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Effects of *Moringa Oleifera* leaf supplementation in lactating sow diets with or without banana peel powder as a fiber source on reproductive performance, fecal moisture content, rectal temperature and hormone profiles

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Abstract

Twenty crossbred (Yorkshire × Landrace) gestating sows were used to determine the effects of *Moringa Oleifera* leaf (MOL) supplementation in lactating sow diets with or without banana peel powder (BPP) on reproductive performance, fecal moisture content, rectal temperature and hormone profile. The treatments were as follows: 1) CON (control diet), 2) M1 (control diet + 0.5% MOL), 3) M2 (control diet + 1.0% MOL), 4) M3 (control diet + 0.75% MOL and 0.75% BPP), and 5) M4 (control diet + 1.5% MOL and 1.5% BPP). The diets was fed from da 107 of gestation to weaning. MOL and BPP supplementation decreased ($p < 0.05$) the fecal moisture content compared with that of the CON at day 0 and 7 after farrowing, and 1.0% supplementation of MOL also decreased ($p < 0.05$) the fecal moisture content at day 14 after farrowing. On farrowing day, MOL supplementation decreased ($p < 0.05$) the blood cortisol and norepinephrine concentrations compared with the CON. In conclusion, the results of this study show that MOL supplementation decreased the fecal moisture content and plasma concentrations of cortisol and norepinephrine, and MOL and BPP supplementation also decreased the fecal moisture content in lactating sows.

Keywords: fecal moisture content, hormones profiles, lactating sow, phytobiotics additives, reproductive performance

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Introduction

Moringa oleifera leaf (MOL), which is most widely cultivated species of the *Moringaceae* family. *Moringaceae* are native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. These plants possess antimicrobial activity (Caceres et al., 1991), while the seeds reportedly have the potential of antispasmodic, antiinflammatory and diuretic activities (Caceres et al., 1992). According to analysis reported by Makkar and Becker (1996), the calcium and protein content of MOL were four times and two times compared with milk, respectively, the potassium content is three times that of banana, the iron content is three times that of spinach, the vitamin C content is seven times that of citrus and the vitamin A content is four times that of carrot. Moringa contains 17 different amino acids, 20.49% of the total plant content, with glutamic acid making up 14.25% of the total amino acids (Moyo et al., 2011). Moringa dried leaf extract is known to be an effective inhibitor of the central nervous system (Caceres et al., 1992). Moringa root is mainly used for curing flatulence and promoting intestinal regularity (Karadi et al., 2006).

Banana peel, is the outer covering of the banana fruit which contain 6 to 9% of crude protein, 43.2 to 49.7% of total dietary fiber, 3.8 to 11% of crude fat, and vitamins (Mohapatra et al., 2010). It could use as feedstock for livestock and poultry. Additionally, it was found that the peels possess valuable medicinal potential and as a possible immunostimulant for livestock since the biological and pharmacological effects such as antibacterial, antihypertensive, anti-diabetic and anti-inflammatory activities (Chabuck et al., 2013; Rattanavichai and Cheng, 2015).

We hypothesize that the ingestion of MOL and banana peel can relieve stress due to decrease concentrations of hormones profiles, improve intestinal tract function and enhance reproductive performance in lactating sows. The present study was conducted to evaluate the effects of dietary supplementation of different levels of MOL with or without banana peel powder (BPP) on reproductive performance, fecal moisture content, rectal temperature and hormones profiles in lactating sows.

Materials and Methods

The experimental use of animals and the procedures followed in this study were approved by the Animal Care and Use of Committee of Dankook University.

Animals, Experimental Design, Diets, and Housing

Twenty crossbred (Yorkshire × Landrace) sows were used to determine the effects of plant extract supplementation on reproductive performance, fecal moisture content, rectal temperature and hormones profiles concentrations in lactating sows. This experiment was conducted with pigs for one sow per pen and four pens per treatment in a randomized complete block design.

Dietary treatments include: CON, control diet; M1, CON + 0.5% MOL; M2, CON + 1.0% MOL; M3, CON + 0.75% MOL and 0.75% BPP; and M4, CON + 1.5% MOL and 1.5% BPP. The ingredient of MOL and BPP was shown in Table 1. The experiment began from day 107 of gestation to weaning on day 21 after parturition.

At day 107 of gestation, the sows were relocated to farrowing crates in environmentally regulated farrowing house, and allotted to 1 of 5 dietary treatments. The mean parity of the sows was 2.7 ± 0.3. Sows were fed on a commercial gestation (2.6 kg) diet from day 107 to farrowing (Table 2). All diet were formulated to meet or exceed the NRC (2012) and provided in mash form and divided into 2 daily meals. Throughout the experimental all pigs were provided with ad libitum access to water.

