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Effects of *Moringa oleifera* Leaf Meal on Plasma Ghrelin and Insulin-like Growth Factor-1 Levels in Swine and its Potential Role in Improving Sow Productivity

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Two experiments were conducted to determine the possible role of *Moringa oleifera* on sow productivity and ghrelin – growth hormone (GH)/insulin-like growth factor-1 (IGF-1) metabolic pathway. In the first experiment, the effects of 15% *M. oleifera* leaf meal (MoLM) inclusion in the diet on the plasma ghrelin and IGF-1 levels were determined using four sexually mature gilts with indwelling catheter. Results showed that plasma ghrelin and IGF-1 levels between the control and experimental groups were not statistically different, although pigs fed with MoLM had apparently lower baseline and higher amplitude of plasma ghrelin levels particularly a few hours right after feeding time. On the other hand, IGF-1 level in pigs fed with MoLM seemed down regulated. The second experiment aimed to know the effects of supplementing 15% MoLM on body condition and sow productivity during the week of gradual feed withdrawal prior to farrowing. A total of 16 sows with 141 piglets were used. Although the experimental group registered higher values, the difference in backfat losses from the control group is not statistically significant. The same results were observed for the corresponding piglets; despite higher weaning weight and average daily gain, the control group was not statistically different from the experimental group. The results showed the potential of 15% MoLM in improving sow productivity through ghrelin – GH/IGF-1 metabolic pathway. Further studies are recommended with longer duration of supplementation. These are also recommended to isolate the potential component in MoLM that improves milk production because the high fiber in MoLM possibly contradicts ghrelin release in the stomach.

Key Words: ghrelin, growth hormone, IGF-1, MoLM, sow productivity

Abbreviations: ADG – average daily gain, BCS – body condition score, EDF – expected date of farrowing, GH – growth hormone, IGF-1 – insulin-like growth factor-1, MoLM – *Moringa oleifera* leaf meal, pGH – porcine growth hormone, WW – weaning weight

INTRODUCTION

In the Philippines, poor sow productivity is one of the major problems of its swine industry and negatively affects farm productivity and profitability (Vega 2010b; Huynh et al. 2012). Sow productivity can be equated to the number of piglets produced by a single sow per year (Koketsu et al. 2017) or throughout its lifetime (Vega et al. 2010a). Several factors may affect productivity which include conception rate, return to estrus, caloric efficiency, feed conversion rates, and animal health and condition (Dors et al. 2013).

One intervention to possibly improve sow reproductive performance is by assisting hormone-mediated pathways, specifically the growth hormone (GH)/insulin-like growth factor-1 (IGF-1) axis (Luxford et al. 1998; Daftary and Gore 2005). According to Lucy (2008), GH and IGF-1 control growth, gestation, and lactation in swine. However, a good supply of protein and energy is necessary to promote GH/IGF-1 axis as food deprivation, which may include poor voluntary feeding, has altered the actions of hormones (Simmen et al. 1998; Moller and Jorgensen 2009). To promote voluntary feeding while enhancing nutrient intake, activation and

increase of ghrelin levels is a consideration.

Studies by Salfen et al. (2004) and Scrimgeour et al. (2008) suggest that ghrelin regulates appetite and feeding behavior among pigs. Ghrelin is an endogenous ligand of the growth hormone secretagogue receptor (GHS-R) and a potential orexigenic agent in monogastrics and ruminants (Roche et al. 2008). Moreover, ghrelin secretion is highly affected by the type of nutrients the animal has digested (Verhulst and Depoortere 2012). Infusion of amino acids enhanced the plasma ghrelin level in sheep while glucose and insulin had no effect on ghrelin secretion (Zhang et al. 2007; Sugino et al. 2010).

Several studies concluded that malunggay (*M. oleifera*) has the capacity to stimulate the acinar cells of the stomach to release ghrelin (Tadeo 2015; Tomoe et al. 2017). For this reason, the use of *M. oleifera*, which is a highly adaptable tropical shrub known for its high protein content and milk-boosting properties (Acda et al. 2010), can be explored.

