 2019b), which may indicate a higher oxygen availability and hence may be another mechanism for the improvements in piglet vitality. There are some reports that HMB supplementation may impact the immune system, as was previously shown in calves (Wójcik et al., 2014); however, this remains to be further elucidated in pigs and is not supported by the highest pre-weaning mortality rate in piglets born to HMB sows in the current study. As mentioned in section 3.1, colostrum production may be influenced by changes in P2 backfat thickness of sows during gestation (Decaluwe et al., 2013) and hence these results may have been impacted by the difference in backfat P2 thickness at the start of the current experiment. However, it is unlikely that a difference of ≤ 2 mm backfat between treatments at day 90 of gestation significantly influenced these results. Furthermore, while absolute backfat P2 at day 90 differed, change in P2 backfat from day 90 to 108 of gestation was not significantly different ($P = 0.77$) between dietary treatments in the current study.

Unfortunately, piglets in the current study were not tagged at birth, and hence we could not determine the pre-weaning mortality rates of the different birth weight classes. This would be an interesting follow up from the current study, especially given the improvements we saw in birth weight and number of piglets born <1.1 kg for CAN litters, which would be expected to improve overall litter survival (Akdag et al., 2009; Bergstrom et al., 2011; Cabrera et al., 2012). Furthermore, the potential improvements in placental sufficiency that may be seen with supplementation of these additives has the potential to have the highest impact on smaller piglets in the litter (Feng et al., 2018; van den Bosch et al., 2019a; van den Bosch et al., 2019b; Wan et al., 2016; Wu et al., 2018).

3.4 Sow Performance in Subsequent Reproduction

There was no significant difference (*P* ≥ 0.10) between dietary treatment groups in terms of the proportion of sows that were remated after weaning, or in farrowing rate of sows that were subsequently mated after weaning, although farrowing rate was numerically higher in the CON group than it was for all the experimental diet groups (Table 5). Wean to remate interval (WRI), gestation length, subsequent born alive, or subsequent number of pigs weaned were also not significantly impacted by diet (*P* ≥ 0.10; Table 5).

Throughout the study, including between weaning the experimental litter and the subsequent farrowing, there was no difference between dietary treatment groups in terms of the proportion of sows that died or were removed from the study for health, reproductive, or locomotory reasons (X^2 = 0.98; *P* = 0.91; data not shown).

Generally, these results were unsurprising given the minimal impact of diet on sow body condition (body weight and P2 backfat thickness) over late gestation or lactation in the current study. Similarly, studies have found no impact of feeding ARG (Moreira et al., 2018) or NCG (Liu et al., 2012) in late gestation and/or lactation on subsequent reproductive output of sows. Reports of the impact of HMB or CAN on subsequent reproductive performance could not be found in the current literature. There are a few reports that suggest that NCG supplementation may impact reproductive hormone production and hence the development of ovarian follicles (Feng et al., 2019; Ma et al., 2020), with the possibility of improving pregnancy rates in sows (Wu et al., 2012). The fact that our NCG treatment had the second lowest WRI (numerically) after the ARG treatment may be as a result of this. However, there may be more of an impact on subsequent reproductive performance of sows if NCG were fed throughout lactation as well, or in early gestation. This effect deserves to be further studied.

Table 5: Results for the subsequent reproductive performance of sows fed one of five experimental diets from day 90 of gestation until farrowing (a control diet, CON; or diets supplemented with either 0.5% arginine, ARG; 0.15% ß-hydroxy-ß-methyl butyrate; HMB; 0.15% N-carbamylglutamate; NCG; or 0.1% calcium nitrate, $Ca(NO_3)_2$, CAN).

	Diet					Diet
Parameter	**CON**	**ARG**	**HMB**	**NCG**	**CAN**	***P*-value**
Remating rate (%)[1]	80.2	78.8	86.7	84.6	84.6	0.60
WRI (d)[2]	4.2 ± 0.2	4.2 ± 0.2	3.9 ± 0.2	4.2 ± 0.2	3.9 ± 0.2	0.46
Farrowing rate (%)[1]	86.3	80.6	79.5	80.5	81.8	0.84
Gestation length (d)	115.5 ± 0.2	115.5 ± 0.4	115.9 ± 0.4	115.8 ± 0.4	115.7 ± 0.2	0.88
Born alive (n)	12.8 ± 0.5	11.6 ± 0.8	12.2 ± 0.8	11.8 ±0.8	11.8 ± 0.4	0.61
Number weaned (n)	9.7 ± 0.4	9.8 ± 0.6	9.4 ± 0.6	10.5 ± 0.6	9.3 ± 0.3	0.53

[1] These parameters were analysed as binomial variables using chi-square analysis (X^2 = 2.75 and X^2 = 1.42, respectively).

[2] Wean to remate interval. Only wean to remate intervals of less than 10 days included in the analysis (outliers removed).

4. Application of Research

These results suggest that the additives investigated may be viable alternatives to feeding L-arginine in late gestation sow diets, especially HMB and CAN, as they may improve piglet birth weights, pre-weaning survival and litter weights in lactation. Fed at low doses, these dietary additives offer a cheaper alternative to L-arginine that can be used on farm in a late gestation sow diet. Since there is a common belief that L-arginine has the potential to reduce sow ADFI due to its bitter taste, and owing to the fact that it requires a relatively high inclusion rate to be effective, has a relatively short half-life, and may have an antagonistic effect on the absorption of other amino acids in the diet, there is a requirement to find suitable alternatives in sow gestation diets. Producers may be more inclined to adopt these alternative additives that we have investigated in the current project, as they require lower inclusion rates, and did not seem to reduce sow feed intake (although neither did L-arginine in the current experiment).

4.1 Cost Benefit Analysis

The approximate costs of the additives used at the time of writing were:

- L-arginine - $14 per kg;
- HMB - $36 per kg;
- NCG - $28 per kg;
- Calcium nitrate - $4 per kg.

At an average daily intake of 3 kg/day (not impacted by diet in the current study), a gestation length of 116 days, feeding from day 90 of gestation, and using the inclusion rates adopted in the current study, this equates to an additional cost of:

- L-arginine – $5.46 per sow per lactation (i.e., 26 days feeding x 3 kg/day x 7c/kg of feed);
- HMB – $4.21 per sow per lactation (i.e., 26 days feeding x 3 kg/day x 5.4c/kg of feed);
- NCG – $3.28 per sow per lactation (i.e., 26 days feeding x 3 kg/day x 4.2c/kg of feed);
- Calcium nitrate - $0.27 per sow per lactation (i.e., 26 days feeding x 3 kg/day x 0.35c/kg of feed).

It must be noted that the cost of L-arginine increased from approximately $11/kg to $14/kg in the time between conducting the current experiment and conducting the cost-benefit analysis, due to COVID-19 impacting the price of production and importation from China (import permit costs, freight costs, etc.). Using the values above and additional revenue expected from the improvements in survival and growth performance (Hermesch et al., 2012) observed in the current experiment, HMB and CAN seem to be the most cost effective additives to use in order to maximise reproductive outcomes on farm (Table 6). It should also be noted that these values do not take into account additional storage requirements for additives, or additional labour for sow feeding in late gestation (discussed further in Section 6.3).

Table 6: Cost benefit analysis of each additive according to the inclusion rates and performance improvements observed in the current project.

