Final Report to the

Agricultural Produce Commission

Pork Producers Committee

Can black soldier fly larvae be used as a dietary protein substitute and reduce zinc oxide use in weaner pigs

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Background to Research

Soybean meal (SBM) is a common plant-based protein source used in piglet and weaner feeds in WA, but this product, whilst having an excellent nutritional profile, can be expensive, especially when supply becomes short. Therefore, finding a complete or even a partial substitute to SBM in piglet diets could benefit WA pork producers and add to ingredient flexibility when it comes to diet formulations. Such products would be especially beneficial for young pigs which require diets containing high-quality proteins of high nutritional value to optimise performance and gastrointestinal tract structure and function.

In this regard, there is mounting interest in the use of insect meal products in animal feeds, with a possible alternative to SBM being black soldier fly (BSF) larvae. Globally, BSF is of particular interest due to the limited land use it needs, ease of rearing, and its ability to easily convert organic wastes. The meal from BSF larvae is a new high protein ingredient source in monogastric diets. The larvae are 42% crude protein (CP) and ~ 19 MJ/kg digestible energy (DE), whereas the defatted meal can have CP levels exceeding 60% with ~ 15.5 MJ/kg DE (Wang and Shelomi, 2017; Biasato et al., 2019). This compares to ~ 48% CP and ~ 15.2 MJ DE in SBM, respectively. The prepupae are also rich in their fat content, ranging from 15-49% on a dry matter (DM) basis, with high levels of medium chain fatty acids (MCFA). Furthermore, this fat can contain up 60% lauric acid which is well known for its antibacterial effect on gut bacteria (Biasato et al., 2019), and can promote gut health. The presence of antimicrobial peptides in BSF can further enhance gut health in piglets. These characteristics identify BSF not only as a possible protein substitute but also as an antimicrobial (e.g., zinc oxide; ZnO) replacement in weaner diets.

Weaning is a challenging time for the young pig. It is typically associated with a reduction in feed intake resulting in gastrointestinal disease and dysfunction and a reduction in growth performance (Pluske et al., 1997; Heo et al., 2013). Weaning generally takes place during a developmental window of the immune system and gastrointestinal tract and involves removal of passive immunity from the sow (the sow's milk). Proliferation of *Escherichia coli* (*E. coli*) in the small intestine is often increased, resulting in the release of enterotoxins that encourage the epithelial cells of the small intestine to secrete fluid into the lumen, causing post-weaning diarrhoea (PWD) (Heo et al., 2013). In order to prevent poor performance and reduce the incidence of PWD, antibiotics and (or) minerals such as ZnO are often included in diets. With regard to ZnO, there is growing evidence to suggest that the use of ZnO in pig diets can contaminate the environment causing mineral deposits in the soil and waterways, and can contribute to antimicrobial resistance (Heo et al., 2013). In this light, the EU has implemented a 5-year phase-out period for the pharmacological use of ZnO, commencing in 2022. Assessing alternatives to ZnO in Australia in advance of any possible regulatory interventions and(or) provide other dietary options is therefore warranted.

Objectives of Research

1. Investigate the effect of full fat and partially defatted black soldier fly meals on growth performance in weaner pigs
2. Identify if antimicrobial peptides in black soldier fly meal can minimise diarrhoea in weaner pigs
3. Cost-benefit analysis of substituting soybean meal with black soldier fly meal in the diets of weaner pigs

Hypotheses

i. Partial substitution of soybean meal with black soldier fly meal will not reduce performance in weaner pigs

ii. The black soldier fly meal will reduce incidences of post weaning diarrhoea compared to pigs fed a standard diet containing no additives

Methodology

This experiment was conducted at the Murdoch University animal isolation house and the experimental protocol used in this study was approved by the Murdoch University Animal Ethics Committee (R3223/20). The animals were handled according to the Australian code of practice for the care and use of animals for scientific purposes (NHMRC, 2013).

Animals, housing and diets

At weaning (21 ± 3 days old) 90 male pigs (Landrace × Large White) were weighed and randomly allocated into 18 pens (5 pigs/pen) and 6 treatment groups (3 pens/treatment). Treatment groups included: (i) negative control (NC, no ZnO); (ii) positve control (PC, NC with 0.3% ZnO); (iii) NC + 5% full fat BSF; (iv) NC + 10% full fat BSF; (v) NC + 5% partially defatted BSF; and (vi) NC + 10% partially defatted BSF meal. The percentage of BSF included in the diets (iii-vi) was substituted for SBM. Diets were fed *ad libitum* for 28 days after weaning. SID amino acids were balanced and diets were formulated to contain 21% crude protein, 3400 kcal/kg ME, 1.35% SID Lysine, 0.85% Calcium and 0.62% Phosphorus.