M. oleifera has many advantages. Its propagation is considered a low-cost method of producing protein supplement in livestock and poultry production (Fritz 2000; Mendieta-Araica et al. 2011) with crude protein (CP) content of 25.1 to 43.5% (Makkar and Becker 1996; Moyo et al. 2012). The leaves are very nutritious and contain high amounts of macro- and micronutrients (Olaofe et al. 2013). *M. oleifera* is also a known galactagogue in ruminants. Sanchez et al. (2006) found that feeding the leaves to dairy cattle resulted in increased dry matter intake and milk production. In addition, *M. oleifera* showed negligible amounts of anti-nutritional factors implying that it does not have detrimental components that can harm farm animals (Gidamis et al. 2003).

Information about the nutritive value of MoLM in pig feeding is scarce. Studies on the role of *M. oleifera* on hormones related to important traits of pigs primarily on the regulation of voluntary feed intake, weight gain and milk production of swine through plasma ghrelin and GH/IGF-1 axis are still lacking. On top of these possibilities, the effects of *M. oleifera* on the physiological level of monogastric animals such as pigs are not clear.

Acda et al. (2010) reported that inclusion of 5% MoLM on finisher pigs had an insignificant effect on the feed conversion efficiency, carcass and meat quality, live weight and daily gain while substitution of 10% MoLM has a comparable effect on the average performance in weaning piglets. A study conducted by Valdivié et al. (2017) concluded that 10% MoLM substitution can be done in the pre-fattening category (from 33 to 75 d of age) without affecting the digestive utilization indicators and is still possible up to 20% substitution in growing pigs. In a test conducted by Gonzales and Herrera (2012) as cited by Valdivié et al. (2017), 15% forage meal substitution in combination with molasses yielded excellent results in weight gains of fatteners. On the other hand, high inclusion rates of MoLM in the diet of pigs were found to be detrimental to sow performance (Kambashi et al. 2014). Perez et al. (2001) recorded that partial substitution of up to 30% MoLM decreased pig performance and depressed feed conversion ratio.

The present study aimed to determine if inclusion of 15% MoLM in the diet can enhance sow productivity through the ghrelin – GH/IGF-1 metabolic pathway. Two different experiments were done using 15% MoLM with two different objectives. The first experiment was conducted to determine whether or not partial substitution of MoLM is involved in the nutrition-dependent changes in plasma ghrelin and IGF-1 of sexually mature gilts. The second experiment tested the possible effect of supplementation of MoLM during the 7-d gradual feed withdrawal before farrowing, assuming it will cause significant improvements on the body condition (backfat thickness and score) of the sows and on reproductive performance indicators (piglet growth traits and daily gains).

MATERIALS AND METHODS

MoLM Preparation

M. oleifera used in the preparation of MoLM (Fig. 1) was acquired from commercial sources in Asingan, Pangasinan. About 30–50 kg of leaves was dried in an oven with a maintained temperature of 65–70°C for 72 h. The dried leaves were manually ground then sealed in two 95-L plastic containers and stored at room temperature for 14 d. Commercial desiccants were placed inside the containers to control humidity. The proximate composition

Fig. 1. *Moringa oleifera* leaf meal (MoLM) drying and preparation at the Institute of Animal Science, College of Agriculture and Food Science, University of the Philippines Los Baños.

of MoLM (Table 1) used in this study was analyzed at the Animal Nutrition Analytical Service Laboratory of the Institute of Animal Science (IAS), College of Agriculture and Food Science (CAFS), University of the Philippines Los Baños (UPLB), College, Laguna, Philippines. To ensure voluntary feeding of 15% MoLM partial feed substitution, a 5-d feeding trial was done before the start of the experiments.

Table 1. *Moringa oleifera* leaf meal nutrient composition.

Component*	Amount (%)
Moisture	9.43
Ash	9.47
Crude protein	21.31
Crude fiber	16.33
Crude fat	5.27
Calcium	2.40
Phosphorus, total	0.34
Nitrogen-free extract (NFE)	38.19

*Laboratory tested in the Animal Nutrition Analytical Service Laboratory (ANASL) of the Institute of Animal Science (IAS), College of Agriculture and Food Science (CAFS), University of the Philippines Los Baños, 2015.