Diet	Additive cost ($/kg)	Inclusion rate (%)	Additional cost per sow ($/gestation)*	Survival improvement to weaning (%)*[1]	Growth improvement (kg of weaning weight)*	Additional performance value ($/litter)*[2]	Overall cost benefit ($/litter)*[3]
CON	0	-	-	-	-	-	-
ARG	14	0.50	5.46	1.0	(0.18)	3.04 + (24.31)	(26.73)
HMB	36	0.15	4.21	(1.7)	0.33	(5.16) + 44.57	35.20
NCG	28	0.15	3.28	1.9	0.12	5.77 + 16.21	18.70
CAN	4	0.10	0.27	2.6	0.19	7.90 + 25.66	33.29

*Relative to the control (CON) diet. Parentheses indicate a negative value.

[1] Based on post-foster survival rate.

[2] Calculated using PigEV (Hermesch et al., 2012). First value is the improvement for survival to weaning and second value is the improvement from growth to weaning.

[3] Cost benefit calculated from the additional performance value per litter minus the additional dietary additive cost per sow.

5. Conclusion

In conclusion, supplementation of CAN to sows in late gestation may improve weight of piglets at birth, reduce the proportion of piglets born <1.1 kg, and improve their pre-weaning survival chance. Supplementation of HMB may also improve the growth performance of piglets to weaning (25 days of age). Therefore, these two additives may show promise as alternatives to supplementary L-arginine in late-gestation sow diets. From the cost benefit analysis performed it seems that both HMB and CAN are the most desirable ingredients to use in sow diets commercially, with the potential to create $30-40 additional revenue per litter produced. However, the L-arginine-supplemented diet did not improve piglet performance in the current experiment, as has been seen in previous studies (recently reviewed by Wu et al., 2018). Further work is required to identify the optimum timeframes and inclusion rates for supplementing HMB, NCG, and CAN in gestating sow diets.

6. Limitations/Risks

6.1 Limitations in Experimental Design

One limitation of the experimental design in the current experiment was that there was a difference in mean sow P2 backfat thickness between treatments before dietary treatments were applied. This may have impacted sow reproductive performance in the current study, as sow P2 backfat in late gestation may influence on some farrowing and lactation performance parameters (Decaluwe et al., 2013; Lavery et al., 2019; Maes et al., 2004). However, in the current study, sow P2 backfat at day 90 of gestation before dietary treatments were applied, did not show a strong linear relationship with any of the reproductive parameters measured. Furthermore, sows used in this study were within an 'acceptable' backfat P2 range at day 90 of gestation (non-fat, non-skinny; min = 8.7 mm, max = 35.6 mm) with an even distribution of sows of different P2s between dietary treatment groups. The mean difference between treatments of ≤ 2 mm backfat would not be expected to significantly influence sow performance. Nonetheless, the ARG sows and their litters seemed to be the worst performers of all treatments in the current study, which we may not expect given the previous literature that describes the health and performance benefits of L-arginine supplementation in sows and growing piglets (Wu et al., 2018; Wu et al., 1998). However, in most previous studies, 1% L-arginine seems to be the most common inclusion rate in those that found positive impacts on placental development, piglet growth and survival, which is higher than the level used in the current study. It is therefore important to further compare these novel additives to L-arginine in sow diets to confirm these results.

6.2 Barriers to Commercialisation

All feed additives used in this project are readily available to be purchased commercially; however, some ingredients (NCG and HMB) had to be sourced internationally. At the inclusion rates and the short duration at which they were employed in the current study, their use is cost effective in breeder diets, given that they may enhance the reproductive efficiency of sows and the growth performance and survival chance of their piglets.

Both NCG and HMB are not registered products in Australia but were tested experimentally under controlled conditions. Since all three novel feed ingredients tested are: (i) not classed as drugs or antibiotics, (ii) are synthetic products and therefore are not cultured in a laboratory/do not pose any additional biosecurity risks, (iii) are not prohibited substances that have an anabolic action in the body, and that (iv) three of the four products (ex. NCG) already have existing Australian import permits, we were not required to have an APVMA research permit for this study.

Indications are that both NCG and HMB are likely to require registration as a veterinary chemical if to be used commercially. To be excluded from registration each product will need to satisfy the steps outlined on the APVMA website (APVMA, 2021). There are four tests for an end product in order to be excluded from registration: Ingredients, manufacturing systems, labelling and claims. With respects to the ingredients test the product(s) must not contain certain ingredients, such as antibiotics; and, all ingredients must be on at least one of the specified lists of substances that are generally recognised as safe (the 'GRAS' lists). An initial search indicated that both substances are not listed on the GRAS lists (there are nine lists on the APVMA site to be satisfied). There have been no potential side effects in animals and humans for HMB (reviewed by Muszyński et al., 2016); however, whether side effects may exist for certain levels of NCG or CAN may require further experiments. Further advice from a regulatory consultant would need to be taken to confirm the products do or do not require registration.

Further research may be required to identify the best additive inclusion levels of these additives in order to make further registration claims, as well as recording any side effects that may be seen if these additives are over-fed. For example, there are reports that HMB may reduce tissue insulin sensitivity at high levels, but this phenomenon remains controversial (Hu et al., 2020). Likewise, a number of studies have looked at the safety and effectiveness of nitrate supplementation (e.g. CAN) in ruminants to reduce methane production (Nolan et al., 2016; van Zijderveld et al., 2010, 2011; Yang et al., 2016); however, not so much work has been done in monogastric animals or pigs specifically. On the other hand, NCG is non-toxic and non-genotoxic, with 0.5% being the tolerated dose in animals (Wu et al., 2015) and quite a bit of research has been undertaken in China to determine its effects in mammals to assist with the registration process (Hu et al., 2019). Registering a product is a more expensive route to commercial adoption, and the decision to register these products will be made on the level of response and the expected return on investment.

Commercialisation and adoption of these feed additives, if effective, would require:

i) Determining whether the technology can be patented. This is not a requirement but if possible, allows protection of the technology as further evidence is gathered;

ii) Further economic analysis;

iii) Expression of Interest from a third party (e.g., feed additive company, feed manufacturer) to import the product(s);

iv) Registration of the product(s) with APVMA;

v) Label claims regarding action for improved health and/or performance.

A future commercialisation and adoption strategy may involve recommendations for application of the technology in Australian conditions. This may require further research to refine e.g., dosage and duration. Initially there was a concern that these products may have to be registered before use in breeding pig diets in Australia. It has been noted that NCG is a registered feed additive product in China and manufactured by Animore Sci & Tech Ltd, who have patented the product (CN104206711A); however, the patent does not cover the Australia region and claims involve growing pigs only. Additionally, another patent (CN103202392B) claims NCG improves reproductive performance when fed to sows prenatally to weaning. The advantages of feeding HMB and CAN in particular in the current study indicate that importing these products for use as pig feed additives could have commercial merit. With respect to HMB, the worldwide patent (AU627111B2) expired in August 2019. This patent included claims for increase lean tissue growth in farm animals and does not cover its use in breeding animals.

It is recommended that further research is done on these additives (especially HMB and CAN) to confirm the optimum feeding times and supplementation levels of these additives in sow diets before any commercialisation strategies are undertaken. However, the results from this project and from previous studies look promising for these feed additives.

