DETERMINING THE LYSINE REQUIREMENTS OF IMMUNOCASTRATED MALE PIGS

2A-109

Report prepared for the Co-operative Research Centre for an Internationally Competitive Pork Industry

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September 2011

GOVERNMENT OF WESTERN AUSTRALIA

Department of Agriculture and Food

Pork CRC

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Established and supported under the Australian Government's Cooperative Research Centres Program

Executive Summary

Little is known about the lysine requirements of immunocastrates. Traditionally it has been assumed that the requirements of immunocastrates would be similar to those of females of the same genotype since there is little difference in requirements of physical castrates and females. It has been suggested that the requirements of immunocastrates for lysine are likely to be the same as entire males for up to 2 weeks after the second immunisation, however, beyond this point it is likely that lysine requirements may be decreased. Therefore the main aim of this study was to determine the optimal available lysine/MJ digestible energy ratio (g Av Lys:DE) of immunocastrates and how quickly their requirements change following the second vaccination with the immunocastration vaccine.

A dose titration approach was undertaken using five levels of available lysine to energy ratio from 0.32 to 0.75 g Av Lys:DE comparing the response of entire males and immunocastrated males for 6 weeks after the secondary immunisation. Immunocastrates had a higher daily gain at lower lysine levels compared to entire males. They also had a higher feed to gain at higher levels of lysine compared to entire males.

Entire males had a higher lysine requirement than immunocastrates over the six week study period (60 kgs LW until slaughter). Lysine requirements of immunocastrates are similar to entire males for two weeks after the secondary immunisation and then decrease. The results of this experiment have determined the lysine requirements for a current Australian genotype and provided a proposed feeding strategy to maximise performance whilst minimising feed costs.

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1. Introduction

Payment systems in Australia are based on carcass weight and carcass fatness with producers penalised for carcasses with increased backfat. Entire males are preferred by producers as they have better feed conversion and less subcutaneous fat compared to physically castrated pigs. However, associated with the production of entire males is boar taint. Boar taint describes the unpleasant urine or perspiration like odour and flavour that may be detected when pork from entire males is cooked (Jensen *et al*. 1997). It is largely caused by androstenone and skatole, which are compounds stored in fat (Ellis and McKeith, 1993). Boar taint is detrimental to the consumption and demand of Australian pork. However, the castration of entire male pigs reduces androstenone and skatole fat levels to levels under the cut-off values (1.0 and 0.20 μg g^{-1}, respectively) to detect boar taint (McCauley *et al*. 2003).

Some producers have returned to the practice of physical castration in an effort to improve the quality of Australian pork and to meet the requirements of the Singapore export market who will not accept entire males. However, there is increasing focus from welfare groups on the practice of physical castration and the problem of lower feed efficiencies and increased backfat still remains. An alternative to physical castration is immunocastration which involves vaccination with an incomplete analogue of gonadotropin-releasing factor conjugated to a carrier protein in a low reactogenic-adjuvant system (Dunshea *et al*. 2001). The vaccine consists of two doses, one at approximately 10 weeks of age and the other typically administered at 4-5 weeks prior to slaughter. This allows the pig to grow as an entire boar with the associated positive effects on growth and carcass leanness. After secondary vaccination any taint substances present are progressively metabolised. Furthermore, after the second vaccination, the pig has an increase in feed intake and growth rate (Dunshea *et al*. 2001; Cronin *et al*. 2003). However, there may also be an increase in backfat (Oliver *et al*. 2003).

In 2008 less than 10% of males slaughtered in Australia were immunocastrated, however, this number increased considerably in 2009 as producers recognised that eating quality of pork is a serious issue (Hennessy, 2009). While the current recommendation is to give the second vaccination 4 to 5 weeks prior to slaughter recent research at Medina by Lealiifano *et al*. (2009) suggests that boar taint can

be controlled when given just two weeks prior to slaughter. This has the advantage of reducing the increase in backfat associated with immunocastration (Lealiifano *et al*. 2009).

