

Nutritional manipulation of the somatotropic axis in grower and finisher pigs

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Executive Summary

Porcine somatotropin (pST) has been used in the pig industry for many years to improve daily weight gain, feed efficiency and P2 fat depth, through its actions on promoting lean growth. However, there is growing interest in providing other options to stimulate lean growth. This experiment looked at two nutritional strategies to stimulate the endogenous somatotrophic system; medium chain fatty acid oil (MCFA) supplementation and cysteamine hydrochloride (CSH) supplementation.

The research objective of Phase 1 of the project was to compare the effectiveness of 4 levels (0%, 1%, 3% & 6%) of dietary MCFA supplementation in the diets of Large White x Landrace grower/finisher male pigs from week 10 to week 22 post-partum on: a) increasing circulating somatotropin concentrations; and b) increasing growth and feed efficiency. Daily weight gain, feed intake and feed conversion ratio were not affected by the inclusion of MCFA oil in the diets, at any of the three levels (1%, 3% or 6%), compared to the control diet that contained about 6% canola oil. Gross energy analysis of the diets confirmed that they were fed at iso-energetic and isonitrogenous levels between treatments. Plasma metabolite and metabolic hormone analysis of pooled blood samples taken every 15 mins for 4 hours at week 17 of age (42 days on treatment diets) indicated that MCFA oil supplementation increased both circulating active ghrelin concentrations and somatotropin concentrations, in a dose dependent manner. Findings from Phase 1 showed that there was a significant effect of MCFA supplementation, particularly at the highest level of 6%, in terms of stimulating the somatotrophic system.

In Phase 2, 64 Large White x Landrace female pigs, of approximately 60 kg live weight, were allocated to one of three treatments; control diet (n = 22), MCFA diet (6% w/w in feed; n = 21) and CSH diet (70 mg/kg in feed; n = 21) for a period of approximately 4 to 5 weeks. Blood samples for metabolic analyses were collected from a subset of animals (n ≈ 6 per treatment) 3 weeks into the treatments. Average daily gain and feed conversion were not affected by the dietary treatments, however MCFA pigs had 19% lower ($P < 0.005$) and CSH pigs had 14% lower ($P < 0.05$) P2 backfat depths compared to the controls. Plasma concentrations of somatotropin, IGF-1, insulin other blood metabolites were not affected by the dietary treatments, but the MCFA treatment group had 20% higher plasma concentration of active ghrelin than the control pigs (approaching significance at $P = 0.12$). In conclusion, although there was no stimulation of the endogenous somatotropin, and no effect on weight gain or feed intake, by either the MCFA or CSH treatments, the decreased backfat depth indicated that there was an improvement in lean meat yield. This latter finding encourages further investigation of these nutritional alternatives to pST injections. One may never be able to stimulate somatotropin to sufficient levels with dietary MCFA to compare with a pST injection; however a small benefit in lean growth, that has welfare and labour benefits, might be attractive to some growers/consumers.

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1. Introduction

The need to optimise labour costs and increasing consumer and welfare concerns about daily injections of exogenous hormones, has led to an interest in alternative methods to exogenous porcine somatotropin (pST) treatment to improve growth performance in pigs. Somatotropin, also known as growth hormone (GH), is a protein hormone secreted from the pituitary gland (Etherton and Bauman 1998). Secretion is regulated by two hormones that act to stimulate (via growth hormone-releasing hormone, GHRH) or inhibit (via growth hormone inhibiting hormone, GHIH, aka somatostatin) the release of somatotropin (Etherton and Bauman 1998). The overall effects of somatotropin are to enhance the ability of muscle (growth) to utilize nutrients, while simultaneously coordinating other physiological processes and tissues (such as adipose tissue), in a manner that supports enhanced lean growth (Etherton and Bauman 1998).