The sows and their litters were individually housed in farrowing crates (2.4 m × 1.8 m), which were constructed of 1.95 m^2 of solid floor and 2.37 m^2 of slatted floor. This space included a piglet nest equipped with an infrared lamp (500 W), a piglet drinking nipple and a piglet feeder placed on a dimpled rubber matting to collect any spillage from the feed. The temperature in the farrowing house was maintained at a minimum of 20°C. Drinking nipples provided water ad libitum to the piglets. Litter size at birth varied from 7 to 14 piglets, and was standardized to 10 piglets per litter within day 2 after birth by cross fostering within each batch. All piglets received injections of 1 ml of iron dextran and the males were castrated day 2 after birth. At day 21 of nursery, sows were relocated to a mating room, with the piglets remaining in the pen for one week (weanling pigs). Detection of estrus was conducted twice per day from weaning onward, at 8:30 am and 4 pm every day. A sow was considered to be in estrus when exhibiting a standing response induced by a back pressure test when in the presence of a boar.

Table 1. Ingredient of *Moringa Oleifera* leaf and banana peel powder.

Items	*Moringa Oleifera* leaf	Banana peel powder
Moisture (%)	7.5	3.6
Calories	205	346
Protein (g)	27.1	4
Fat (g)	2.3	2
Carbohydrate (g)	38.2	88
Sugars (g)	8.3	47
Fiber (g)	19.2	10
Minerals (mg)		
Ca	2,003	22
Mg	368	108
P	204	74
K	1,324	1,491
Cu	0.57	0.39
Fe	28.2	1.15
S	870	42
Zn	0.02	0.61
Vitamin (mg)		
Vitamin A - β carotene	16.3	0.29
Vitamin B1 - thiamin	2.64	0.18
Vitamin B2 - riboflavin	20.5	0.24
Vitamin B3 - nicotinic acid	8.2	0.07
Vitamin B6 - pyridoxal phosphate	0.21	0.44
Vitamin C - ascorbic acid	17.3	7.1
Vitamin E - tocopherol acetate	113	0.39
Amino acid (g/16 g N, %)		
Arginine	1.33	-
Histidine	0.61	-
Lysine	1.32	-
Tryptophan	0.43	-
Phenylanaline	1.39	-
Methionine	0.35	-
Threonine	1.19	-
Leucine	1.95	-
Soleucine	0.83	-
Valine	1.06	-

Sampling and Measurements

At farrowing and weaning, the backfat thickness of all sows was measured at 6 cm off the midline at the 10th rib using a real-time ultrasound instrument (Piglot 105, SFK Technology, Herlev, Denmark). All piglets were weighed on born and on the weaning day for body weight (BW) and weight gain. Feed intake of sows was recorded daily at birth and weaning for average daily feed intake (ADFI).

Daily collections of total fecal samples were made at day 0, 7, 14, and 21 after parturition for the determination of moisture. Total moisture was determined by air-drying the total collection at 60℃, followed by an equilibration and moisture determination at 105℃ (Harris, 1970). Rectal temperature was measured at 6 o'clock in the morning during day 0, 7, 14, and 21 of the lactation.

To assess blood characteristics, the sows were bled via puncture of the auricular vein at farrowing and weaning (day 21) to determine the concentrations of epinephrine, norepinephrine and cortisol. Blood samples were collected into non-heparinized tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, USA) to obtain serum, which was separated via 30 min of centrifugation at 4,000 × g at 4℃, the aliquot was stored at - 4℃. The serum were removed and stored at - 20℃ until being used in cortisol analysis. Serum concentrations of cortisol were determined with a standardized solid phase radioimmunoassay

Table 2. Composition of experimental diets (as-fed basis).

Items	Gestation diet	Lactation diet
Ingredient (%)		
Corn	57.10	51.12
Soybean meal	10.65	24.61
Wheat bran	12.00	4.00
Rapeseed meal	3.70	2.50
Rice bran	6.00	5.00
Tallow	3.59	6.05
Molasses	3.60	3.50
Dicalcium phosphate	1.52	1.64
Limestone	0.99	0.76
Salt	0.60	0.50
Lysine, 98%	0.05	0.12
Vitamin and mineral premix[z]	0.20	0.20
Calculated nutrient content (%)		
Metabolizable energy (MJ/kg)	3.19	3.44
Crude protein	13.10	17.10
Crude fat	6.89	9.10
Lysine	0.65	1.00
Calcium	0.87	0.85
Phosphorus	0.76	0.73

From day 107 of gestation until farrowing, sows were fed the gestation diet (additive replaced a portion of the corn), from farrowing to weaning, sows were fed the lactation diet (additive replaced a portion of the corn).