Given the increased interest in immunocastration it is important that the lysine requirements of diets fed to immunocastrates are appropriate as if not correct then this could contribute to an increase in carcass fatness. However, there is no published work on the lysine requirements of immunocastrates (Dunshea, 2009). Under most circumstances it would be assumed that the requirements of immunocastrates would be similar to those of females of the same genotype, since there is little difference in requirements of physical castrates and females. However, recent research by Moore *et al*. (unpub) has found that entire males have higher requirements than females in the finisher period (0.66 vs. 0.63 g AvLys/MJ DE, respectively) and that these requirements are higher then currently used by industry. Dunshea (2009) suggests that requirements of immunocastrates for lysine are likely to be the same as entire males for up to 2 weeks after the secondary immunisation, however, beyond this point it is likely that lysine requirements may be decreased. Determining the lysine requirements of immunocastrates, and the time post second vaccination that they change, will allow establishment of management practices to minimise the associated increase in backfat.

Hypotheses

1. That immunocastrated pigs given the second vaccination 6 weeks pre-slaughter will have an increased growth rate and lower feed conversion ratio as levels of available lysine/MJ digestible energy increase, until a plateau is reached at their genetic potential.
2. Immunocastrated pigs will have a lower optimal available lysine/MJ digestible energy ratio than entire males beyond two weeks after the secondary vaccination.

2. Methodology

The experiment was conducted at the Department of Agriculture and Food Western Australia's (DAFWA) Medina Research Centre. The experimental protocol used was approved by the DAFWA Animal Research Committee and by the Animal Ethics Committee. The animals were handled according to the Australian code of practice for the care and use of animals for scientific purposes (NHMRC, 2004).

A total of four hundred and twenty Large White x Landrace x Duroc entire male and immunocastrated pigs were used in this experiment. The experiment was a 2 x 5 factorial with the main treatments being:
i) sex (entire males and immunocastrates) and;
ii) available lysine to MJ digestible energy (Av Lys/MJ DE) ratio (0.32, 0.43, 0.54, 0.64 and 0.75 g Av Lys/MJ DE).

Allocation and housing

Pigs who had received their priming dose of Improvac® at 10 weeks of age were sourced at approximately 50 kg live weight from a high health status commercial herd whose bloodlines were sourced from the Pig Improvement Company. In order to obtain the required number of pigs it was necessary to bring the pigs to Medina Research Centre in six groups, over a six week period.

Upon arrival at Medina the pigs were identified individually with ear tags, weighed and stratified on their liveweight (LW) before being allocated to treatment. The pigs were housed in groups of 7 in a naturally ventilated grower-finisher facility. All pigs had *ad libitum* access to feed and water.

Diets

The experimental diets were fed for six weeks from 60.1 until slaughter at 107.5 kg LW. The composition of the diets and the ratios used to attain the blended diets using the Feedlogic system are given in Tables 1 and 2, respectively. The second Improvac® vaccination was given six weeks before slaughter.

Table 1: The composition of the diets for the two extreme lysine levels.

Diet	Diet 1 (Low)	Diet 2 (High)
Ingredients (g/kg)		
Barley	517	100
Triticale	50	200
Wheat	255	235
Groats	20.0	100
Mill run	20.0	31.7
Canola meal	10	100
Soyabean meal	50.0	168
Meat meal	20.0	36.2
Tallow	32.9	10
Lysine	0	2.01
Methionine Alimet MHA	0	0.48
Threonine	0	0.80
Minerals and Vitamins	2.50	2.50
Limestone	9.21	6.22
Dical Phosphorus	10.5	5.00
Salt	2.50	2.50
Choline chloride	0.20	0
Nutrient composition[a]		
DE (MJ/kg)	13.5	13.6
Crude protein (%)	12.3	21
Available lysine:DE (MJ/kg)	0.32	0.75

[a] Calculated composition.

Table 2: The blend ratios of the two basal diets to produce the five dietary treatments.