6.3 On Farm Logistics

It must also be noted that our results describe the impact of providing these additives from day 90 of gestation up until the point of farrowing. Given that feeding systems for gestating sows may be limiting for implementation of these late gestation diets on a commercial scale, it would be of interest to conduct further work into whether these additives can be fed just as a pre-farrowing transition diet (i.e. from entry to the farrowing house until the point of farrowing) to reduce the requirement for further on-farm storage and adaptation of existing feeding systems.

Depending on the feeding systems utilised on an individual farm basis, feeding sows from day 90 of gestation until farrowing may not be practical. For example, in the Rivalea system at the R&I/Module 1 facility used for the current experiment, sows are housed in static groups from soon after mating throughout gestation until about day 108 when they are transferred into the farrowing house. In these group-housed gestation pens, sows are fed from an ESF feeder that is fed from one or two silos only, which are used to feed a whole shed worth of gestating sows, all at several different days of gestation. Furthermore, within that pen, the sows may reach day 90 of gestation on different days of the week. Therefore, an additional diet to be fed at exactly day 90 for sows at different periods of gestation would require further infrastructure (silos, feed lines, etc.) to accommodate the additional diet. Moreover, from entry into the farrowing house it is common for sows to receive a lactation (rather than a gestation) diet, and hence on-farm storage may be limiting for an additional pre-farrowing ‘transition’ diet with the additives included. Top-dressing may be an option for late gestation sows in the dry sow facility and/or the farrowing house; however, there are increased labour costs with this method, still requiring further storage of the top-dress, and in group housed sows this may result in unequal amounts being distributed amongst sows. These are all aspects that must be taken into consideration at a commercial level when implementing targeted sow late gestation diets.

7. Recommendations

As a result of the outcomes in this study the following recommendations have been made:

- It is recommended that further advice from a regulatory consultant be sought to confirm the products do or do not require registration;
- It is recommended that follow up studies are first conducted looking further at feeding HMB and CAN in late gestation sow diets, to further evaluate them as an alternative to l-arginine in these diets;
- With this caveat, it is recommended from the results of the current experiment that HMB or CAN be incorporated into late gestation sow diets from day 90 of gestation in order to improve the proportion of piglets born <1.1 kg (CAN), piglet pre-weaning survival chance (CAN), and/or piglet average weight at weaning (at 25 days of age; CAN and HMB). Both of these offer more cost-effective alternatives to L-arginine in late gestation sow diets;
- Supplementation of NCG during late gestation is also recommended over L-arginine, although acquisition of this feed additive currently requires importation from China and the cost benefit of feeding this additive is less than that of HMB or CAN.

8. References

Akdag, F., Arslan, S., & Demir, H. (2009). The effect of parity and litter size on birth weight and the effect of birth weight variations on weaning weight and pre-weaning survival in piglet. *Journal of Animal and Veterinary Advances, 8*(11), p. 2133-2138.

Alba-Roth, J., Müller, O. A., Schopohl, J., & Werder, K. V. (1988). Arginine Stimulates Growth Hormone Secretion by Suppressing Endogenous Somatostatin Secretion*. *The Journal of Clinical Endocrinology & Metabolism, 67*(6), p. 1186-1189. doi:10.1210/jcem-67-6-1186

APVMA. (2021). Stockfeed and petfood regulation. Retrieved from https://apvma.gov.au/node/13021 accessed on 25th July.

Bass, B., Bradley, C., Johnson, Z., Boyd, R., Usry, J., Maxwell, C., & Frank, J. (2011). Influence of dietary L-arginine supplementation to sows during late gestation on sow and litter performance during lactation. *Res. Ser, 597*, p. 151-155.

Bergstrom, J., Potter, M., Nelssen, Tokach, M., Henry, S., Dritz, S., Goodband, & Derouchey, J. M. (2011). The association of sow and litter characteristics with piglet birth weight and the implications for growth, survival, and carcass characteristics of pigs on a commercial farm. In.

Bird, I. M., Zhang, L., & Magness, R. R. (2003). Possible mechanisms underlying pregnancy-induced changes in uterine artery endothelial function. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 284*(2), p. R245-R258. doi:10.1152/ajpregu.00108.2002

Blicharski, T., Tomaszewska, E., Dobrowolski, P., Hułas-Stasiak, M., & Muszyński, S. (2017). A metabolite of leucine (β-hydroxy-β-methylbutyrate) given to sows during pregnancy alters bone development of their newborn offspring by hormonal modulation. *PloS one, 12*(6), p. e0179693. doi:10.1371/journal.pone.0179693

Burnol, A., Loizeau, M., & Girard, J. (1990). Insulin receptor activity and insulin sensitivity in mammary gland of lactating rats. *American Journal of Physiology-Endocrinology and Metabolism, 259*(6), p. E828-E834.

Cabrera, R. A., Lin, X., Campbell, J. M., Moeser, A. J., & Odle, J. (2012). Influence of birth order, birth weight, colostrum and serum immunoglobulin G on neonatal piglet survival. *Journal of animal science and biotechnology, 3*(1), p. 1-10. doi:10.1186/2049-1891-3-42

Cai, S., Zhu, J., Zeng, X., Ye, Q., Ye, C., Mao, X., Zhang, S., Qiao, S., & Zeng, X. (2018). Maternal N-carbamylglutamate supply during early pregnancy enhanced pregnancy outcomes in sows through modulations of targeted genes and metabolism pathways. *Journal of Agricultural and Food Chemistry, 66*(23), p. 5845-5852.

Cao, W., Xiao, L., Liu, G., Fang, T., Wu, X., Jia, G., Zhao, H., Chen, X., Wu, C., Cai, J., & Wang, J. (2016). Dietary arginine and N-carbamylglutamate supplementation enhances the antioxidant statuses of the liver and plasma against oxidative stress in rats. *Food & Function, 7*(5), p. 2303-2311. doi:10.1039/C5FO01194A

Chang, K., & Lubo, Z. (2008). Review Article: Steroid Hormones and Uterine Vascular Adaptation to Pregnancy. *Reproductive Sciences, 15*(4), p. 336-348. doi:10.1177/1933719108317975

Che, L., Yang, P., Fang, Z., Lin, Y., & Wu, D. (2013). Effects of dietary arginine supplementation on reproductive performance and immunity of sows. *Czech J. Anim. Sci, 58*(4), p. 167-175.