Effects of MoLM on Plasma Ghrelin and Insulin-Like Growth Factor-1 in Gilts

The first experiment was conducted from March to April 2015 at the IAS, CAFS, UPLB. A completely randomized design was employed where four gilts were randomly distributed to two treatments: treatment 1 (100% commercial diet) and treatment 2 (85% commercial diet + 15% MoLM). Blood sampling was done in 12 different periods for two consecutive days to monitor possible pre-prandial and post-prandial effects of MoLM on plasma ghrelin and IGF-1 levels.

Care and Management of Gilts

Four 7-month-old pedigreed crossbred (Landrace x Large White) gilts acquired from an accredited breeder farm were used in the experiment. Each gilt was confined in an individual metabolic cage and allowed to acclimatize for 7 d before the 2-d feed substitution and blood sampling.

Before the acclimatization period, all gilts were inserted with a catheter by an accomplished licensed veterinarian. The gilts were fasted 12 h (overnight) before implanting the catheter; each gilt was anesthetized by injecting Atropine® (0.5 mg/kg body weight; Hizon Laboratory, Inc.) intramuscularly, followed by Xylazine® (2.2 mg/kg body weight; Lloyd Laboratories, U.S.A.) and Zoletil® (4 mg/kg body weight; Virbac Laboratory), respectively. Each medicine was given at 10-min interval. Then, the catheter was surgically fitted in the external jugular vein. Terramycin® (Pfizer Inc., New York, USA) was applied topically on the wounds to avoid infection. The catheters were kept functional by flushing commercially available 0.9% saline and heparin solution (China Chemical and Pharmaceutical Co. Ltd., Taiwan) twice daily at 0600 h and 1700 h.

A standard commercial diet was given to both treatments during the adaptation period. The commercial diet contained 16% crude protein, 7% crude fiber, 3% crude fat, 0.75% calcium, and 12% moisture content. The gilts had free access to water using nipple drinkers and the diets were given twice daily at 0800 h and 1600 h. On the 2-d supplementation and sampling period, the treatment 2 diet was shifted to 15% MoLM substitution. Temperature and relative humidity in the room were also monitored. The cages and the floor were washed twice daily with water and the lights were kept open for 12 h during the dark period.

Blood Sampling

Blood samples were collected at 0700, 0800, 0900, 1100, 1400, 1600, 1800, and 2300 h on the first day, and at 0700 h, 0800 h, 0900 h, and 1100 h of the following day during the experimental period. The collected blood samples were placed in a tube with EDTA to avoid coagulation. The samples were centrifuged at 1,500 rpm for 20 min and stored at -30°C prior to assay. Catheters were flushed every sampling period with saline and Heparin solution.

Plasma Hormone Assay

The total plasma ghrelin level was quantitatively measured using a commercially available Porcine Ghrelin ELISA kit (Cusabio Biotech Co., Ltd.). The detectable range of this kit was from 62.5 to 4000 pg/mL. The intra-assay and inter-assay CV were <8% and <10%. All the procedures, except from the plasma dilution of the blood samples, were done based on the protocol of the manufacturer.

The same procedure was used for the analysis of plasma IGF-1 using Porcine IGF-1 ELISA kit (Cusabio Biotech Co., Ltd.). The kit had a detectable range of 6.25 to 400 ng/mL. The intra-assay and inter-assay CV were also <8% and <10%, respectively. All processed samples were then measured using ELISA READER (Microplate Autoreader EL311, Biotek Instruments).

Analysis of variance was performed to check for interaction and main effects of MoLM level (with and

without) and time of sampling. Significance was set at 5% alpha. Statistical analysis was done using the Statistical Analysis Software (SAS) 9.3 computer program.

Effects of MoLM on Backfat Thickness and Sow Productivity

The second experiment was approved by the IAS, CAFS UPLB. The study was conducted from April to June 2015 at an accredited sow breeder farm in Sitio Halang, Brgy. Macamot, Binangonan, Rizal, Philippines. A total of 16 gestating sows were used in the experiment following completely randomized design and distributed equally into two treatments: 8 sows without MoLM (T1) and 8 sows with MoLM (T2).