[z] Provided per kilogram of complete diet: vitamin A, 10,000 IU; vitamin D3, 2,000 IU; vitamin E, 48 IU; vitamin K_3, 1.5 mg; riboflavin, 6 mg; niacin, 40 mg; d-pantothenic, 17 mg; biotin, 0.2 mg; folic acid, 2 mg; choline, 166 mg; vitamin B_6, 2 mg; and vitamin B_{12}, 28 µg, Fe (as $FeSO_4 \cdot 7H_2O$), 90 mg; Cu (as $CuSO_4 \cdot 5H_2O$), 15 mg; Zn (as $ZnSO_4$), 50 mg; Mn (as MnO_2), 54 mg; I (as KI), 0.99 mg; and Se (as $Na_2SeO_3 \cdot 5H_2O$), 0.25 mg.

kit (Diagnostic Products Corporation, Los Angeles, USA). Norepinephrine (NE) and epinephrine (EPI) were assayed using an ion exchange purification procedure followed by liquid chromatography with electrochemical detection, as described previously by Hay and Mormède (1997). In brief, the samples were loaded onto cationic columns, and the catecholamines were eluted with boric acid. The eluates were assayed via HPLC (Shimadzu Scientific Instruments, Inc., Colombia, USA) with electrochemical detection with an oxidizing potential of + 0.65 Volt. The intra- and inter-assay cyclic voltammetry (CV) were 7.0 and 7.1% for NE and 6.5 and 11.6% for EPI, respectively.

Statistical Analysis

All data in this experiment were analyzed in accordance with a completely randomized design using the GLM procedure (SAS Institute Inc., Cary, USA). The individual sow or litter of piglets was used as the experimental unit. For the blood profile data, the initial data was used as a covariate. Differences among treatment means were determined via Turkey's multiple range test, and a probability level of $p < 0.05$ was regarded as statistically significant.

Results

Reproductive Performance of Sows

No effect ($p > 0.05$) was observed on reproductive performance parameters such as backfat thickness, back fat loss, ADFI, days to estrus, piglet BW, and weight gain by the supplementation of MOL with or without BPP (Table 3).

Fecal Moisture Content

At farrowing, 1.5% MOL and 1.5% BPP inclusion decreased ($p < 0.05$) fecal moisture content compared with CON (Table 4). At day 7 after farrowing, plant extract along with banana powder supplementation decreased ($p < 0.05$) fecal moisture content compared with CON and M1. However, fecal moisture content was decreased ($p < 0.05$) by the inclusion of 1.0% MOL at day 14 after farrowing.

Table 3. Effect of *Moringa Oleifera* leaf (MOL) supplementation with or without banana peel powder (BPP) on reproductive performance in lactating sows and piglets.

Items	CON	M1	M2	M3	M4	SE	p-value
Backfat thickness (mm)							
Farrowing	25.5	25.2	25.0	25.5	25.2	2.60	0.524
Weaning	15.8	19.3	16.5	16.3	20.5	2.20	0.621
Backfat loss	9.7	5.8	8.5	9.2	4.7	2.69	0.410
ADFI (kg)	6.93	6.11	5.72	6.38	5.65	0.47	0.362
Days to estrus	5.0	5.7	5.0	5.3	6.0	0.6	0.254
Piglet BW (kg)							
Birth BW	1.34	1.60	1.49	1.61	1.43	0.12	0.632
Weaned BW	6.56	7.26	6.51	7.15	6.86	0.38	0.412
Weight gain	5.22	5.65	5.02	5.54	5.42	0.44	0.246

CON, control diet; M1, CON + 0.5% MOL; M2, CON + 1.0% MOL; M3, CON + 0.75% MOL and 0.75% BPP; M4, CON + 1.5% MOL and 1.5% BPP; ADFI, average daily feed intake; BW, body weight; SE, standard error.

Rectal Temperature

As shown in Table 5, the rectal temperature was decreased ($p < 0.05$) by the inclusion of 1.5% MOL and 1.5% BPP compared with 1.0% MOL inclusion at day 7 after farrowing. However, at initial, day 14, and day 21 no significant effects were observed among the treatments.

Table 4. Effect of *Moringa Oleifera* leaf (MOL) supplementation with or without banana peel powder (BPP) on fecal moisture content in lactating sows.