Chew, B. P., Eisenman, J. R., & Tanaka, T. S. (1984). Arginine Infusion Stimulates Prolactin, Growth Hormone, Insulin, and Subsequent Lactation in Pregnant Dairy Cows. *Journal of Dairy Science, 67*(11), p. 2507-2518. doi:10.3168/jds.s0022-0302(84)81607-0

Cieslak, D. G., & Nieradko-Iwanicka. (2018). β-Hydroxy β-methylbutyrate (HMB) supplementation during pregnancy and perinatal period in animals studies andpossible application in humans. *Journal of Education, Health and Sport, 8*(3), p. 11-18. doi:10.5281/zenodo.1183841

Costa, K. A., Marques, D. B. D., De Campos, C. F., Saraiva, A., Guimarães, J. D., & Guimarães, S. E. F. (2019). Nutrition influence on sow reproductive performance and conceptuses development and survival: A review about l-arginine supplementation. *Livestock Science, 228*, p. 97-103. doi:10.1016/j.livsci.2019.08.010

Dallanora, D., Marcon, J., Walter, M. P., Biondo, N., Bernardi, M. L., Wentz, I., & Bortolozzo, F. P. (2017). Effect of dietary amino acid supplementation during gestation on placental efficiency and litter birth weight in gestating gilts. *Livestock Science, 197*, p. 30-35. doi:10.1016/j.livsci.2017.01.005

Dasgupta, T., Hebbel, R., & Kaul, D. (2006). Protective effect of arginine on oxidative stress in transgenic sickle mouse models. *Free Radical Biology and Medicine, 41*(12), p. 1771-1780. doi:10.1016/j.freeradbiomed.2006.08.025

Davenport, G. M., Boling, J. A., & Schillo, K. K. (1995). Growth and endocrine responses of lambs fed rumen-protected ornithine and arginine. *Small Ruminant Research, 17*(3), p. 229-236. doi:10.1016/0921-4488(95)00685-E

De Vos, M., Che, L., Huygelen, V., Willemen, S., Michiels, J., Van Cruchten, S., & Van Ginneken, C. (2014). Nutritional interventions to prevent and rear low-birthweight piglets. *Journal of animal physiology and animal nutrition, 98*(4), p. 609-619. doi:10.1111/jpn.12133

Decaluwe, R., Maes, D., Declerck, I., Cools, A., Wuyts, B., De Smet, S., & Janssens, G. P. J. (2013). Changes in back fat thickness during late gestation predict colostrum yield in sows. *Animal, 7*(12), p. 1999-2007. doi:10.1017/S1751731113001791

Duan, Y., Li, F., Li, Y., Tang, Y., Kong, X., Feng, Z., Anthony, T. G., Watford, M., Hou, Y., & Wu, G. (2016). The role of leucine and its metabolites in protein and energy metabolism. *Amino acids, 48*(1), p. 41-51.

Duan, Y., Zhang, L., Li, F., Guo, Q., Long, C., Yin, Y., Kong, X., Peng, M., & Wang, W. (2018a). ß-Hydroxy-ß-methylbutyrate modulates lipid metabolism in adipose tissues of growing pigs. *Food & Function, 9*(9), p. 4836-4846. doi:10.1039/C8FO00898A

Duan, Y. H., Zeng, L. M., Li, F. N., Kong, X. F., Xu, K., Guo, Q. P., Wang, W. L., & Zhang, L. Y. (2018b). ß-hydroxy-ß-methyl butyrate promotes leucine metabolism and improves muscle fibre composition in growing pigs. *Journal of animal physiology and animal nutrition, 102*(5), p. 1328-1339. doi:10.1111/jpn.12957

Feng, T., Bai, J., Xu, X., Guo, Y., Huang, Z., & Liu, Y. (2018). Supplementation with N-carbamylglutamate and vitamin C: improving gestation and lactation outcomes in sows under heat stress. *Animal Production Science, 58*(10), p. 1854-1859. doi:10.1071/AN15562

Feng, T., DeVore, A. A., Perego, M. C., Morrell, B. C., & Spicer, L. J. (2019). Effects of N-carbamylglutamate and arginine on steroidogenesis and proliferation of pig granulosa cells in vitro. *Animal Reproduction Science, 209*, p. 106138. doi:10.1016/j.anireprosci.2019.106138

Flummer, C., Kristensen, N. B., & Theil, P. K. (2012a). Body composition of piglets from sows fed the leucine metabolite ß-hydroxy ß-methyl butyrate in late gestation. *Journal of Animal Science, 90*(suppl_4), p. 442-444.

Flummer, C., & Theil, P. K. (2012b). Effect of ß-hydroxy ß-methyl butyrate supplementation of sows in late gestation and lactation on sow production of colostrum and milk and piglet performance. *Journal of Animal Science, 90*(suppl_4), p. 372-374.

Flynn, N. E., Meininger, C. J., Haynes, T. E., & Wu, G. (2002). The metabolic basis of arginine nutrition and pharmacotherapy. *Biomedicine & Pharmacotherapy, 56*(9), p. 427-438. doi:10.1016/S0753-3322(02)00273-1

Foxcroft, G., Dixon, W., Dyck, M., Novak, S., Harding, J., & Almeida, F. (2009). *Prenatal programming of postnatal development in the pig.* Paper presented at the Control of Reproduction VIII: Proceedings of the Eighth International Conference on Pig Reproduction.

Frank, J. W., Escobar, J., Nguyen, H. V., Jobgen, S. C., Jobgen, W. S., Davis, T. A., & Wu, G. (2007). Oral N-Carbamylglutamate Supplementation Increases Protein Synthesis in Skeletal Muscle of Piglets. *The Journal of Nutrition, 137*(2), p. 315-319. doi:10.1093/jn/137.2.315

Gao, K., Jiang, Z., Lin, Y., Zheng, C., Zhou, G., Chen, F., Yang, L., & Wu, G. (2012). Dietary l-arginine supplementation enhances placental growth and reproductive performance in sows. *Amino acids, 42*(6), p. 2207-2214. doi:10.1007/s00726-011-0960-9

Hermesch, S., Ludemann, C. I., & Amer, P. R. (2012). *PigEV–a new tool to derive economic values for pigs.*

Holanda, D. M., Marcolla, C. S., Guimarães, S. E. F., Neves, M. M., Hausman, G. J., Duarte, M. S., Abreu, M. L. T., & Saraiva, A. (2019). Dietary L-arginine supplementation increased mammary gland vascularity of lactating sows. *Animal, 13*(4), p. 790-798. doi:10.1017/s1751731118002069

Hong, J., Fang, L. H., Jeong, J. H., & Kim, Y. Y. (2020). Effects of L-Arginine Supplementation during Late Gestation on Reproductive Performance, Piglet Uniformity, Blood Profiles, and Milk Composition in High Prolific Sows. *Animals, 10*(8), p. 1313. doi:10.3390/ani10081313

Hu, L., Kristensen, N. B., Krogh, U., & Theil, P. K. (2020). Net Absorption and Metabolism of β-Hydroxy- β-Methyl Butyrate during Late Gestation in a Pig Model. *Nutrients, 12*(2), p. 561. doi:10.3390/nu12020561

Hu, Y., Shao, D., Wang, Q., Xiao, Y., Zhao, X., Shen, Y., Zhang, S., Tong, H., & Shi, S. (2019). Effects of dietary N-carbamylglutamate supplementation on growth performance, tissue development and blood parameters of yellow-feather broilers. *Poultry Science, 98*(5), p. 2241-2249. doi:10.3382/ps/pey591

Hułas-Stasiak, M., Jakubowicz-Gil, J., Dobrowolski, P., Tomaszewska, E., & Muszyński, S. (2019). Maternal β-hydroxy-β-methylbutyrate (HMB) supplementation during pregnancy affects early folliculogenesis in the ovary of newborn piglets. *Theriogenology, 128*, p. 91-100. doi:10.1016/j.theriogenology.2019.02.003

Ishida, M., Hiramatsu, Y., Masuyama, H., Mizutani, Y., & Kudo, T. (2002). Inhibition of placental ornithine decarboxylase by DL-α-difluoro-methyl ornithine causes fetal growth restriction in rat. *Life Sciences, 70*(12), p. 1395-1405. doi:10.1016/S0024-3205(01)01510-7

Jones, A. M. (2014). Influence of dietary nitrate on the physiological determinants of exercise performance: a critical review. *Applied Physiology, Nutrition, and Metabolism, 39*(9), p. 1019-1028.