Care and Management of Sows

The test sows were housed in a tunnel ventilated facility to control the external temperature and eliminate potential heat stress. Ten days before the expected date of farrowing (EDF), the test sows were transferred to an air-conditioned farrowing facility where each of the farrowing pens were kept clean and washed twice daily. The average temperature of the facility was monitored at 22–24°C and the test sows were acclimatized for 72 h. Seven days before EDF, all sows in treatment T1 (control) were gradually feed-restricted from 3,000g (3 kg) to 500 g (minus 500 g/day) of commercial feeds towards farrowing. Likewise, sows in treatment 2 (experimental) went through the same feeding regimen but with an additional 450 g of MoLM per day. The 450 g MoLM was the computed 15% of the 3 kg standard amount of feed offered per day. The additional MoLM was pre-mixed with the commercial feeds. Free water access was provided through nipple drinkers.

Sow Farm Data

For sow data, body score and backfat thickness were taken 7 d before EDF and another during piglet weaning. The backfat thickness was measured using Renco Lean-Meater Digital Backfat Indicator by Renco®, Minneapolis, MN, USA. To determine sow body condition scores (BCS), the guideline by Coffey et al. (1999) was used.

For the piglets of the test sows, litter size, birth weight (BW), number of piglets weaned, weaning weights (WW), and average daily gain (ADG) from birth to weaning were recorded. Weaning was done on the 28th day of survival and ADG was computed using the formula:

$$ADG = (WW - BW)/28\ d$$

Statistical Analysis

T-test was performed to check for the possible effects of MoLM on sow backfat thickness and BCS, and their corresponding piglet growth traits (BW, WW, and ADG) between treatments. The level of confidence was set at $\alpha = 0.05$, such that at $p < 0.05$, the alternative hypothesis that there were significant differences between the means of the data collected from the two treatment groups was accepted. All statistical analyses were performed using SAS University Edition.

RESULTS AND DISCUSSION

Experiment 1. Effects of *M. oleifera* (MoLM) on Plasma Ghrelin and Insulin-Like Growth Factor-1 in Gilts

Effects of MoLM Supplementation on Plasma Ghrelin in Gilts

In the first experiment, plasma ghrelin levels from both treatments were variable throughout the 12 h sampling period following a similar pattern. Figure 2 shows that the plasma ghrelin rose predominantly after feeding. Plasma ghrelin of gilts without MoLM (control) peaked at 0900 h (744.50 ± 53.03 pg/mL), 1600 h (809.50 ± 300.52 pg/mL), and at 0900 h of the following day (867.00 pg/mL). Similarly, plasma ghrelin of gilts treated with MoLM peaked at 0900 h (677.00 ± 322.82 pg/mL), 1800 h (983.67 ± 632.16 pg/mL), and at 0900 h of the following day (1073.67 ± 748.08 pg/mL). On the other hand, the lowest measurements of plasma ghrelin level from both treatments were recorded several hours before feeding. Control gilts had the lowest measurements at 0700 h (637.00 ± 56.57 pg/mL), 1100 h (552.00 ± 21.21 pg/mL), and at 0700 h of the following day (649.50 ± 123.74 pg/mL) while MoLM gilts had the lowest measurements at 0800 h (552.00 ± 175.04 pg/mL), 1100 h (448.67 ± 105.61 pg/mL), 2300h (533.67 ± 184.44 pg/mL), and 1100 h of the following day (538.67 ± 76.02 pg/mL). However, there were no significant differences in the plasma ghrelin levels between the two treatment groups.