Items (%)	CON	M1	M2	M3	M4	SE	p-value
Day 0	72.10a	71.56ab	70.59ab	71.65ab	70.50b	0.44	0.021
Day 7	72.33a	72.44a	71.05ab	70.17b	69.86b	0.54	0.013
Day 14	71.35a	69.14ab	67.54b	69.87a	69.88a	0.64	0.019
Day 21	73.00abc	73.58ab	70.60c	75.16a	71.73bc	0.73	0.009

CON, control diet; M1, CON + 0.5% MOL; M2, CON + 1.0% MOL; M3, CON + 0.75% MOL and 0.75% BPP; M4, CON + 1.5% MOL and 1.5% BPP; SE, standard error.
a - c: Means in the same row with different superscripts differ ($p < 0.05$).

Table 5. Effect of *Moringa Oleifera* leaf (MOL) supplementation with or without banana peel powder (BPP) on rectal temperature in lactating sows.

Items (℃)	CON	M1	M2	M3	M4	SE	p-value
Day 0	39.0	39.4	39.0	39.1	38.9	0.19	0.501
Day 7	39.9ab	39.8ab	40.2a	39.5ab	39.2b	0.26	0.027
Day 14	39.3	39.0	39.1	39.0	39.0	0.20	0.187
Day 21	38.8	38.9	38.8	38.7	38.5	0.29	0.285

CON, control diet; M1, CON + 0.5% MOL; M2, CON + 1.0% MOL; M3, CON + 0.75% MOL and 0.75% BPP; M4, CON + 1.5% MOL and 1.5% BPP; SE, standard error.
a, b: Means in the same row with different superscripts differ ($p < 0.05$).

Table 6. Effect of *Moringa Oleifera* leaf (MOL) supplementation with or without banana peel powder (BPP) on hormones profiles concentration in lactating sows.

Items	CON	M1	M2	M3	M4	SE	p-value
Cortisol (μg/dL)							
Farrowing	15.10a	13.70b	12.23b	12.75ab	13.73ab	2.63	0.022
Weaning	4.50	4.05	3.60	3.70	3.87	1.99	0.145
Epinephrine (pg/mL)							
Farrowing	62.10	26.25	35.20	60.05	40.67	11.17	0.321
Weaning	27.40	24.20	41.77	15.95	39.77	16.08	0.095
Norepinephrine (pg/mL)							
Farrowing	134.6a	86.5b	99.7b	126.1a	131.7a	4.07	0.024
Weaning	108.5	207.1	156.9	64.7	250.3	112.57	0.217

CON, control diet; M1, CON + 0.5% MOL; M2, CON + 1.0% MOL; M3, CON + 0.75% MOL and 0.75% BPP; M4, CON + 1.5% MOL and 1.5% BPP; SE, standard error.
a, b: Means in the same row with different superscripts differ ($p < 0.05$).

Hormones Profiles

At farrowing, treatment M1 and M2 were showed significantly decrease ($p < 0.05$) in the blood cortisol concentration compared with CON (Table 6), at the same time, norepinephrine concentrations in M1 and M2 were significant ($p < 0.05$) lower than other treatments.

Discussion

The deleterious effects of stress on pigs have become recognized as commercially important. Transport and vaccination of pigs, and their introduction to new surroundings can be a frightening and stressful experience, which detrimentally affect farrowing and weaning. When a pig is in the condition of stress, it will be more susceptible to sickness, may leading to eat less feed and grow slower. Thus, it is important to minimize stress status. All hormones affect all measurable behaviors. Therefore, all hormones modulate stress, anxiety and depression, but the effects they have will depend on where, when, how long, and at what concentration they are present.

The tree has been advocated as an outstanding indigenous source of highly digestible protein, calcium, iron, Vitamin C and carotenoids suitable for utilization in many of the so-called “Developing” regions of the world where undernourishment is a major concern. According to Fuglie (2001), the many uses for MOL include alley cropping, animal forage, biogas, domestic cleaning agent, blue dye, fencing, fertilizer, foliar nutrient, green manure, gum, honey- and sugar cane juice-clarifier, honey, medicine, ornamental plantings, biopesticide, pulp, rope, tannin for tanning hides and water purification. The benefits for the treatment or prevention of disease or infection that may accrue from either dietary or topical administration of MOL preparations (e.g., extracts, decoctions, poultices, creams, oils, emollients, salves, powders, porridges) are not clear (Palada, 1996).