To address the issues related to daily injections of pST, researchers have started to investigate dietary means of increasing endogenous somatotropin levels. Numerous studies have found that dietary inclusion of the sulfhydryl compound cysteamine hydrochloride (CSH) increases somatotropin secretion in rats, sheep and fish (see Dunshea 2007). The increase in somatotropin secretion is presumably due to the inhibitory effect of CSH on somatostatin (GHIH) release. Yang et al. (2005) found that dietary supplementation of CSH at 30 mg/kg and 50 mg/kg live weight resulted in positive effects on average daily gain in finisher pigs, with an optimal response occurring at 30 mg/kg (+13.8% gain), but had no effect on plasma concentrations of somatotropin. Dunshea (2007) found that dietary supplementation of CSH at 70 mg/kg feed in finisher gilts caused a modest increase in daily gain (+ 7.4%), but somatotropin levels were not measured in this study. McElwain et al. (1999) found that intravenous injection of 25, 50 or 75 mg CSH per kg live weight actually decreased plasma somatotropin concentrations in a dose dependent manner.

Data from Phase 1 indicated that the use MCFA supplementation in the diet of grower/finisher pigs increased circulating somatotropin levels, presumably as a consequence of increases in the bio-activation of the metabolic hormone, ghrelin. Ghrelin is a somatotropin-releasing peptide that was first isolated from the stomach of rats (Vizcarra et al. 2007), and it has been reported to have a stimulating effect on somatotropin release from the pituitary gland (Dong et al. 2009). Salfen et al. (2004) showed that ghrelin infusion for 5 days positively influenced weight gain and concomitantly increased somatotropin and insulin secretion in weaner pigs. The major active form of ghrelin is a 28 amino acid peptide containing an octanoic (C8) fatty acid on the third serine in the peptide, a modification that is essential for biological activity to allow binding to its receptor (Nishi et al. 2005). Recently it was reported that ingested MCFAs (containing high levels of C8) are directly utilised for the bio-activation of ghrelin in rats (Nishi et al. 2005).

The study of Phase 1 demonstrated in grower/finisher pigs that a 6% MCFA dietary incorporation was optimum (compared to 1% and 3%) for increasing the bio-

activation of ghrelin and increasing circulating somatotropin concentrations. However, there was no effect on growth performance in this study. A possible reason for the lack of effect may have been that the small sample size (n=15) in this dose-response study was insufficient to pick up modest effects on growth performance of less than about 100 g/day. Another possible reason for the lack of effect may have been that the level of somatotropin stimulation by the 6% MCFA dietary treatment (2-fold increase compared to the control, to about 14 ng/ml) may not have been sufficient to affect growth significantly. Hansen et al. (1997) found that a daily pST injection in grower pigs that raised endogenous somatotropin concentrations to about 14 ng/ml had no effect on muscle protein accretion, whereas Klindt et al. (1992) found that pST implants that raised endogenous somatotropin concentrations in castrated male pigs to about 30 ng/ml resulted in a 22% increase in average daily gain. The effect of the level of somatotropin stimulation and resulting growth performance with MCFA and CSH dietary supplementation has not been studied.

The purpose of Phase 2 of the study was to compare the effectiveness of added dietary MCFA at 6% and a cysteamine hydrochloride (CSH) dietary supplement in the grower/finisher pig from week 16 to week 21 post-partum on: circulating somatotropin, IGF-1, insulin and ghrelin concentrations; growth and feed efficiency data; commercial carcass attributes (P2 fat depth, dressing percentage).

Hypotheses:

1. The addition of 6% MCFA to the diets of grower/finisher pigs will increase endogenous somatotropin levels and increase the rate of weight gain and improve feed efficiency.
2. The addition of 70 mg/kg feed of CSH to the diets of grower/finisher pigs will increase endogenous somatotropin levels and increase the rate of weight gain and improve feed efficiency.