The post-prandial rise and the pre-prandial fall in

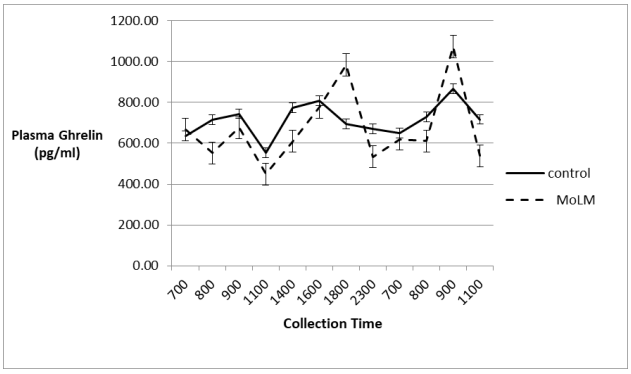

Fig. 2. Comparison of plasma ghrelin concentrations in gilts. Plasma ghrelin concentrations were measured as values of mean ± S.E.M.

plasma ghrelin from both treatment groups can be attributed to the type of nutrients bioavailable for the stimulation and release of this hormone during digestion (Zhang et al. 2007). Tests performed in sheep by Sugino et al. (2002) showed that infusion of amino acids and proteins enhances plasma ghrelin levels. Moreover, tryptophan had increased the plasma ghrelin and its mRNA expression in the duodenum of weaning piglets (Zhang et al. 2007). Hence, if amino acids can enhance ghrelin secretion, then infusing additional proteins from *M. oleifera* can increase its availability.

In tests conducted by Olaofe et al. (2013), it was observed that *M. oleifera* has high concentrations of essential amino acids such as isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. The MoLM used in this study had a crude protein content of 21.31% compared with only 16% in the commercial diet. At 15% substitution, higher protein availability resulting in increasing ghrelin levels of the MoLM-treated gilts is expected. This apparently manifested as MoLM registered lower baseline and higher amplitude levels (Fig. 2) in MoLM-treated gilts compared with the control group. However, elevations in ghrelin levels were not maintained throughout the experiment. One possible cause is that the overall nutrient uptake of the group fed with MoLM was not high enough to sustain increases in ghrelin secretions. Consequently, low nutrient uptake will eventually result in lower protein acquisition. This can be due to high fiber content of MoLM that may affect the digestibility of *M. oleifera*.

Comparing the commercial diet given with the control which contains 7% crude fiber (CF), MoLM contains CF as high as 16.33%. This coincides with the findings of Fritz (2000), Joshi and Mehta (2010), and Melo et al. (2013) who obtained 11.3% to 12.1% CF from the samples in their research. By drying the leaves and stems of *M. oleifera*, the fiber content increases its concentration in dry matter (DM) (Agoreyo et al. 2011). High fiber diet with high nitrogen content can be efficiently used by ruminants as the microorganisms in their rumen synthesize microbial proteins. However, for monogastrics like pigs, utilization of high fiber diet is not that efficient due to the absence of some endogenous enzymes needed to digest some plant cell wall components such as cellulose (Kambashi et al. 2014; Perez Hernandez and Torres Porras 2001). For this reason, the digestibility of MoLM may have affected ghrelin levels in treated sows. In a study by Kambashi et al. (2014), MoLM feeding tests in sows were mildly successful as high inclusion rates were found detrimental to sow performance. Perez Hernandez and Torres Porras (2001) recorded that partial substitution of MoLM up to 30% in the diet of growing pigs decreased pig performance and depressed feed conversion ratio.

Another possibility which caused lower plasma ghrelin levels in gilts fed with MoLM is the slow consumption. This may be caused by the low palatability of *M. oleifera* (Mendrieta-Araica et al. 2009). Commercial diet was consumed by the gilts within 15 min after offering while the gilts fed 15% MoLM had fully consumed their diet several hours after 0800 h morning feeding. Since nutritional status and amount of nutrient uptake influence plasma ghrelin secretion (Zhang et al. 2007; Sugino et al. 2010), it is suggested that poor and slow intake, as well as high fiber content of MoLM diet, may have resulted in lower ghrelin levels in MoLM-treated gilts.