Pigs were housed in three different rooms, in pens of metal construction and plastic floors, allowing at least 0.41 m^2 per pig. The pens were fitted with a nipple drinker, a five-space feeder, and plastic bottles for enrichment purposes.

Growth performance

Pig weight and pen feed intake were measured weekly at weaning (d0), d7, d14, d21 and d28 to determine average daily gain, average daily feed intake and feed conversion ratio.

Blood sampling and analysis

Blood samples were collected at d14 of the experiment from 3 pigs per pen (n=54) using vena cava puncture into a 9 mL lithium heparin tube with a 20-gauge, 38 mm needle and vacutainer. Blood from the lithium heparin tubes was processed by centrifugation at 2000 ×

g for 15 min to separate the plasma, which was later stored at -20° C until analysis. Haptoglobin in plasma was determined using a method based on Comparative Hematology International (Eckersall et al., 1999). Plasma creatine, urea nitrogen (PUN) and alkaline phosphatase (ALP) were analyzed using Beckman Coulter/Olympus Reagent kits (OSR6178, OSR6134 and OSR6004, respectively).

Statistics

All performance indicators and blood measures were analyzed using a one-way analysis of variance using SPSS v. 24 (IBM SPSS, USA). The percentage of pigs that developed diarrhoea (score 4 for ≥2 consecutive days) during the experiment was analyzed using Chi-square in SPSS.

Results

Table 1. Analyzed feed results for a 48% soybean meal, the supplied full fat black soldier fly (BSF) and the defatted BSF

	Soybean meal	Full Fat BSF	Defatted BSF
Dry Matter, %	90.8	94.7	94.3
Crude protein, %	46.7	42.5	59.5
Fat, %	1.4	35.6	18.3
Ash, %	7.1	4.1	2.9
Moisture, %	10	5.3	5.7
Amino acid, %			
Isoleucine	2.20	1.98	3.11
Leucine	3.69	3.10	4.99
Lysine	3.04	2.79	4.48
Methionine	0.65	0.81	1.43
Phenylalanine	2.49	1.93	3.20
Tryptophan	0.66	0.64	0.96
Threonine	1.89	1.73	2.65
Valine	2.31	2.77	4.19
Alanine	2.08	2.76	3.79
Aspartic acid	5.47	4.15	5.98

Diarrhoea scores

A significant difference was observed in post weaning diarrhoea and the 6 dietary treatments (P=0.045). The percentage of PWD for each dietary treatment group was; negative control had 46.7%, positive control had 20%, 5% full fat had 0%, 10% full fat had 13.3%, 5% defatted had 20% and 10% defatted had 33.3% PWD.

Performance data

The ADG and ADFI did not differ significantly between the dietary treatment groups at any time of the experiment. ADFI in week 4 tended to be higher in pigs fed a positive control diet compared to a negative control diet (Table 2). However, FCR was significantly lower in pigs fed the NC and NC + 10% defatted diet compared to the PC diet during the d21-28 period (1.30, 1.20 and 1.69 respectively; P=0.023; Table 2).

Plasma analysis

Plasma urea, creatine and haptoglobin did not differ significantly between treatments (Table 3). However, ALP in PC pigs was significantly higher than pigs fed a NC + 10% full fat diet (P=0.035) and tended to be higher than pigs fed the NC + 5% full fat diet (P=0.076).

Table 2. The effect of the 6 experimental diets on average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR)

	Negative control	Positive control	NC+5%Full Fat	NC+10%Full Fat	NC+5% Defatted	NC+10% Defatted	SEM	*P* value
ADG (g)								
D0-7	26	37	25	18	24	19	6.1	0.959
D8-14	178	208	158	199	226	221	9.2	0.256
D15-21	388	390	369	386	399	431	11.2	0.731
D22-28	548	606	571	588	614	528	14.3	0.478
D0-27	285	324	281	298	316	300	8.1	0.620
ADFI (g)								
D0-7	97	107	91	94	87	97	5.7	0.939
D8-14	248	274	242	262	278	284	10.2	0.798
D15-21	529	559	483	536	566	535	13.4	0.564
D22-28	714[e]	1017[f]	869[ef]	854[ef]	907[ef]	789[ef]	24.0	0.051
D0-27	397	473	421	437	460	426	12.5	0.567
FCR (g/g)								
D0-7	2.40	2.57	2.66	3.48	4.02	2.56	1.12	0.607
D8-14	1.40	1.32	1.43	1.32	1.24	1.24	0.03	0.486
D15-21	1.37	1.45	1.39	1.39	1.42	1.24	0.04	0.802
D22-28	1.30[a]	1.69[b]	1.51[ab]	1.49[ab]	1.48[ab]	1.20[a]	0.03	0.023
D0-27	1.39	1.46	1.49	1.47	1.45	1.27	0.02	0.321