2. Methodology

This study was approved by the Animal Ethics Committees at both Murdoch University and the WA Department of Agriculture and Food to ensure compliance with the guidelines of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Experimental design (Phase 1)

The initial aim was to compare the effectiveness of 3 levels of dietary MCFA in the grower/finisher pig. From week 14 post-partum, male pigs (n=15/group) received: standard commercial diet (control), or one of three MCFA dietary incorporation rates (1%, 2% & 4% - fed at iso-caloric levels), till week 22 post-partum. All diets contained adequate energy, protein and lysine (based on wheat and soybean meal) to allow an endogenous pST increase to have an effect. At week 17 post-

partum, a subgroup of pigs (n=6 per treatment) selected based on growth rates to reflect the group average, had temporary catheters inserted in the vein maxillaris by means of insertion through an ear vein (auricularis lateralis or auricularis rostralis). The ear vein cannulation technique is similar to that described in detail by Zanella & Mendl (1992). Catheters were kept patent in between blood samplings by flushing with approximately 1 ml of 5 IU/ml heparin solution in sterile saline (0.9% w/v Baxter Healthcare, QLD, Australia code # AHF7123). The first 2 ml of blood withdrawn at each sampling was removed to avoid dilution of the sample with the previous flush, and replaced prior to the subsequent flush by means of a three-way tap. The following day, repeated blood samples (1 ml) were collected at 15 min intervals for 4 hours into EDTA-tubes. The tubes were immediately centrifuged at 12,000g for 10 minutes, and aliquots of the plasma were pooled for each animal and immediately stored at -20°C. Pooled aliquots from these samples were used to measure somatotropin, active ghrelin, insulin, IGF-I and glucose. Body weight and feed intake was recorded weekly.

Experimental design (Phase 2)

The experiment initially involved 64 crossbred (Large White x Landrace) female pigs, which were acclimatised and grown in individual pens from an approx. age of 10 weeks at the Medina Research Centre. The pigs were weighed, ear-tagged, and allocated at random to treatments in a randomised complete block design having 3 treatments: consisting of a control group (commercial diet); a 6% MCFA dietary supplement group (chosen from findings of Phase 1); and a CSH (cysteamine hydrochloride) 70 mg/kg feed dietary supplement group, with 21 pigs allocated to each treatment (22 in control group). At 19 weeks of age blood samples were taken to assess circulating somatotropin, active ghrelin, IGF-1 and other markers of metabolic status.

Experimental Diets (Phase 2)

Dietary treatments commenced from 16 weeks of age and continued until approx week 19 to 22 post-partum. All diets (Appendix 1) were designed to be iso-energetic (by the addition of canola oil to the control and CSH diets), and contained adequate protein and lysine (i.e. 0.65 g available lysine/MJ) to allow an endogenous pST increase to have an effect on muscle protein synthesis (pers. comm. Prof Frank Dunshea, University Melbourne). Live weight was recorded weekly, and feed refusals were monitored daily. At the end of the experiment the pigs were sent to a commercial abattoir where basic information on carcass characteristics was obtained (e.g. P2 fat depth, carcass weight, dressing percentage).

Dietary gross energy (GE) content was determined using a ballistic bomb calorimeter (SANYO Gallenkamp, Loughborough, Leics, UK).

Blood Sampling (Phase 2)

A sub-group of pigs from the 3 treatment groups (n=19: 6 control; 7 MCFA; and 6 CSH), selected based on growth rates to reflect the group average, had temporary

catheters inserted in the vein maxillaris by means of insertion through an ear vein (auricularis lateralis or auricularis rostralis) at week 19. The following day, repeated blood samples (1 ml) were collected at 15 min intervals for 4 hours into EDTA-tubes. The tubes were immediately centrifuged at 12,000g for 10 minutes, and aliquots of the plasma were pooled for each animal and immediately stored at -20°C. Pooled aliquots from these samples were used to measure somatotropin, IGF-I, active-ghrelin, insulin, glucose, urea, glycerol, triglycerides and free fatty acids.

Analysis of Blood Samples (Phase 2)

Pooled plasma samples were assayed in duplicate for IGF-I using a commercial ELISA kit (Human IGF-I DG100, Lot 267244, R & D Systems, Minneapolis USA). The kit was previously validated for use with porcine samples by running porcine standards and testing for parallelism. The sensitivity of the assay was 0.026 ng/ml with an intra-assay precision of 3.5% (CV).