Kao, M., Columbus, D. A., Suryawan, A., Steinhoff-Wagner, J., Hernandez-Garcia, A., Nguyen, H. V., Fiorotto, M. L., & Davis, T. A. (2016). Enteral β-hydroxy-β-methylbutyrate supplementation increases protein synthesis in skeletal muscle of neonatal pigs. *American Journal of Physiology-Endocrinology and Metabolism, 310*(11), p. E1072-E1084. doi:10.1152/ajpendo.00520.2015

Kim, S. W., Mateo, R. D., Yin, Y.-L., & Wu, G. (2007). Functional amino acids and fatty acids for enhancing production performance of sows and piglets. *Asian Australasian Journal of Animal Sciences, 20*(2), p. 295.

Kim, S. W., & Wu, G. (2009). Regulatory role for amino acids in mammary gland growth and milk synthesis. *Amino acids, 37*(1), p. 89-95.

Krakowski, L., Krzyżanowski, J., Wrona, Z., Kostro, K., & Siwicki, A. K. (2002). The influence of nonspecific immunostimulation of pregnant sows on the immunological value of colostrum. *Veterinary Immunology and Immunopathology, 87*(1), p. 89-95. doi:10.1016/S0165-2427(02)00004-1

Krogh, U., Oksbjerg, N., Storm, A. C., Feyera, T., & Theil, P. K. (2017). Mammary nutrient uptake in multiparous sows fed supplementary arginine during gestation and lactation. *Journal of Animal Science, 95*(6), p. 2517-2532. doi:10.2527/jas.2016.1291

Laarveld, B., Christensen, D. A., & Brockman, R. P. (1981). The Effect of Insulin on Net Metabolism of Glucose and Amino Acids by the Bovine Mammary Gland*. *Endocrinology, 108*(6), p. 2217-2221. doi:10.1210/endo-108-6-2217

Lavery, A., Lawlor, P. G., Magowan, E., Miller, H. M., O'Driscoll, K., & Berry, D. P. (2019). An association analysis of sow parity, live-weight and back-fat depth as indicators of sow productivity. *Animal, 13*(3), p. 622-630. doi:10.1017/S1751731118001799

Lin, C.-C., Tsai, W.-C., Chen, J.-Y., Li, Y.-H., Lin, L.-J., & Chen, J.-H. (2008). Supplements of l-arginine attenuate the effects of high-fat meal on endothelial function and oxidative stress. *International Journal of Cardiology, 127*(3), p. 337-341. doi:10.1016/j.ijcard.2007.06.013

Liu, X. D., Wu, X., Yin, Y. L., Liu, Y. Q., Geng, M. M., Yang, H. S., Blachier, F., & Wu, G. Y. (2012). Effects of dietary L-arginine or N-carbamylglutamate supplementation during late gestation of sows on the miR-15b/16, miR-221/222, VEGFA and eNOS expression in umbilical vein. *Amino acids, 42*(6), p. 2111-2119.

Luise, D., Bertocchi, M., Bosi, P., Correa, F., Spinelli, E., & Trevisi, P. (2020). Contribution of L-Arginine supplementation during gestation on sow productive performance and on sow microbial faecal profile. *Italian Journal of Animal Science, 19*(1), p. 330-340.

Ma, Y., Zhou, S., Lin, X., Zeng, W., Mi, Y., & Zhang, C. (2020). Effect of dietary N-carbamylglutamate on development of ovarian follicles via enhanced angiogenesis in the chicken. *Poultry Science, 99*(1), p. 578-589. doi:10.3382/ps/pez545

Maes, D., Janssens, G., Delputte, P., Lammertyn, A., & de Kruif, A. (2004). Back fat measurements in sows from three commercial pig herds: relationship with reproductive efficiency and correlation with visual body condition scores. *Livestock Production Science, 91*(1-2), p. 57-67.

Mateo, R. D., Wu, G., Bazer, F. W., Park, J. C., Shinzato, I., & Kim, S. W. (2007). Dietary l-Arginine Supplementation Enhances the Reproductive Performance of Gilts. *The Journal of Nutrition, 137*(3), p. 652-656. doi:10.1093/jn/137.3.652

Mateo, R. D., Wu, G., Moon, H. K., Carroll, J. A., & Kim, S. W. (2008). Effects of dietary arginine supplementation during gestation and lactation on the performance of lactating primiparous sows and nursing piglets1. *Journal of Animal Science, 86*(4), p. 827-835. doi:10.2527/jas.2007-0371

Meininger, C. J., & Wu, G. (2002). [24] Regulation of endothelial cell proliferation by nitric oxide. In *Methods in Enzymology* (Vol. 352, p. 280-295): Academic Press.

Mo, W., Wu, X., Jia, G., Zhao, H., Chen, X., Tang, J., Wu, C., Cai, J., Tian, G., Wang, J., & Liu, G. (2018). Roles of dietary supplementation with arginine or N-carbamylglutamate in modulating the inflammation, antioxidant property, and mRNA expression of antioxidant-relative signaling molecules in the spleen of rats under oxidative stress. *Animal Nutrition, 4*(3), p. 322-328. doi:10.1016/j.aninu.2018.02.003

Moreira, R. H. R., Lanferdini, E., Fonseca, L. D. S., Chaves, R. F., Garbossa, C. A. P., Saraiva, A., Nogueira, E. T., & Abreu, M. L. T. D. (2018). Arginine improves nutritional quality of sow milk and piglet performance. *Revista Brasileira de Zootecnia, 47*(0). doi:10.1590/rbz4720170283

Muszyński, S., Tomczyk, A., Dobrowolski, P., Tomaszewska, E., & Hułas-Stasiak, M. (2016). Preliminary study of time-dependent influence of maternal nutrition with addition of ß-hydroxy-ß-methylbutyrate on the body weight and selected organs weight in the new-born spiny mice (Acomys cahirinus) offspring. *Electr J Pol Agric Univ, 19*(4), p. 08.