Effects of MoLM Supplementation on Plasma IGF-1 Levels of Gilts

In Figure 3, plasma IGF-1 levels of the gilts from the two treatment groups expressed positive relationship with changes in plasma ghrelin levels in Figure 2. Control gilts recorded high IGF-1 levels at 0900 h (131.43 ± 18.9 ng/mL), 1800 h (212.50 ± 160.10 ng/mL), and at 0800 h of the following day (131.07 ± 17.68 ng/mL), while MoLM-treated gilts recorded peak IGF-1 levels at 0900 h (120.24 ± 47.63 ng/mL), and at 1600 h (158.81 ± 60.76 ng/mL). On the other hand, the drops in measurements of plasma IGF-1 level from both treatments were recorded several hours after feeding. The control group recorded the lowest values at 1100 h (93.22 ± 1.52 ng/mL), and at 2300 h (Control: 83.93 ± 1.52 ng/mL). In the same manner, the MoLM-treated group recorded dropping values at 0800 h (78.57 ± 27.18 ng/mL), 1100 h (80.48 ± 21.11 ng/mL), 1800 h (61.07 ± 57.08 ng/mL), and at 1100 h of the following day (59.76 ± 14.74 ng/mL).

Recent discoveries of ghrelin that is expressed in the stomach, hypothalamus and in the blood of mammals suggest that this molecule is involved in the hormonal

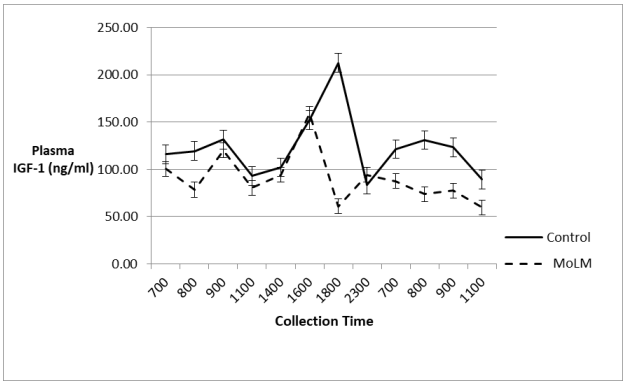

Fig. 3. Comparison between plasma IGF-1 levels of gilts. Plasma IGF-1 concentrations were measured, and values are mean ± S.E.M.

regulation of GH release (Kojima and Kangawa 2005; Ceranowicz et al. 2010; Lewitt 2013). The presence of ghrelin in the stomach suggests that it may play a role in the nutritional regulation of the GH/IGF axis (Roelfsema and Clark 2001). Therefore, the post-prandial effects in plasma IGF-1 are a possible result of the stimulation of the plasma ghrelin.

Toogood et al. (1996) and Berryman et al. (2008) concluded that GH and IGF-1 have synergistic effects and that as GH secretion declines, there is also a concomitant decrease in IGF-1 levels. Berryman et al. (2008) stated that a reduction in the GH/IGF-1 axis is correlated with increased percentage of total body and visceral fat, decreased muscle mass, decreased physical fitness, decreased immune function, and physiological decline in estrogen and androgen concentrations. However, about 97% of the IGF in blood bind with high specificity and affinity to a family of six binding proteins, called IGFBPs (1 to 6) that modulate their bioavailability (Martinelli et al. 2008). The binding of IGF-I to IGFBP limits the bioactivity of IGF-I, as bound IGF-I probably cannot activate the IGF-I receptor (Roelfsema and Clark 2001). In this study, a noticeable down regulation in IGF-1 levels starting from 1800 h of the first day sampling period to 1100 h of the following day was observed. This inhibitory action is more evident in MoLM-treated gilts as the lowest IGF-1 levels recorded (Fig. 2) occurred during the period when ghrelin levels peaked (Fig. 1). Earlier studies found that increase in ghrelin eventually results in increases in GH availability (Kojima and Kangawa 2005; Ceranowicz et al. 2010; Lewitt 2013). Since GH influences IGF-1 in liver through insulin-like growth factor binding protein-1 (IGFBP-1), while IGFBP-1 has an endocrine role to inhibit IGF-1 availability (Heijboer et al. 2006), enhanced increases in GH may cause higher inhibition of IGF-1 bioavailability. In this study, however, the difference in IGF-1 levels between the MoLM-treated and the control group are not statistically significant ($P > 0.05$).