Pooled plasma samples were assayed in duplicate for somatotropin using a commercial ELISA kit (Active Mouse/Rat GH DSL 10-72100, Lot 891185, Diagnostic Systems Laboratories Inc., Texas USA). The kit was previously validated for use with porcine samples by running porcine standards and testing for parallelism.

Pooled plasma samples were assayed in duplicate for active-ghrelin using a commercial ELISA kit (Human Ghrelin (active) EZGRA - 88K, Lot 1635980, Millipore, Billerica MA USA). The kit was previously validated for use with porcine samples by running porcine standards and testing for parallelism. The sensitivity of the assay was 25 pg/ml with an intra-assay precision of 3.86% (CV).

Pooled plasma samples were assayed in duplicate for insulin using a commercial ELISA kit (Porcine Insulin 10-1200-02, Lot 15719, Mercodia AB, Uppsala SWEDEN). The sensitivity of the assay was 0.062 µg/ml with an intra-assay precision of 3.5% (CV).

Pooled plasma samples were analysed for glycerol, triglycerides, glucose and free fatty acids levels by an enzymatic colorimetric method using Glycerol kinase (Roche Diagnostics, Indianapolis, IN, USA), Lipase and Glycerol kinase (Roche Diagnostics, Indianapolis, IN, USA), Hexokinase (Roche Diagnostics, Indianapolis, IN, USA) and acyl-CoA synthetase (WAKO NEFA-C Kit, Novachem Pty Ltd, Collingwood, Vic, Australia), respectively.

Statistical Analysis (Phase 2)

The sample size of 21 (Phase 2) was estimated using Power calculations based on standard deviation values taken from similar experiments, and an expected modest level of daily weight gain deemed significant at 65g/day. Data were analysed by using the General Linear Model procedures of SAS according to a randomised design having three treatments. The somatotropin data was found not to be in a normal distribution, hence it was log transformed to achieve normality prior to analysis. A level of probability of less than 0.05 indicated a statistical

difference between treatments. Treatment means were compared using post-hoc analysis using Fisher's-protected least significant difference test. Pigs that were removed (n = 2 from the control treatment) from the experiment due to health issues were not included in the analyses. Starting live weight was used as a covariate.

3. Outcomes

Phase 1

During the whole trial daily weight gain, feed intake and feed conversion ratio were not affected by the inclusion of MCT in the diets, at any of the three levels (1%, 3% & 6%), compared to the control diet that contained 6% canola oil. Plasma metabolite and metabolic hormone analysis of pooled blood samples taken every 15 mins for 4 hours at week 17 of age (42 days on treatment diets) indicated that MCT inclusion increased both circulating active ghrelin concentrations, GH and insulin concentrations, in a dose dependent manner (Fig 1). MCT inclusion in the diet had no effect on circulating IGF-I levels (Fig. 1).

The deliverables from Phase 1 of the trial were that we indeed found a significant effect of MCT inclusion, particularly at the highest level of 6%, on circulating ghrelin and GH concentrations. The lack of difference in growth performance may have been related to the lack of IGF-1 response, or to a more subtle effect that wasn't able to be picked up with the animal numbers (designed to pick up a difference in ADG to the control of 100 g/day). Findings were used in the design of Phase 2 in that the 6% dose of MCT was chosen as the most effective.