Treatment	Diet 1	Diet 2	MJ DE/kg	Av. Lys/MJ DE
1	100	0	13.5	0.32
2	75	25	13.5	0.43
3	50	50	13.5	0.54
4	25	75	13.5	0.64
5	0	100	13.5	0.75

Six weeks after the diets were implemented the pigs were individually tattooed, removed from feed overnight and transported to a commercial abattoir (approx. 90 minute transport time). The pigs were stunned using a carbon dioxide, dip-lift stunner set at 85% CO_2 for 1.8 minutes (Butina, Denmark). Exsanguination, scalding, dehairing and evisceration were performed using standard commercial procedures. Hot carcase weight (AUSMEAT Trim 13; head off, fore trotters off, hind trotters on; AUS-MEAT Ltd, South Brisbane, Qld, Australia) and P2 backfat depth, 65 mm from the dorsal midline at the point of the last rib (PorkScan) were measured approximately 35 minutes after exsanguination, prior to chiller entry (2ºC, airspeed 4 m/second).

Blood analysis

Blood samples were taken from all pigs in the pen of every second batch (21 pigs) at the commencement of the experimental diets (Day 0), Day 3, 7, 10, 14, 21, 28, 35 and 42. The blood samples were centrifuged at 2,000 g for 10 minutes at 5°C to recover plasma and were stored at -20°C until analysed. Plasma urea nitrogen was quantified using a commercial kit (Olympus Kit Cat. No. OSR6134). The assay was performed on an automated analyser according to the manufacturer's instructions (Olympus AU400; Olympus UK Ltd, Hertfordshire, United Kingdom).

Statistical analysis

Two-way analysis of variance (ANOVA) was performed with the Genstat 12 program to analyse the main effects of sex and diet. Position within the shed was used as a block in the analysis. Linear and quadratic effects for overall daily gain, feed:gain and P2 were also determined using polynomial orthogonal contrasts. Plasma urea nitrogen was analysed by repeated measures analysis of variance. A

level of probability of less than 0.05 was used to determine statistical difference between treatments.

3. Outcomes

Pigs were an average of 60.1 kg LW at the start of the experiment with no difference between entire males and immunocastrates (P>0.05). Immunocastrates were significantly heavier than the entire males (P=0.001) when the experiment ended. The immunocastrates had a higher average daily gain (P=<0.001), increased feed intake (P=<0.001), increased feed to gain (P=0.019), heavier carcass weight (P<0.001), lower dressing percentage (P=0.007) and a higher P2 (P<0.001) compared to the entire males (Table 3 and 4, Figure 1 and 2). These findings are in agreement with Moore *et al*. (2009) and Rikard-Bell *et al*. (2009).

There was no significant difference in initial LW between lysine treatments, however, those on the higher levels of lysine were significantly heavier than those on the lower levels at the end of the experimental period (P=0.375 and P<0.001, respectively). Increasing the level of lysine increased both daily gain and feed to gain in a linear and quadratic manner (P<0.001 and P<0.001, respectively). Pigs that received the lowest levels of lysine grew slower (P<0.001), ate less feed (P=0.026), had a higher feed to gain (P<0.001), lower carcass weight (P<0.001), lower dressing percentage (P<0.001) and a higher P2 (P<0.001) compared to pigs that received the higher levels of lysine. P2 decreased linearly (P<0.001) as the lysine level increased (<0.001).

Immunocastrates had a higher ADG at lower lysine levels compared to entire males (P<0.001). In addition, at higher levels of lysine immunocastrates had a higher F:G compared to entire males (P<0.001).

Table 3: Growth performance and carcass characteristics for entire male and immunocastrated male pigs fed varying levels of Av Lys/MJ DE from 60.1 to 107.5 kgs (n=6).