Although plasma ghrelin and IGF-1 levels of MoLM-supplemented sows showed larger discrepancies, suggesting greater inhibitory actions of the two hormones, the poor and slow intake as well as digestibility issues of MoLM may have affected the bioavailability of proteins necessary to trigger desirable and sustained effects in the ghrelin – GH/IGF-1 axis. It must also be remembered that for this experiment, MoLM-treated gilts were given their substituted diets for 2d only, which may not be enough to provide a more conclusive result. Nevertheless, the results have relevance in terms of hormone action, as hormones stay in baseline level unless a trigger is stopped (Ranabir and Reetu 2011).

Experiment 2. Effects of Supplementing MoLM 7 d before Expected Date of Farrowing (EDF) on Sow Body Condition and Piglet Growth Traits

Effects of MoLM Supplementation on Sow Backfat Thickness and Body Condition Score

Back fat thickness is a parameter used to determine the amount of energy reserves a pig has in a certain span of time (Boyd et al. 2002; Teagas 2011). This parameter is significant in determining sow reproductive performance (Roongsitthichai and Tummaruk 2014). Aside from this, body condition score (BCS) can also be used to determine the adequacy of gestation feeding levels and lactation feed intakes (Coffey et al. 1999). Results in Table 2 showed that higher backfat losses were observed in MoLM-treated sows (2.75 ± 0.796 mm) compared with the control group (1.50 ± 0.567 mm). However, these differences in backfat loss, together with BCS at weaning, were not statistically significant.

According to Delhanty and Van der Lely (2011), ghrelin is essential for blood glucose control in starvation. These metabolic effects are mediated in part by a central stimulatory effect of ghrelin on appetite and GH release and in part by peripheral actions on insulin secretion and

Table 2. Comparison of sow reproductive performance indicators.

Sow Reproductive Performance Indicators	T1 – Control[1]	T2 – MoLM[1]	*P*-value[2]
Backfat thickness (mm)			
Before EDF	17.00 ± 1.052	16.38 ± 0.778	0.640
At weaning	15.50 ± 0.886	13.63 ± 1.068	0.198
Mean difference	1.50 ± 0.567	2.75 ± 0.796	0.222
Sow body score at weaning[3]	3.50 ± 0.189	3.13 ± 0.125	0.120
Average litter size born alive	9.00 ± 0.926	9.13 ± 1.109	0.932
Average birth weight of piglets (kg)	1.48 ± 0.043	1.40 ± 0.037	0.172
Average pigs weaned	8.75 ± 1.031	8.88 ± 1.172	0.937
Average weaning weight (kg)	7.85 ± 0.361	8.59 ± 0.391	0.189
ADG (kg)	0.23 ± 0.012	0.26 ± 0.014	0.138

[1]Values are mean ± SEM.
[2]Different letter superscripts per row (a b) are significantly different ($p < 0.05$).
[3]Body condition scoring guidelines from Coffey et al. (1999): 1 - emaciated, 2 - thin, 3 - ideal, 4 - fat, and 5 - overly fat

insulin sensitivity, as well as hepatic glucose production (Sun et al. 2008; Zhao et al. 2010). Earlier studies by Poretsky et al. (1996) concluded that an increase in GH levels has the capacity to diminish insulin action on adipocytes. Insulin, on the other hand, inhibits glucose metabolism and promotes lipid uptake among cells. Similar results by Magri et al. (1990) also verified that porcine growth hormone (pGH) elicits several metabolic effects in porcine adipocytes which collectively reduce the rate of lipogenesis, and thereby contribute to decrease in lipid deposition. Recent studies of Sasaki-Suzuki et al. (2009) also demonstrated that chronic pGH pretreatment of adipocytes impairs glucose transport activity in the plasma membrane of the cell. This may explain the higher backfat losses incurred by MoLM-supplemented sows as *M. oleifera* showed its potential in affecting ghrelin levels previously noted in the first experiment in gilts. This additional fat loss is seemingly an influential action of porcine ghrelin to stimulate pGH, and in turn inhibit glucose uptake in adipose cells.

