Short note [Nota corta]

EFFECT OF DIETARY INCLUSION OF *Brosimum alicastrum Swartz* LEAF MEAL ON DIARRHEA CONTROL IN PIGLETS†

[EFECTO DE LA INCLUSIÓN DIETETICA DE HARINA DE HOJAS DE *Brosimum alicastrum Swartz* SOBRE EL CONTROL DE DIARREAS EN LECHONES]

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SUMMARY

Piglet mortality due to diarrhea is the most important economic loss in the pig industry. However, the use of antibiotics is been banned nowadays and the nutraceutical products are getting importance. The objective of this study was to evaluate the effect of *Brosimum alicastrum Swartz* leaf meal (BLM) in the diet of piglets, as a nutraceutical additive for reducing diarrhea. The study consisted in fed piglets with pre-starter commercial feed under two treatments, either without (T1) or with (T2) inclusion of 20 g/kg BLM. Ninety-eight piglets were used per treatment, with an average initial live weight (LW) of 2.6 ± 0.42 kg. The test lasted from 5 to 27 days-old, the pigs were fed *ad libitum*. Offer and rejection of food were recorded daily, as was the presence of diarrhea; live weight was recorded weekly. Data on productive measures were analyzed by T-test; contingency tables, X^2 tests and calculation of relative risk (RR) for the incidence of diarrhea were applied. Daily weight gain (DWG) in T2 was higher than in T1 (273 ± 0.12 vs 220 ± 0.09 g/day) (P = 0.003). The true incidence rate of diarrhea was 3% and 1% (P <0.001) for T1 and T2 respectively. The cumulative incidence rate of diarrhea was lower (P <0.001) in T2 (25%) than in T1 (54%). The risk of developing diarrhea was higher in T1 (RR = 0.67, 95% CI, 0.39-1.13). The inclusion of BLM in the diet of piglets reduced the incidence of diarrhea and increased DWG.

Key words: Feed intake; daily weight gain; *Brosimum alicastrum* leaf meal; relative risk.

RESUMEN

La mortalidad de lechones por diarrea es la pérdida económica más importante en la industria porcina. Sin embargo, el uso de antibióticos está prohibido hoy en día y los productos nutracéuticos están adquiriendo importancia. El objetivo de este estudio fue evaluar el efecto de la harina de hojas de *Brosimum alicastrum Swartz* (BLM) en la dieta de lechones, como un aditivo nutracéutico para reducir la diarrea. El estudio consistió en alimentar a los lechones con alimento comercial pre-iniciador bajo dos tratamientos, ya sea sin (T1) o con (T2) inclusión de 20 g / kg de BLM. Se utilizaron noventa y ocho lechones por tratamiento, con un peso vivo inicial promedio (PL) de 2.6 ± 0.42 kg. La prueba duró de los 5 a 27 días de edad, los cerdos fueron alimentados a voluntad. La oferta y el rechazo de los alimentos se registraron diariamente, al igual que la presencia de diarrea; El peso en vivo fue registrado semanalmente. Los datos sobre las medidas productivas se analizaron mediante la prueba T; se aplicaron tablas de contingencia, pruebas de X^2 y cálculo de riesgo relativo (RR) para la incidencia de diarrea. El aumento de peso diario (DWG) en T2 fue mayor que en T1 (273 ± 0.12 vs 220 ± 0.09 g / día) (P = 0.003). La tasa verdadera de incidencia de diarrea fue del 3% y del 1% (P <0,001) para T1 y T2, respectivamente. La tasa de incidencia acumulada de diarrea fue menor (P <0,001) en T2 (25%) que en T1 (54%). El riesgo de desarrollar diarrea fue mayor en T1 (RR = 0,67; IC del 95%, 0,39-1,13). La inclusión de BLM en la dieta de los lechones redujo la incidencia de diarrea y aumentó la ganancia diaria de peso.

Palabras clave: Ingesta de alimento; ganancia diaria de peso; harina de hojas de *Brosimum alicastrum*; riesgo relativo.

INTRODUCTION

Pre-weaning mortality is one of the causes of significant economic losses in the pork industry, with rates of 10 to 20% (Maldonado, 2008). At birth, piglets face a great challenge of adaptation for obtaining good nutrition and passive immunity that allows them to survive (Kim *et al*., 2006). Antibiotics are often use in the feed at sub-therapeutic level, causing residues in animal products and so, public health problems, due to resistance by pathogenic microorganisms, and reducing beneficial organisms in the pig intestine (Naqid *et al*., 2015).

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On the other hand, prebiotics are slightly digestible feed ingredients that stimulate the selective growth and metabolic activity of bifidobacterial and lactobacilli, which will act as probiotics that inhibit the growth of pathogens, stimulating the immune system (Modesto *et al*., 2011). For those reasons, it is necessary the search for local alternatives as prebiotics.