[a-b] Mean values within a row that have different superscript are significantly different *(p<0.05)*

[e-f] Mean values within a row that have different superscripts tend to be different (p<0.1)

Table 3. The effect of the 6 experimental diets on plasma urea, creatine, alkaline phosphatase (ALP) and haptoglobin collected after 14 days of diet

	Negative control	Positive control	NC+5% Full Fat	NC+10% Full Fat	NC+5% Defatted	NC+10% Defatted	SEM	*P* value
Urea (mmol/L)	4.73	4.96	4.62	4.84	4.81	4.88	0.19	0.997
Creatinine (umol/L)	88.0	87.7	82.3	88.3	85.6	82.4	1.82	0.859
ALP (U/L)	$321^{ab,ef}$	$385^{a,e}$	$273^{ab,f}$	$263^{b,ef}$	$334^{ab,ef}$	$351^{ab,ef}$	11.0	0.020
Haptoglobin (mg/mL)	0.69	0.40	0.78	0.62	0.59	0.45	0.05	0.173

[a-b] Mean values within a row that have different superscript are significantly different (p<0.05)

[e-f] Mean values within a row that have different superscripts tend to be different (p<0.1)

Discussion and Conclusions

The first hypothesis tested if partial substitution of soybean meal with black soldier fly meal will not reduce performance in weaner pigs. This study found that 5 or 10% substitution of either full fat or partially defatted BSF did not reduce average daily gain and feed conversion ratio compared to pigs fed commercial diets, in the immediate post weaning period. Similarly, Biasato et al. (2019) found that supplementing weaner pigs with 5 and 10% defatted BSF meals did not influence growth performance. The same study found that feed intake was higher in pigs supplemented with 10% defatted BSF between days 24 and 61 post weaning but not in the immediate post weaning period (days 1 to 23) (Biasato et al., 2019). The author attributed this to the increased palatability of the insect meal. Therefore the results from this experiment and other researchers show that supplementing or partially substituting SBM with BSF meals does not negatively affect growth performance in weaner pigs.

Plasma urea nitrogen (PUN) is used to measure the amount of waste product produced by the liver as the body breaks down proteins (Sterndale et al., 2022). BSF meal in this study did not alter PUN suggesting the supplementation had no negative impact on muscle protein break down or nitrogen utilization. Similarly plasma creatine, that is measured to indicate kidney function, was not affected by BSF supplementation. Haptoglobin, an acute phase protein that increases during an inflammatory response, did not significantly differ in pigs fed the six different diets (Tizard, 2013). These results highlight that BSF supplementation had no detrimental effects on protein utilization, kidney function or acute phase proteins in weaner pigs. Alkaline Phosphatase (ALP) measures the amount of enzymes in the blood. Levels that are too high can indicate liver or gall bladder disease and concentrations too low may indicate malnutrition. In this experiment, ALP was significantly higher in pigs fed the ZnO diet compared to pigs fed 10% Full fat BSF diet. This effect should be further investigated to determine if the lower levels on ALP had a negative effect on the pigs.

The second hypothesis tested if the black soldier fly meal would reduce incidences of post weaning diarrhoea compared to pigs fed a standard diet containing no additives. This study found that all diets containing BSF had lower incidences of diarrhoea than pigs fed a standard diet with no additives. Interestingly it was also found that pigs fed 5 and 10% full fat BSF meals had less PWD than pigs fed a diet containing a pharmaceutical dose of ZnO. These results indicate that using insect meals or oils in pig diets as an antimicrobial substitute should be further investigated.

In conclusion, this experiment showed that BSF supplementation had no negative impacts on weaner pigs and could be used as a possible protein substitute. However due to the current price of BSF meals, using BSF as a protein substitute on a commercial farm is not economically feasible. Nevertheless, further investigation should be conducted to determine if BSF oil can be used as a ZnO replacement.

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