	Lysine level (g Av Lys/MJ DE)					SED[a]	P-value		
	0.32	0.43	0.54	0.64	0.75		Level	Sex	LxS
Initial LW (kg)									
Male	60.1	60.2	59.9	60.8	60.0	0.694	0.735	0.748	0.790
Immunocastrate	59.9	59.8	60.3	60.3	60.3				
Final LW (kg)									
Male	95.4	102.7	107.7	110.5	110.2	1.344	<0.001	<0.001	<0.001
Immunocastrate	103.8	111.1	109.6	112.3	111.7				
ADG 0 -2 weeks (g)									
Male	754	982	1025	1129	1193	97	<0.001	0.274	0.076
Immunocastrate	863	1027	1022	1182	1107				
ADG 0-3 weeks (g)									
Male	816	982	1052	1136	1178	42	<0.001	<0.001	0.185
Immunocastrate	930	1149	1114	1229	1202				
ADG 0-4 weeks (g)									
Male	831	1018	1078	1161	1211	41	<0.001	<0.001	0.049
Immunocastrate	1005	1196	1152	1221	1259				
ADG 3-6 weeks (g)									
Male	883	1070	1258	1261	1247	43	<0.001	<0.001	<0.001
Immunocastrate	1194	1328	1265	1278	1276				
FI 0 -2 weeks (kg)									
Male	2.21	2.32	2.32	2.41	2.34	0.065	0.039	0.135	0.606
Immunocastrate	2.31	2.41	2.37	2.40	2.32				
FI 0-3 weeks (kg)									
Male	2.31	2.41	2.41	2.49	2.44	0.067	0.013	<0.001	0.720
Immunocastrate	2.51	2.66	2.57	2.67	2.56				
FI 0-4 weeks (kg)									
Male	2.38	2.49	2.49	2.59	2.53	0.074	0.010	<0.001	0.782
Immunocastrate	2.71	2.86	2.77	2.89	2.79				
FI 3-6 weeks (kg)									
Male	2.74	2.83	2.84	3.01	3.01	0.117	0.009	<0.001	0.838
Immunocastrate	3.33	3.53	3.37	3.56	3.58				
FCR 0 -2 weeks									
Male	2.95	2.38	2.28	2.14	1.97	0.09	<0.001	0.254	0.044
Immunocastrate	2.69	2.35	2.32	2.04	2.09				
FCR 0-3 weeks									
Male	2.85	2.47	2.31	2.20	2.08	0.072	<0.001	0.125	0.147

Immunocastrate	2.71	2.32	2.31	2.18	2.15				
FCR 0-4 weeks									
Male	2.89	2.45	2.32	2.23	2.08	0.068	<0.001	0.429	0.005
Immunocastrate	2.70	2.40	2.41	2.37	2.21				
FCR 3-6 weeks									
Male	3.10	2.66	2.26	2.40	2.41	0.097	<0.001	<0.001	<0.001
Immunocastrate	2.80	2.65	2.67	2.80	2.82				

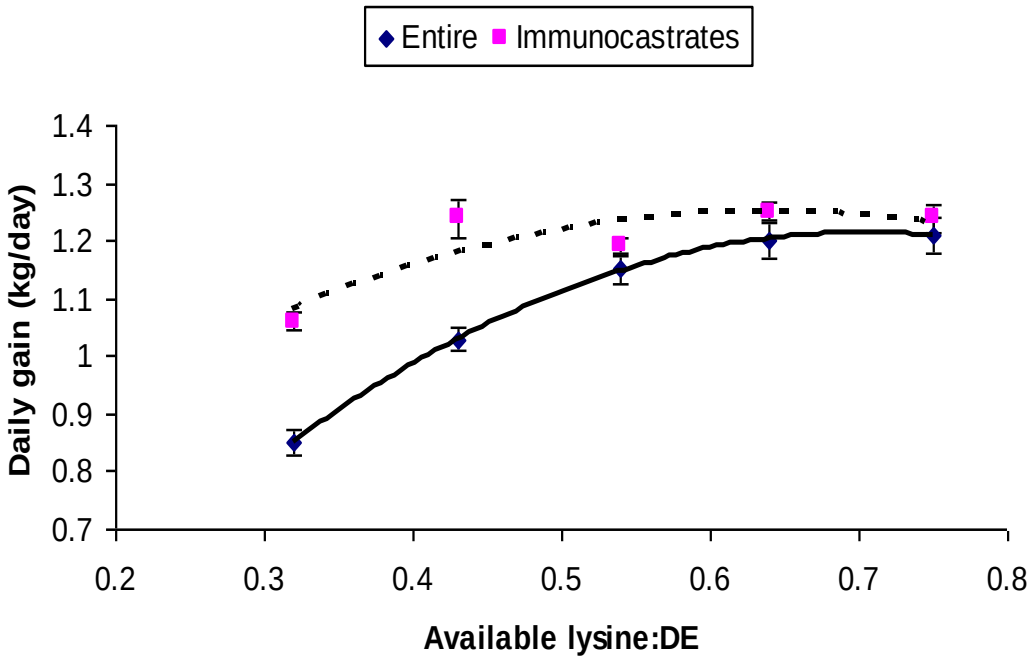


Figure 1: Effect of available lysine on daily gain for immunocastrates and entire males (± SEM) 60 to 107 kg LW.