Ramón (*Brosimum alicastrum Swartz*) is a very valuable tropical tree for livestock production, since its forage contains no secondary metabolites; in addition to the amount of amylopectin and soluble fiber such as pectin that its seed contains (Zamora-Crescencio *et al*., 2009; Pérez- Pacheco *et al*., 2015). Soluble fiber can favorably stimulate the microbial community of the gastrointestinal tract and decrease digestive problems, then it may be an option to decrease diarrhea in piglets (Lindberg, 2014). However, there are no studies of the effect of Ramón leaf meal on either the production parameters or the rate of reduction the incidence of diarrhea in piglets. The objective of this study was to evaluate the inclusion of *B. alicastrum* leaf meal (BLM) in the diet of piglets, as a factor that reduces diarrhea.

MATERIALS AND METHODS

The present study was carried out in the pig production farm of the Faculty of Veterinary Medicine and Animal Science of the Universidad Autónoma de Yucatán, México (FMVZ-UADY).

Animals

Twenty litters (9.8 piglets in average) of the L337 genetic line from the pig farm were used; they were divided into two groups of 98 piglets each (5 day-old and 2.6 ± 0.42 Kg initial live weight). All animals were identified. The number of animals per treatment was estimated using the EpiSample program, for an experimental cohort study, using a 95% confidence level, 80% test power and a relative risk of 2.5 (Segura-Correa, 2008). The animals were used from 5 to 27 days-old.

The experiment was divided in three periods: the first, from the 5th to the 15th day of piglets life; the second period from the 16th to the 21st day of life; and the third period, from the 22nd to the 27th day of life. During the first two periods, the animals were confined in elevated metal cages with a grating floor. Each elevate cage had a feeder and automatic nipple drinker for the sow; and a collective feeder and wooden box on the front of the cage for the piglets. In the third period, piglets were housed in the weaning area, in raised cages with floor and walls of bars, each cage had a collective feeder of 1.2 m long, located in the lateral part of the cage and an automatic nipple drinker.

Brosimum alicastrum leaf meal

The BLM was obtained from trees located at FMVZ-UADY, having six months of regrowth; the forage was dried in an electric oven at 60 °C for 48 hours, then, it was ground in an electric mill with a 5 mm screen. The chemical composition of BLM determined according to AOAC (2000) was 4.2 % humidity, 14.17% crude protein, 40.38% Acid detergent fiber and 49.07% Neutral detergent fiber.

Experimental diet

Two treatments were used: a control group (T1) using a commercial concentrate feed for piglets (pre-starter NUPIG UNO composed according to the manufacturer: 12% humidity, 20% crude protein, 4% Fat, 3.5% Crude fiber, 7% Ash and 53.5 % free-nitrogen extract) and the treated group (T2), where BLM was mixed at 20 g/kg with the same control feed as feed basis (980 g of control diet and 20 g BLM). The feed was offered *ad libitum* in a collective feeder per litter; the rejected feed was weighed on a daily basis.

Feed consumption

Feed intake was recorded daily per litter, and it was estimated as the difference between feed offered and rejected.

Daily weight gain (DWG)

DGW was estimated by the difference of the initial and the final weight in each period; dividing this difference by the days each period lasted.

Incidence of diarrhea

The animals with diarrhea were monitored and registered daily. Monitoring consisted on post-feeding observation of the litters for ten minutes, three times a day until 3:00 pm.

Time at Risk (TAR)

The TAR was estimated according to the following formula:
TAR = number of piglets at the beginning + ½ income of animals - ½ outputs of animals - ½ cases x period of observation

Cumulative incidence rate

Obtained from the following formula:
Number of new cases / Number of animals at risk at the beginning of the period.

Analysis of data

For the variables of feed intake per litter and DGW per piglet, analysis of variance was performed according to a completely randomized design; using the T-test (Excel, 2010). The records of prevalence and incidence of diarrhea per litter were analyzed using non-parametric statistics tool and the program Win Episcope 2.0 for obtaining the incidence of diarrhea was used.

RESULTS

The measures of productive performance in both groups of piglets is showed in Table 1. Table 2 shows the results of true incidence rate of diarrhea per period and per day.

Table 1. Average and standard deviation for productive performance in piglets of genetic line L337, for periods of study, supplemented with and without 2% *Brosimum alicastrum* leaf meal (BLM) in the diet.