Plant extracts such as essential oils (EO) (Yan et al., 2010), *Eugenol, Cinnamaldehyde* (Yan and Kim 2012), *Houttuynia cordata, Taraxacum officinale* (Yan et al., 2011b; 2012b), and *Saururus chinensis* (Ao et al., 2011) were widely used as alternatives to antibiotics in weaning and grower-finisher pigs because of their benefits. In this study, dietary administration of MOL alone or along with BPP decreased the concentrations of cortisol and norepinephrine during some periods, which was consistent with the theory and with previous research findings. *Moringaceae* possesses antimicrobial activity (Caceres et al., 1991), while the seeds reportedly have antispasmodic, anti-inflammatory and diuretic activities (Caceres et al., 1992). MOL is an effective inhibitor of the central nervous system (Caceres et al., 1992). Anti-stress plant drugs have been reported earlier to produce non-specifically increased resistance in animal models of stress (Brekhman and Dordymov, 1969; Singh et al., 1978; Bhargava and Singh, 1985; Singh, 1986,). These agents reduce the stress-related alarm reaction in animals and emotional stress in man. The latter causes a variety of intestinal disturbances including hyper-motility of the gastrointestinal tract. In Ayurveda, gastrointestinal disorders are related to a variety of human ailments. The benefits observed with bananas may reflect their content of tryptophan, an amino acid that can be converted to serotonin, which is associated with improved mood. Furthermore, bananas are high in potassium, which helps normalize the heartbeat and regulate the body's water balance. During periods of high stress, our body's potassium levels tend to be rapidly depleted; eating bananas is a healthy way to rebalance them without using drugs. In contrast, herb ranging from 0.05 - 0.2% were not capable to alleviate cortisol concentration in blood (Wang et al., 2008), but increase lymphocyte count, red blood cells (RBC), and white blood cells (WBC) in weaning and growing pigs (Yan et al., 2011a, 2012a).

The previous study conducted by our team (Wang et al., 2008) indicated that supplementation with phytogenic substances was not able to improve backfat during farrowing and weaning at 0.04%, which agree with current results of this study. This is clearly an area where the preponderance of evidence – both classical scientific and extensive anecdotal evidence – is overwhelming. The scientific evidence has been available for over 50 years, although much of it is completely unknown to Western scientists. In the late 1940's and early 1950's, a team from the University of Bombay, Travancore University, and the Department of Biochemistry at the Indian Institute of Science in Bangalore identified a compound called pterygospermin (Anderson and Bell, 1986). The compound readily dissociates into two molecules of benzyl isothiocyanate (Kurup and Narasimha Rao, 1952, 1954; Narasimha Rao and Kurup, 1953; Das, 1954, 1957; Anwar and Bhanger, 2003). Benzyl isothiocyanate was already understood at that time to have antimicrobial properties. This group not only identified pterygospermin, but performed extensive and elegant characterization of its mode of antimicrobial action in the middle of 1950's. They identified the tree from which the substance was isolated as "*Moringa pterygosperma*," now regarded as an archaic designation for "*M. oleifera*."). Others showed that pterygospermin and extracts of Moringa plants from which it was isolated are antibacterial against a variety of microbes. Yet, the identity of pterygospermin has been challenged as an artifact of isolation or structural determination (Eilert et al., 1981).

Normal fecal material is a semisolid containing up to approximately 70% moisture. The moisture is bound in such a way that it cannot be separated from the solid material by centrifugation at 22,000 × g. It appears that there is a saturation point of the fecal material at around 70 - 73% moisture; above this percentage, loose stools are observed. Percentage moisture could possibly be used as an index to severity of diarrhea. Presently, the fecal moisture content of sows whose diet was supplemented with MOL and/or BE decreased in certain periods. *Moringa oleifera* is a small or medium sized tree that is cultivated throughout India. The tender pods are esteemed as a vegetable. Seeds are used as purgative, antipyretic and anti-inflammatory (Warrier and Nambiar, 1993).That might be a factor to decrease diarrhea. Similar results were demonstrated that herbs could prevent diarrhea incidence in weaning piglets (Cho et al., 2012). Due to its fiber content, BE help restore normal bowel function. In addition, diarrhea usually depletes the body of important electrolytes (of which the most important is potassium, which is enriched in bananas). They also contain pectin, a soluble fiber (hydrocolloid) that can help normalize movement through the digestive tract. Moringa root is mainly used for curing flatulence and promoting intestinal regularity (Karadi et al., 2006). Like Moringa root, Phytoncide was proved to elevate nutrient digestibility and improve the fecal Lactobacillus counts in weaning pigs (Zhang et al., 2012).

Conclusion

In conclusion, this study indicates that administration of MOL with or without BPP can decrease fecal moisture content, and that the administration of MOL can decrease the secreted concentrations of hormones profiles.

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