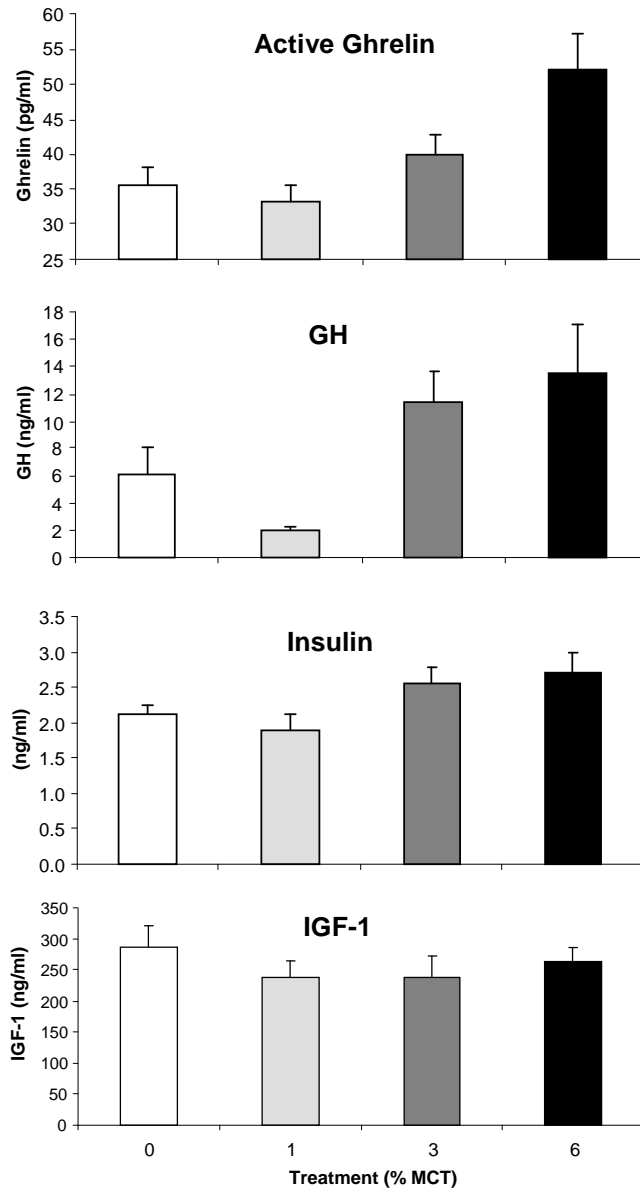


Figure 1: Circulating plasma concentrations of active ghrelin, growth hormone (GH), insulin and insulin-like growth factor-1 (IGF-1) for grower/finisher pigs supplemented with 0, 1, 3 or 6% dietary MCT.

Phase 2

Growth Performance

Table 1: Mean values of the Performance data collected from the experimental pigs over the length of the experiment (n = 62; control n = 20, MCFA n = 21, CSH n = 21).

Treatment	Control	MCFA	CSH	SEM	P-value
LW start, kg	61.6	62.2	62.2	0.47	0.59
LW end, kg	92.8	92.1	93.6	0.89	0.45
ADFI, g/d	2.81	2.67	2.79	0.05	0.11
ADG, g/d	1061	944	1030	38.5	0.10
FCR, kg/kg	2.75	2.89	2.74	0.09	0.46
P2 backfat, mm	13.1 ^a	10.5 ^b	11.2 ^b	0.59	0.01
HSCW, kg	62.4	61.2	61.8	0.72	0.54

LW start = live weight at the start of the treatments, LW end = live weight at the end of the treatments, ADFI = average daily feed intake, ADG = average daily gain, FCR = feed conversion ratio, P2 = the position of backfat measurement 65 mm down the left side from the midline at the level of the head of the last rib, HSCW = hot standard carcass weight, SEM = Standard error of the mean. Values without the same superscript differ significantly, $P < 0.05$.

There was no difference between treatments for starting live weight, final live weight, feed conversion ratio, or hot standard carcass weight (Table 1). There was a trend towards overall significance between treatments for average daily feed intake ($P = 0.11$) with the MCFA pigs consuming about 5 % less than the control pigs ($P = 0.05$), and a trend towards overall significance between treatments for average daily gain ($P = 0.10$) with the MCFA gaining 11% less than the control pigs ($P = 0.04$).

There was an overall significant difference between treatments for P2 backfat ($P < 0.01$) with the MCFA pigs having a 19% lower ($P < 0.005$) and the CSH pigs having a 14% lower backfat score compared to the control pigs (Table 1).

There was no difference in the number of days (in the experiment) to reach slaughter weight between the three treatments (Control = 37.8 ± 1.69 ; MCFA = 37.1 ± 1.86 ; CSH = 36.2 ± 1.47 days).