Parameter	Treatment		*P*
Live weight (Kg)	CONTROL	BLM	
15 day old	5.1 ± 1.6	5.3 ± 1.4	0.485
21 day old	6.5 ± 1.7	6.6 ± 1.4	0.663
27 day old	7.8 ± 1.9	8.2 ± 1.9	0.120
Intake/day/litter (g)	CONTROL	BLM	
From 5 to 15 days	65 ± 40	74 ± 50	0.932
From 16 to 21 days	149 ± 90	233 ± 24	0.393
From 22 to 27 days	2030 ± 40	1781± 68	0.336
DGW (g/d)	CONTROL	BLM	
From 5 to 15 days	250 ± 15	257 ± 14	0.944
From 16 to 21 days	228 ± 9	219 ± 15	0.677
From 22 to 27 days	220 ± 9	273 ± 12	0.003

P=probability

A higher weight gain was observed in the third period by including BLM in the diet (53g above control group) (P = 0.003, Table 1). Table 2 shows the difference in the incidence rate of diarrhea during the second (P = 0.013) and third periods of the experiment (P = 0.001); the true incidence for T1 (3%) is higher than in T2 (1%) of low risk animals with daily diarrhea (P = 0.0001). The cumulative incidence of diarrhea were 54% and 25.5% for T1 and T2 respectively during the experiment (P = 0.0001). The RR indicate that animals consuming only commercial feed were 0.67 times more exposed to diarrhea than those consuming BLM as feed additive (Table 2).

DISCUSSION

This is the first study using *B. alicastrum* as a prebiotic in piglets. BLM is an additive high in insoluble fiber and the results of the present study indicate that the inclusion of BLM did not affect feed consumption but increased DWG from 22 to 27 days of pig age and decreased the diarrhea incidence in the piglets.

BLM as prebiotic

Chiquieri *et al*. (2006) found that there were no significant effects on productive behavior by using prebiotics and probiotics on piglets, although, Brambillasca *et al*. (2010) reported a favorable effect on DWG using inulin and citrus pulp. These authors refer to diets that have highly fermentable fructans (inulin) and oligofructose, which stimulate the growth of bifidobacteria, accordingly, the reduction of pathogenic bacteria (Clostridium, Coccoides-Eubacterium) and decrease of gastroenteric problems was observed. Beccaccia *et al*. (2015) used citrus pulp containing pectin as a prebiotic in pigs to improve DWG and observed fewer incidence of diarrhea. Lindberg (2014) mentioned that soluble fiber reduced transit time and provided substrates that are slowly degraded by microbiota in the distal large intestine and modulate intestinal morphology by increasing villus length; thus, improving nutrient absorption. Guallichico (2011) reported a positive effect on DWG using mannano-oligosaccharide in piglets. Also, the inclusion of prebiotics, pastures or fodder in low doses should not cause changes in animal consumption and may benefit DWG (Cajarville *et al*., 2011; Rondón *et al*., 2013).

BLM and diarrhea control

Pérez-Pacheco *et al*. (2015) described that *B. alicastrum* possesses greater amount of soluble dietary fiber as pectin and also cellulose. Lin *et al*. (2011) and Lindberg (2014) mentioned that soluble dietary fiber ferments in the gastrointestinal tract, consequently the volatile fatty acids which are produced inhibit certain pathogenic bacteria and stimulate bacteria like bifidobacteria and lactobacillus. In this way, the reduction in the incidence of diarrhea in this experiment could be attributed to the water-soluble dietary fiber (pectin) and in minor extent to the insoluble fiber from BLM. Piglets consuming BLM probably had a healthy gastrointestinal tract, improving nutrient absorption and leading to higher DWG. Similarly, Zhao and Kim (2014) reported that the supply of *L. plantarum* reduces the incidence of diarrhea in piglets at weaning by stimulating interleukin-6; which elevates the levels of immunoglobulins of the B lymphocytes. Naqid *et al*. (2015) did not observe diarrhea when using lactulose and Lactobacillus in piglets with *S. typhimurium.*

Table 2. True incidence rate of diarrhea in L337 genetic line piglets fed pre-starter feed with or without the addition of *Brosimum alicastrum* leaf meal (BLM).

Age	Control					BLM					
Days	ABR	NC	ATRP	ATRD	IR	ABR	NC	ATRP	ATRD	IR	*P*
5 to 15	98	27	84.5	845	0.032	98	18	89	89	0.020	0.126
16 to 21	71	11	65.5	393	0.028	80	3	78.5	78.5	0.006	0.013
22 to 27	60	15	52.5	315	0.048	77	4	75	75	0.009	0.001
TIR RR 0.67		53	202.5	1553	0.034		25	242.5		0.014	0.0001

ABR: Animals at risk at the beginning of the period, NC: New cases, ATRP: Animal time at risk per period, ATRD: Animal time at risk per day, IR: Incidence rate, TIR: True incidence rate; RR: relative risk; IC: Interval confidence (0.39-1.13), P: Probability

These results confirm previous findings by Rojas-Schroeder *et al*. (2017) that forage from BA has favorable nutritional values for the feeding of domestic productive species while adding knowledge on the usefulness of BLM as an alternative prebiotic for the control of diarrhea and to reduce feeding and therapeutic costs, caused by diarrhea, in pig production

CONCLUSION

It can be concluded that the incidence of diarrhea was reduced in the piglets of the L337 genetic line consuming 2% of *Brosimum alicastrum* leaf meal, also, DWG increased in the d 22 to 27 of age, without affecting consumption.

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