National Health and Medical Research Council. (2013). *Australian code for the care and use of animals for scientific purposes. 8th edition.* Canberra, ACT, Australia: National Health and Medical Research Council

Nissen, S., Faidley, T. D., Zimmerman, D. R., Izard, R., & Fisher, C. T. (1994). Colostral milk fat percentage and pig performance are enhanced by feeding the leucine metabolite ß-hydroxy-ß-methyl butyrate to sows. *Journal of Animal Science, 72*(9), p. 2331-2337. doi:10.2527/1994.7292331x

Nolan, J. V., Godwin, I. R., de Raphélis-Soissan, V., & Hegarty, R. S. (2016). Managing the rumen to limit the incidence and severity of nitrite poisoning in nitrate-supplemented ruminants. *Animal Production Science, 56*(8), p. 1317-1329. doi:10.1071/AN15324

Nuntapaitoon, M., Muns, R., Theil, P. K., & Tummaruk, P. (2018). l-arginine supplementation in sow diet during late gestation decrease stillborn piglet, increase piglet birth weight and increase immunoglobulin G concentration in colostrum. *Theriogenology, 121*, p. 27-34. doi:10.1016/j.theriogenology.2018.08.012

O'Quinn, P. R., Knabe, D. A., & Wu, G. (2002). Arginine catabolism in lactating porcine mammary tissue. *Journal of Animal Science, 80*(2), p. 467-474. doi:10.2527/2002.802467x

Oksbjerg, N., Krogh, U., Jensen, J. A., Møller, H. S., Ramaekers, P., Rasmussen, M. K., Therkildsen, M., & Theil, P. K. (2019). Supplementation of sows with L-Arginine during gestating and lactation affects muscle traits of offspring related with postnatal growth and meat quality: From conception to consumption. *Meat Science, 152*, p. 58-64. doi:10.1016/j.meatsci.2019.02.004

Omar, S. A., Webb, A. J., Lundberg, J. O., & Weitzberg, E. (2016). Therapeutic effects of inorganic nitrate and nitrite in cardiovascular and metabolic diseases. *Journal of Internal Medicine, 279*(4), p. 315-336. doi:10.1111/joim.12441

Père, M.-C., & Etienne, M. (2000). Uterine blood flow in sows: effects of pregnancy stage and litter size. *Reproduction Nutrition Development, 40*(4), p. 369-382. doi:10.1051/rnd:2000105

Pérez Laspiur, J., & Trottier, N. L. (2001). Effect of dietary arginine supplementation and environmental temperature on sow lactation performance. *Livestock Production Science, 70*(1), p. 159-165. doi:10.1016/S0301-6226(01)00209-3

Quesnel, H., Quiniou, N., Roy, H., Lottin, A., Boulot, S., & Gondret, F. (2014). Supplying dextrose before insemination and L-arginine during the last third of pregnancy in sow diets: Effects on within-litter variation of piglet birth weight1. *Journal of Animal Science, 92*(4), p. 1445-1450. doi:10.2527/jas.2013-6701

Rekiel, A., Więcek, J., Batorska, M., & Kulisiewicz, J. (2014). Effect of sow prolificacy and nutrition on preand postnatal growth of progeny–a review. *Annals of Animal Science, 14*(1), p. 3-15.

Sun, Y. K., Yan, X. G., Ban, Z. B., Yang, H. M., Hegarty, R. S., & Zhao, Y. M. (2017). The effect of cysteamine hydrochloride and nitrate supplementation on in-vitro and in-vivo methane production and productivity of cattle. *Animal Feed Science and Technology, 232*, p. 49-56. doi:10.1016/j.anifeedsci.2017.03.016

Szcześniak, K. A., Ostaszewski, P., Fuller, J. C., Ciecierska, A., & Sadkowski, T. (2015). Dietary supplementation of β-hydroxy-β-methylbutyrate in animals - a review. *Journal of animal physiology and animal nutrition, 99*(3), p. 405-417. doi:10.1111/jpn.12234

Tan, B., Yin, Y., Kong, X., Li, P., Li, X., Gao, H., Li, X., Huang, R., & Wu, G. (2010). l-Arginine stimulates proliferation and prevents endotoxin-induced death of intestinal cells. *Amino acids, 38*(4), p. 1227-1235. doi:10.1007/s00726-009-0334-8

Tatara, M. R., Śliwa, E., & Krupski, W. (2007). Prenatal programming of skeletal development in the offspring: Effects of maternal treatment with β-hydroxy-ß-methylbutyrate (HMB) on femur properties in pigs at slaughter age. *Bone, 40*(6), p. 1615-1622. doi:10.1016/j.bone.2007.02.018

Trottier, N. L., Shipley, C. F., & Easter, R. A. (1997). Plasma amino acid uptake by the mammary gland of the lactating sow2. *Journal of Animal Science, 75*(5), p. 1266-1278. doi:10.2527/1997.7551266x

van den Bosch, M., Wijnen, J., Van De Linde, I. B., Van Wesel, A. A. M., Melchior, D., Kemp, B., Clouard, C., & Van Den Brand, H. (2019a). Effects of maternal dietary nitrate supplementation during the perinatal period on piglet survival, body weight, and litter uniformity. *Translational Animal Science, 3*(1), p. 464-472. doi:10.1093/tas/txy137

van den Bosch, M., Wijnen, J., van de Linde, I. B., van Wesel, A. A. M., Melchior, D., Kemp, B., van den Brand, H., & Clouard, C. (2019b). Effects of maternal dietary nitrate supplementation on farrowing and placental characteristics, level of asphyxiation at birth and piglet vitality. *Theriogenology, 129*, p. 1-7. doi:10.1016/j.theriogenology.2019.01.033

van Zijderveld, S. M., Gerrits, W. J. J., Apajalahti, J. A., Newbold, J. R., Dijkstra, J., Leng, R. A., & Perdok, H. B. (2010). Nitrate and sulfate: Effective alternative hydrogen sinks for mitigation of ruminal methane production in sheep. *Journal of Dairy Science, 93*(12), p. 5856-5866.

van Zijderveld, S. M., Gerrits, W. J. J., Dijkstra, J., Newbold, J. R., Hulshof, R. B. A., & Perdok, H. B. (2011). Persistency of methane mitigation by dietary nitrate supplementation in dairy cows. *Journal of Dairy Science, 94*(8), p. 4028-4038.

Wan, H., Zhu, J., Su, G., Liu, Y., Hua, L., Hu, L., Wu, C., Zhang, R., Zhou, P., Shen, Y., Lin, Y., Xu, S., Fang, Z., Che, L., Feng, B., & Wu, D. (2016). Dietary supplementation with ß-hydroxy-ß-methylbutyrate calcium during the early postnatal period accelerates skeletal muscle fibre growth and maturity in intra-uterine growth-retarded and normal-birth-weight piglets. *British journal of nutrition, 115*(8), p. 1360-1369. doi:10.1017/s0007114516000465

Wan, H., Zhu, J., Wu, C., Zhou, P., Shen, Y., Lin, Y., Xu, S., Che, L., Feng, B., & Li, J. (2017). Transfer of ß-hydroxy-ß-methylbutyrate from sows to their offspring and its impact on muscle fiber type transformation and performance in pigs. *Journal of animal science and biotechnology, 8*(1), p. 2.