Metabolic Effects

Table 2: Hormonal assay data collected from a subset (n = 19) of experiment pigs at week 19 (control n = 6, MCFA n = 7, and CSH n = 6).

Treatment	Control	MCFA	CSH	SEM	P-value
IGF-1 ng/ml	1.29	1.36	1.39	0.12	0.89
Ghrelin pg/ml	213 ^b	259 ^b	184 ^a	20.2	0.05
Insulin µg/l	0.12	0.12	0.16	0.02	0.24
Glucose (mmol/L)	5.22	5.12	5.28	0.17	0.93
Triglyceride (mmol/L)	0.70	0.85	0.60	0.11	0.28
FreeFattyAcids (mmol/L)	0.14	0.12	0.10	0.02	0.30
Glycerol (µmol/L)	16.4	13.6	13.1	2.04	0.52

SEM = Standard error of the mean. Values without the same superscript differ significantly, P < 0.05

There was an overall significant difference (P = 0.05) between treatments in the mean plasma concentration of active ghrelin in the pigs (Table 2). Specifically, the MCFA pigs had 40% higher levels of active ghrelin than the CSH pigs (P < 0.05), and 20% higher levels than the control pigs, approaching significance at P = 0.12. All other metabolic markers (IGF-1, insulin, glucose, triglyceride, free fatty acids, and glycerol) had no difference between treatment groups.

Somatotropin Effects

Table 3: Transformed somatotropin data that was collected at week 19 (total n = 19; control n = 6, MCFA n = 7, and CSH n = 6).

	Control	MCFA	CSH
GH ng/ml	10.0	8.27	10.4
95% conf.	(4.1 -24.8)	(3.3-20.4)	(4.2-25.6)

There was no difference between the treatment groups for mean plasma concentration of somatotropin (Table 2.3).

Discussion

The hypothesis, that addition of 6% MCFA or CSH to the diets of grower/finisher pigs would increase endogenous somatotropin levels, increase the rate of weight gain and improve feed efficiency, was not supported. There was no effect of the MCFA or CSH diet on plasma somatotropin concentrations, ADG or FCE (feed conversion efficiency). However, the MCFA diet did increase circulating levels of active ghrelin and both the MCFA and CSH diets decreased the P2 fat depth. The reason for the lack of full activation of the somatotropic system is not known, however the lack of somatotropin stimulation agrees with numerous studies looking into pST treatment of pigs, in that increases in plasma somatotropin concentrations, to about 25 ng/ml, are needed to see an increase in ADG and FCE (Etherton et al. 1987). Possible reasons for the lack of a somatotropin response in the MCFA and CSH supplemented animals could include: 1) the proposed mechanism of stimulation of somatotropin did not operate in these pigs; 2)

insufficient stimulation to cause an increase in somatotropin levels; 3) insufficient numbers of animals to pick up a modest increase in somatotropin and/or growth stimulation. It is also possible that our MCFA and CSH treatments may have initially increased somatotropin levels via the proposed mechanisms at the beginning of the experiment, but didn't sustain somatotropin levels by the time of the blood sampling period.

There was a significant reduction in P2 backfat depth with the MCFA and CSH treatments. As stated above, this was not accompanied by an increase in somatotropin levels, so a somatotropin-stimulated increase in lipolysis, as suggested by researchers using pST injections (Vestergaard et al. 2008), appears not to account for this effect. There are a number of possibilities for the reduction in P2 backfat. Firstly, the reduction in adiposity may be a result of the non-significant decrease in food intake. However, as this was not a significant effect it is unlikely to be responsible for the 19% decrease in P2 backfat depth. Secondly, the ghrelin increase in the MCFA group may have directly stimulated lipolysis. Ghrelin has also been demonstrated to be capable of stimulating lipolysis in rodents and dairy cows (Vestergaard et al. 2008; Roche et al. 2008). Thirdly, the MCFA and CSH treatments may have increased the sensitivity of the adipose tissue to the somatotropic/adrenergic system (Houseknecht et al. 1995). Somatotropin is a chronic, homeorhetic effector of adipose tissue metabolism that has been shown to enhance the lipolytic response to an adrenaline challenge in lactating cows, growing steers, and growing pigs (Houseknecht et al. 1995). It is possible that in our study, even though circulating somatotropin levels were not altered, that adrenaline sensitivity of the adipose tissue may have been altered, leading to the decline in P2 fat depth. Finally, Yang and colleagues (2005), studying CSH effects in pigs, also found a decrease in backfat without effects on somatotropins. They stated that the CSH effects on backfat could have been mediated through the anabolic effects of glucagon and thyroid hormone, which increased in their study, on the skeletal muscles and catabolic effects on the adipose tissue. Glucagon and thyroid hormone weren't measured in the present study. These scenarios warrant further investigation.