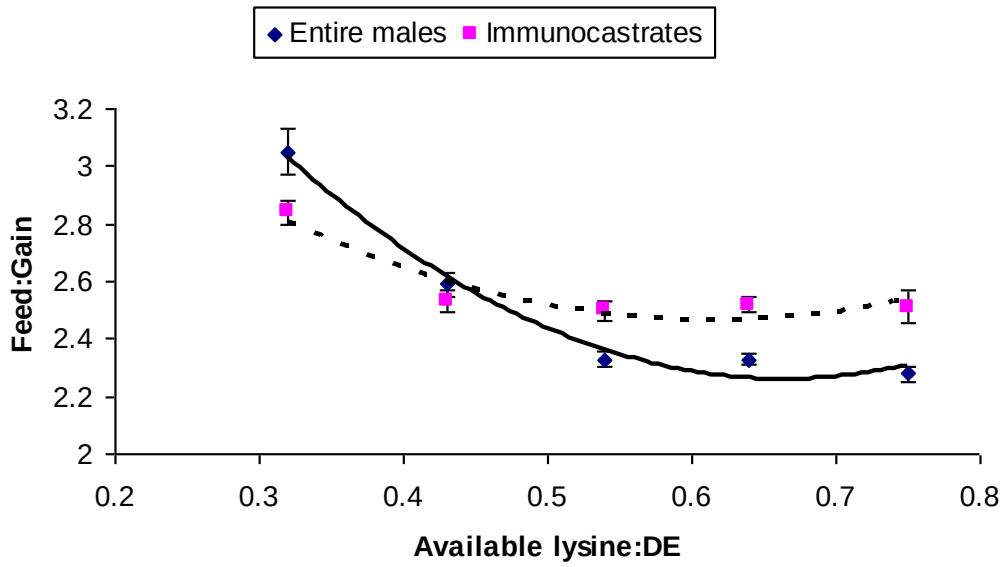


Figure 2: Effect of available lysine on feed to gain for immunocastrates and entire males (± SEM) 60 to 107 kg LW.

Table 4: Carcass characteristics for entire male and immunocastrated male pigs fed varying levels of Av Lys/MJ DE from 60.1 to 107.5 kgs (n=6).

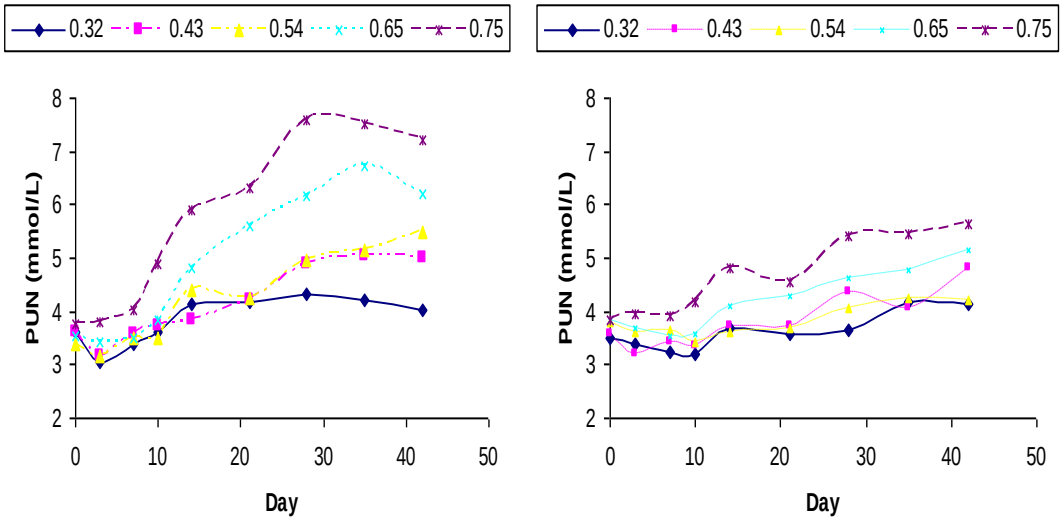
	Lysine level (g Av Lys/MJ DE)					SED[a]	P-value		
	0.32	0.43	0.54	0.64	0.75		Level	Sex	LxS
Carcass weight (kg)									
Male	63.2	69.2	73.2	74.7	73.9	0.959	<0.001	<0.001	<0.001
Immunocastrate	68.4	73.8	74.3	75.0	74.3				
Dressing percentage									
Male	66.2	67.4	68.0	67.6	66.7	0.392	<0.001	0.007	0.483
Immunocastrate	65.8	66.4	67.8	66.9	66.5				
P2 (mm) [b]									
Male	13.0	12.9	11.1	10.4	9.86	0.757	<0.001	<0.001	0.298
Immunocastrate	15.1	13.2	12.6	11.8	11.2				