Wang, T., Yao, W., Xia, J., Li, J., Shao, Y., & Huang, F. (2019). Dietary supplementation with garcinol during late gestation and lactation facilitates acid-base balance and improves the performance of sows and newborn piglets1. *Journal of Animal Science, 97*(11), p. 4557-4566. doi:10.1093/jas/skz292

Wei, S., Yang, Y., Xiao, Y., Liu, Y., Tian, J., Spicer, L. J., & Feng, T. (2020). Effects of N-carbamylglutamate on steroidogenesis and relative abundances of mRNA transcripts in pig placental trophoblasts. *Animal Reproduction Science, 221*, p. 106569. doi:j.anireprosci.2020.106569

Wilson, G. J., Wilson, J. M., & Manninen, A. H. (2008). Effects of beta-hydroxy-beta-methylbutyrate (HMB) on exercise performance and body composition across varying levels of age, sex, and training experience: A review. *Nutrition & Metabolism, 5*(1), p. 1. doi:10.1186/1743-7075-5-1

Wójcik, R., Małaczewska, J., Siwicki, A. K., Micińskim, J., & Zwierzchowski, G. (2014). The effect of ß-hydroxy-ß-methylbutyrate (HMB) on selected parameters of humoralimmunity in calves. *Polish journal of veterinary sciences, 17*(2), p. 357-359. doi:10.2478/pjvs-2014-004

Wu, G., Bazer, F. W., Cudd, T. A., Jobgen, W. S., Kim, S. W., Lassala, A., Li, P., Matis, J. H., Meininger, C. J., & Spencer, T. E. (2007). Pharmacokinetics and Safety of Arginine Supplementation in Animals. *The Journal of Nutrition, 137*(6), p. 1673S-1680S. doi:10.1093/jn/137.6.1673s

Wu, G., Bazer, F. W., Cudd, T. A., Meininger, C. J., & Spencer, T. E. (2004a). Maternal nutrition and fetal development. *The Journal of Nutrition, 134*(9), p. 2169-2172. doi:10.1093/jn/134.9.2169

Wu, G., Bazer, F. W., Johnson, G. A., & Hou, Y. (2018). BOARD-INVITED REVIEW: Arginine nutrition and metabolism in growing, gestating, and lactating swine. *Journal of Animal Science, 96*(12), p. 5035-5051. doi:10.1093/jas/sky377

Wu, G., Bazer, F. W., Satterfield, M. C., Li, X., Wang, X., Johnson, G. A., Burghardt, R. C., Dai, Z., Wang, J., & Wu, Z. (2013). Impacts of arginine nutrition on embryonic and fetal development in mammals. *Amino acids, 45*(2), p. 241-256. doi:10.1007/s00726-013-1515-z

Wu, G., Bazer, F. W., Wallace, J. M., & Spencer, T. E. (2006). Board-invited review: intrauterine growth retardation: implications for the animal sciences. *Journal of Animal Science, 84*(9), p. 2316-2337. doi:10.2527/jas.2006-156

Wu, G., Knabe, D. A., & Kim, S. W. (2004b). Arginine Nutrition in Neonatal Pigs. *The Journal of Nutrition, 134*(10), p. 2783S-2790S. doi:10.1093/jn/134.10.2783s

Wu, G., & Morris, S. M. (1998). Arginine metabolism: nitric oxide and beyond. *Biochemical Journal, 336*(1), p. 1-17. doi:10.1042/bj3360001

Wu, W. Z., Wang, X. Q., Wu, G. Y., Kim, S. W., Chen, F., & Wang, J. J. (2010a). Differential composition of proteomes in sow colostrum and milk from anterior and posterior mammary glands. *Journal of Animal Science, 88*(8), p. 2657-2664. doi:10.2527/jas.2010-2972

Wu, X., Ruan, Z., Gao, Y., Yin, Y., Zhou, X., Wang, L., Geng, M., Hou, Y., & Wu, G. (2010b). Dietary supplementation with L-arginine or N-carbamylglutamate enhances intestinal growth and heat shock protein-70 expression in weanling pigs fed a corn-and soybean meal-based diet. *Amino acids, 39*(3), p. 831-839.

Wu, X., Wan, D., Xie, C., Li, T., Huang, R., Shu, X., Ruan, Z., Deng, Z., & Yin, Y. (2015). Acute and sub-acute oral toxicological evaluations and mutagenicity of N-carbamylglutamate (NCG). *Regulatory Toxicology and Pharmacology, 73*(1), p. 296-302. doi:10.1016/j.yrtph.2015.07.009

Wu, X., Yin, Y. L., Liu, Y. Q., Liu, X. D., Liu, Z. Q., Li, T. J., Huang, R. L., Ruan, Z., & Deng, Z. Y. (2012). Effect of dietary arginine and N-carbamoylglutamate supplementation on reproduction and gene expression of eNOS, VEGFA and PlGF1 in placenta in late pregnancy of sows. *Animal Reproduction Science, 132*(3), p. 187-192. doi:10.1016/j.anireprosci.2012.05.002

Yang, C., Rooke, J. A., Cabeza, I., & Wallace, R. J. (2016). Nitrate and inhibition of ruminal methanogenesis: microbial ecology, obstacles, and opportunities for lowering methane emissions from ruminant livestock. *Frontiers in Microbiology, 7*, p. 132.

Ye, C., Zeng, X., Zhu, J., Liu, Y., Ye, Q., Qiao, S., & Zeng, X. (2017). Dietary N-Carbamylglutamate Supplementation in a Reduced Protein Diet Affects Carcass Traits and the Profile of Muscle Amino Acids and Fatty Acids in Finishing Pigs. *Journal of Agricultural and Food Chemistry, 65*(28), p. 5751-5758. doi:10.1021/acs.jafc.7b02301

Yonamine, C. Y., Teixeira, S. S., Campello, R. S., Gerlinger-Romero, F., Rodrigues Jr, C. F., Guimarães-Ferreira, L., Machado, U. F., & Nunes, M. T. (2014). Beta hydroxy beta methylbutyrate supplementation impairs peripheral insulin sensitivity in healthy sedentary Wistar rats. *Acta Physiologica, 212*(1), p. 62-74. doi:10.1111/apha.12336

Zeng, X., Huang, Z., Zhang, F., Mao, X., Zhang, S., & Qiao, S. (2015). Oral administration of N-carbamylglutamate might improve growth performance and intestinal function of suckling piglets. *Livestock Science, 181*, p. 242-248. doi:10.1016/j.livsci.2015.09.004

Zeng, X., Mao, X., Huang, Z., Wang, F., Wu, G., & Qiao, S. (2013). Arginine enhances embryo implantation in rats through PI3K/PKB/mTOR/NO signaling pathway during early pregnancy. *Reproduction, 145*(1), p. 1-7.

Zhang, B., Che, L., Lin, Y., Zhuo, Y., Fang, Z., Xu, S., Song, J., Wang, Y., Liu, Y., Wang, P., & Wu, D. (2014). Effect of Dietary N-Carbamylglutamate Levels on Reproductive Performance of Gilts. *Reproduction in Domestic Animals, 49*(5), p. 740-745. doi:10.1111/rda.12358

Zhang, F., Zeng, X., Yang, F., Huang, Z., Liu, H., Ma, X., & Qiao, S. (2013). Dietary N-Carbamylglutamate Supplementation Boosts Intestinal Mucosal Immunity in Escherichia coli Challenged Piglets. *PloS one, 8*(6), p. e66280. doi:10.1371/journal.pone.0066280

Zhang, H., Sun, L., Wang, Z., Deng, M., Nie, H., Zhang, G., Ma, T., & Wang, F. (2016). N-carbamylglutamate and L-arginine improved maternal and placental development in underfed ewes. *Reproduction, 151*(6), p. 623-635. doi:10.1530/rep-16-0067

Zhang, H., Zhao, F., Peng, A., Dong, L., Wang, M., Yu, L., Loor, J. J., & Wang, H. (2018). Effects of Dietary l-Arginine and N-Carbamylglutamate Supplementation on Intestinal Integrity, Immune Function, and Oxidative Status in Intrauterine-Growth-Retarded Suckling Lambs. *Journal of Agricultural and Food Chemistry, 66*(16), p. 4145-4154. doi:10.1021/acs.jafc.8b00726

Zhu, C., Guo, C.-Y., Gao, K.-G., Wang, L., Chen, Z., Ma, X.-Y., & Jiang, Z.-Y. (2017). Dietary arginine supplementation in multiparous sows during lactation improves the weight gain of suckling piglets. *Journal of Integrative Agriculture, 16*(3), p. 648-655. doi:10.1016/s2095-3119(16)61426-0

Zhu, J., Zeng, X., Peng, Q., Zeng, S., Zhao, H., Shen, H., & Qiao, S. (2015). Maternal N-carbamylglutamate supplementation during early pregnancy enhances embryonic survival and development through modulation of the endometrial proteome in gilts. *The Journal of Nutrition, 145*(10), p. 2212-2220.