4. Application of Research

Application of the research findings in the commercial world.

Commercial application of the dietary supplements of both MCFA or CSH would theoretically be easily achievable. Indeed, CSH is already commercially offered as the product Porcinin, and commercial trials using palm oil (another source of C8 fatty acids) are currently being assessed.

Opportunities uncovered by the research

There is the possibility that the modest benefit in lean growth, that's welfare, consumer and labour friendly, might be attractive to some growers/consumers.

Commercialization/Adoption Strategies

- The significant 19% reduction in P2 backfat with the MCFA diet, and the 14% reduction with the CSH diet, compared to the control pigs, would have potential benefits to cost of production in terms of improving lean growth.
- The ease of adoption by producers in using an MCFA-like nutritional supplement would relate to the cost of C8-rich feed additives such as palm and coconut oil, the level of supplementation needed, and the cost of infrastructure to incorporate the supplement into diets.
- Further understanding of the mechanism of action may lead to future application for commercial patenting.

5. Conclusion

Although there was no effect of MCFA on growth performance or stimulation of the somatotropic axis, there was also no effect of the commercial CSH product (Porcinin), indicating that either the design was insufficient to pick up modest changes in somatotropin levels and weight gain, or that the effects of MCFA and CSH on backfat are acting independently of somatotropic action. The MCFA and CSH treatments did increase lean growth, and this therefore encourages further investigation of these nutritional alternatives to pST injections. One may never be able to stimulate somatotropin to sufficient levels with dietary MCFA to compare with a pST injection; however a modest benefit in lean growth, that's welfare, consumer and labour friendly, might be attractive to some growers/consumers.

6. Limitations/Risks

Further testing of the exact mechanistic action of MCFA and CSH dietary supplements is advisable, using robust experimental designs.

7. Recommendations

As a result of the outcomes in this study, it is evident that MCFA and CSH are not as effective as pST in stimulating lean tissue growth rate in finishing pigs and cannot therefore be recommended as replacements at the present time. Nevertheless, the marked decrease in P2 backfat may be of national interest.

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Appendices

Appendix 1: Table 3.2: Feed composition (calculated) of the experimental diets

Ingredient %	Diets		
	Control	MCFA	CSH
Barley	41.292	42.892	41.292
Wheat	12.705	9.310	12.635
Lupins	15.000	15.000	15.000
Soybean meal	18.451	19.817	18.451
Meat Meal	6.626	5.492	6.626
Canola Oil	5.000	0.000	5.000
MCFA Oil	0.000	6.000	0.000
Porcinin	0.000	0.000	0.070
Lysine	0.0054	0.0000	0.0054
Methionine	0.0515	0.0532	0.0515
Threonine	0.000	0.0009	0.000
Minerals + Vitamins	0.070	0.0700	0.070
Limestone	0.100	0.665	0.100
Di-calcium Phosphate	0.500	0.5000	0.500
Salt	0.200	0.2000	0.200

Calculated content	Diets		
	Control	MCFA	CSH
DE, MJ/kg	14.40	14.479	14.40
Fat, %	7.715	8.587	7.715
Crude fibre, %	5.519	5.538	5.519
Protein, %	21.70	21.545	21.70
Lysine, %	1.144	1.146	1.144