[a] SED for level × sex

[b] Carcass weight used as a covariate for P2.

Plasma urea nitrogen (PUN) concentration was significantly affected by sex, level of lysine and time (P<0.001, P<0.001 and P<0.001, respectively) (Figure 3). Immunocastrated males had an increased PUN concentration compared to entire

males from Day 10 to Day 42 (P<0.001). PUN concentration also increased as the level of lysine increased from Day 3 to Day 42. From Day 21 to Day 42 there was a sex by lysine interaction with immunocastrated males having a higher PUN concentration at the increased lysine levels than entire males (P=0.058, P=0.036, P=0.002, and P=0.028). These results are in agreement with McCauley *et al.* (2003) who found increased PUN concentrations 14 days after the second immunisation. Claus *et al.* (2007) and Bauer *et al.* (2009) found PUN levels were elevated within 10 days after the second immunisation.



a) Immunocastrates b) Entire males

Figure 3: Change in plasma urea nitrogen (PUN) concentration over six weeks for (a) immunocastrated males and (b) entire males from 60.1 to 107 kgs LW fed increasing levels of available lysine (n=21).

4. Application of Research

This project aimed to determine the available lysine/MJ digestible energy requirements of immunocastrated male pigs after the second immunisation. When the second immunisation was given at 60 kg LW (6 weeks prior to slaughter) it appears from the growth performance outcomes that 0.43 g Av Lys/MJ DE is sufficient over this period. However when carcass characteristics were taken into

account, it is estimated that the required level to minimize the increase in carcass fatness while maximizing growth performance was approximately 0.54 g Av Lys/MJ DE. The lysine level indicated is lower than that for entire males and females (0.66 and 0.63 g Av Lys/MJ DE, respectively) recently found by Moore *et al*. (2010), however, this level was determined over a weight range from 50 to 100 kgs LW.

It was also hypothesized that immunocastrated pigs would have a lower optimal available lysine/MJ digestible energy ratio than entire males beyond two weeks after the second immunisation. When daily gain and FCR were broken down over the six week feeding period it shows the required g Av Lys:DE is similar for entire males and immunocastrates until 3 weeks after the second immunisation. However, the PUN levels show that this change is beginning to occur by 10 days after the second immunisation. PUN levels are an indicator of the amount of excess protein (lysine) in the diet. Therefore, it is suggested that the lysine level of diets fed to immunocastrates should remain equivalent to that of entire males until 2 weeks after the second immunisation, when it can then be decreased.

5. Conclusion

There are no known published studies on the lysine requirements of immunocastrated male pigs. The findings of this study suggest that the lysine requirements of immunocastrates are lower than entire males over the 6 week study period when the second immunisation was given at 60 kg LW. They also suggest that the lysine requirements begin to decrease within 2 weeks after the second immunisation.

6. Limitations/Risks

The successful implementation of these findings relies on the producer being able to change diets at targeted intervals which may not be possible. The predicted lysine requirement is for the PIC genotype and the optimum level may need to be adjusted for other genotypes and production strategies.

7. Recommendations

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

1. Immunocastrated male pigs require a lower lysine level than entire males following the second immunisation.
2. That immunocastrated male pigs remain on the same lysine levels as entire males until at least two weeks after the second immunisation. At this time the available lysine to energy requirement in the diet may be decreased to 0.43 g Av Lys:DE.

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