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Appendices

Appendix 1: Supplementary Tables and Figures

Table S1: Number of sows in each parity group per dietary treatment.

n	Parity Group			
Diet	1,2	3,4	5,6	≥7
CON	40	26	25	17
ARG	37	24	28	12
HMB	40	30	26	11
NCG	43	34	22	14
CAN	38	28	28	14

Table S2: Number of sows in each total born (TB) group per dietary treatment.

n	TB Group			
Diet	≤12	13,14	15,16	≥17
CON	33	26	19	29
ARG	30	23	22	17
HMB	25	39	21	17
NCG	27	24	26	26
CAN	32	27	27	19

Table S3: Results from the pairwise comparisons of the diet x parity group interaction (LSD method with a Bonferroni adjustment for multiple comparisons) for litter number at day 25 of lactation. Comparisons were only made within the 2nd (parities 3 and 4) and 4th (parities ≥ 7) groups between CON vs. other diets and ARG vs. other diets (NCG, HMB, or CAN) with a total of 14 comparisons made.

Pairwise Comparisons[a]

Par Grp	(I) TMT	(J) TMT	Mean Difference (I-J)	Std. Error	df	Sig.[c]	95% Confidence Interval for Difference[c]	
							Lower Bound	Upper Bound
Parities 3,4	CON	ARG	-.803	.592	386	1.00	-1.966	.361
		HMB	-.604	.542	386	1.00	-1.670	.463
		NCG	-1.626*	.529	386	0.028	-2.666	-.586
		CAN	-.783	.554	386	1.00	-1.873	.307
	ARG	CON	.803	.592	386		-.361	1.966
		HMB	.199	.574	386	1.00	-.930	1.327
		NCG	-.823	.563	386	1.00	-1.930	.283
		CAN	.019	.587	386	1.00	-1.135	1.174
	HMB	CON	.604	.542	386		-.463	1.670
		ARG	-.199	.574	386		-1.327	.930
		NCG	-1.022*	.510	386		-2.025	-.019
		CAN	-.179	.537	386		-1.235	.876
	NCG	CON	1.626*	.529	386		.586	2.666
		ARG	.823	.563	386		-.283	1.930
		HMB	1.022*	.510	386		.019	2.025
		CAN	.843	.520	386		-.181	1.866
	CAN	CON	.783	.554	386		-.307	1.873
		ARG	-.019	.587	386		-1.174	1.135
		HMB	.179	.537	386		-.876	1.235
		NCG	-.843	.520	386		-1.866	.181
Parities ≥ 7	CON	ARG	-.798	.800	386	1.00	-2.370	.775
		HMB	1.745*	.799	386	0.42	.174	3.317
		NCG	.826	.778	386	1.00	-.704	2.355
		CAN	1.795*	.756	386	0.25	.308	3.282
	ARG	CON	.798	.800	386		-.775	2.370
		HMB	2.543*	.854	386	0.042	.865	4.222
		NCG	1.623	.834	386	0.73	-.016	3.263
		CAN	2.593*	.816	386	0.028	.989	4.197

HMB	CON	-1.745*	.799	386		-3.317	-.174
	ARG	-2.543*	.854	386		-4.222	-.865
	NCG	-.920	.835	386		-2.561	.721
	CAN	.050	.815	386		-1.552	1.652
NCG	CON	-.826	.778	386		-2.355	.704
	ARG	-1.623	.834	386		-3.263	.016
	HMB	.920	.835	386		-.721	2.561
	CAN	.970	.794	386		-.592	2.531
CAN	CON	-1.795*	.756	386		-3.282	-.308
	ARG	-2.593*	.816	386		-4.197	-.989
	HMB	-.050	.815	386		-1.652	1.552
	NCG	-.970	.794	386		-2.531	.592

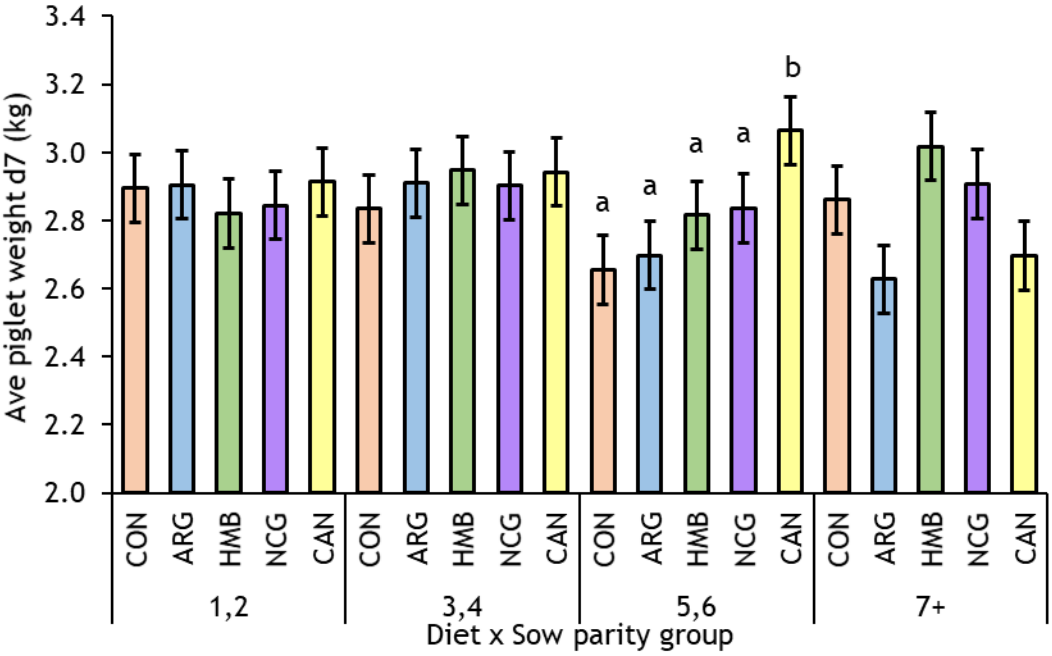
Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Dependent Variable: LITTERNO_25D.

c. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Figure S4: Average piglet weight at day 7 of lactation each parity group. Results are shown as the interaction (least squares) means from the linear mixed model analysis. Different superscripts denote a significant (*P* < 0.05) pairwise difference between two dietary treatments within a parity group with a Bonferroni adjustment for multiple comparisons (only CON vs. other diets and ARG vs. other diets comparisons were made). See text for dietary treatment details.


Ave piglet weight d7 (kg)
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
a
a
a
a
b
CON
ARG
HMB
NCG
CAN
1,2
3,4
5,6
7+
Diet x Sow parity group