For sows about to farrow, higher fat reserve is undesirable because it can cause several complications such as mastitis, constipation, and agalactia (Lynch 1989). This will also reduce lactation feed intake as the sows use body fat and mass to compensate milk yield. In any case, when a sow gets off lactation feed early, any nutrient restriction in this period will significantly influence sow fertility. This will greatly affect reproductive performance even if early weaning is done (Thaker and Bilkei 2005). For these reasons, commercial swine raisers gradually withdraw gestational feeds a week before the expected date of farrowing (EDF). This diet management system keeps the sow leaner while pushing them to keep feeding while lactating.

The inability of MoLM to significantly promote additional backfat loss can still be attributed to the duration of its supplementation, which in this experiment is only given 7d prior to EDF. The amount of protein uptake from *M. oleifera* may not be enough to cause sustained increases in porcine ghrelin, which may result in lower pGH production and uninhibited lipid uptake (Magri et al. 1990; Sasaki-Suzuki et al. 2009). Although backfat loss is natural during lactation as sows also use body reserves to compensate for lactation requirements (Boyd et al. 2002; Teagas 2011), it can be inferred that extending supplementation of 15% MoLM may have better potentials for further reducing backfat thickness to promote voluntary lactation feeding. Another factor which can be considered from the results of the first experiment is the digestibility of *M. oleifera* since its high fiber content is not suitable for monogastric digestion and nutrient conversion especially in high inclusions. This can eventually influence porcine ghrelin levels since nutritional status and amount of nutrient uptake directly affects its availability, and in turn affects GH levels and glucose transport in adipocytes.

Effects of Sow MoLM Supplementation on Piglet Growth Traits

It is hypothesized that MoLM can also increase milk production in sows through plasma ghrelin and GH/IGF-1 axis. A study by Lucy (2008) explained that this somatotropic axis in sows remains coupled during lactation where both GH and IGF-I are elevated in their blood. This suggests that proportional influences of GH/IGF-1 axis are involved in milk production. This is supported by findings of Brown et al. (2005) where administration of GH improved the mammary gland tissues in heifers, suggesting that IGF-1 is involved in the regulation of milk production. Bao et al. (2016) discussed a model where IGF-I from stromal cells in a paracrine fashion stimulates proliferation and branching of alveolar epithelial cells. Prosser et al. (1991), Walden et al. (1998), and Molento et al. (2002) concluded that subcutaneous injection of GH increased IGF-1 mRNA expression, while insulin infusion increased the positive effect of GH on the concentrations of IGF-I in milk and other milk parameters such as milk yield, protein yield, and casein yield. For instance, administration of bovine growth hormone (bGH) increased milk production in lactating cows (Ahmad and Sarwar 2002).

In this experiment, MoLM-supplemented sows were expected to produce larger amounts of milk. This can be measured indirectly based on changes in the daily gains of piglets since nursing piglets get all the nourishment from their mothers for the first 2–3 wk of their lives (Young et al. 2004). Of the piglets produced by the test sows, only 141 piglets were weaned. Comparison on piglet growth traits in Table 2 showed that the BW, WW, and ADG between the treatments were not significantly different ($P > 0.05$). However, WW and ADG of piglets from MoLM-supplemented sows recorded larger gains which imply that piglet milk intake was higher. These data reflect the potential of MoLM to improve lactation performance through ghrelin – GH/IGF-1 axis. It is still possible that the duration of MoLM feeding and the bioavailable protein of M. oleifera in this experiment was not enough to cause significant effects in milk production.

CONCLUSION AND RECOMMENDATION

This study investigated the effects of MoLM on sow productivity through the ghrelin – GH/IGF-1 hormone-mediated pathway. Although the results were not statistically significant, supplementation of 15% MoLM seems to influence the baseline and amplitude of plasma

ghrelin level, and the down regulation of plasma IGF-1 among MoLM-treated pigs. In addition, 15% MoLM supplementation to sows about to farrow positively showed potentials in improving productivity as shown in higher WW and ADG of its corresponding piglets. It is presumed that high fiber content of MoLM and the duration of supplementation have affected the results. It is further recommended that a similar study be conducted using higher dose of MoLM at longer duration, and that identification, isolation, and characterization be done on the component in *M. oleifera* that is involved in stimulating the stomach – anterior pituitary – liver axis associated with milk production.

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