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Reproduction performance of sows inseminated with stress gene-free semen given probiotic-supplemented feed

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ABSTRACT

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This study generally aimed to assess the effect of supplementation with various levels of a novel product containing probiotic and yeast cells, a feed enzyme, short-chain oligosaccharides, and herbal extracts (Farmer Peck's Performance Booster®) and the use of halothane free gene semen on two successive parities in sows in selected farms in Leyte. Randomized complete block design (RCBD) with treatment used in T_0 - (in-feed antibiotics, 0g probiotic/kilogram feed and AI using semen from farm's boar); T_1 - (0 antibiotics, 2g probiotic/kilogram feed and AI with halothane free gene semen) and T_2 - (0 antibiotics, 3g probiotic/kilogram feed and AI with halothane free gene semen). The study results showed that sow-litter performance of artificially inseminated sows using halothane free gene semen in two farrowing seasons was significantly higher in T_1 and T_2 groups than that of T_0 group as affected by probiotic supplementation. Probiotic supplementation, both at 2g kg^{-1} (T_1) and 3g kg^{-1} (T_2) of feed is effective in improving both litter and sow performance. The cost of using halothane free gene semen for AI is less as compared to using semen from the farm's boar, and the cost of using probiotic at two levels as feed additive is relatively lower than using antibiotics based on the pre-weaning mortality, litter size, and litter weight at weaning. The use of halothane free gene semen can now be widely used in the different piggery farms. Including the use of probiotic supplement for both the sows and piglets.

Keywords: probiotic supplementation, artificial insemination, halothane free gene semen, swine production

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INTRODUCTION

At present, the fastest and cheapest way to achieve heterosis through crossbreeding is by artificial insemination (AI). As with other animals, AI in pigs offers many advantages in different fields the greatest of which is gaining access to superior genetics (Knox 2000).

The stress gene is a recessive gene found in some breeds of pigs that affects the animal's susceptibility to stress and lead to porcine stress syndrome (PSS). It is also called halothane gene because it can be tested by exposing the pigs to the anesthetic halothane (Zhang et al 1992). The stress susceptible HAL n allele is associated with reduced body fat in pigs (Kathirvel & Archibald 2001). The swine stress gene (*hal*) is located in chromosome 6 (p1.1-q2.1) and codes for ryanodine receptors, which are Ca^{2+} release channels of skeletal muscle sarcoplasmic reticulum. Comparison of sequence of full-length *hal* cDNA (Genbank M91451) from PSS susceptible and PSS non-susceptible pigs revealed 18 single nucleotide polymorphisms between these two types of pigs. One of the polymorphisms involves the substitution of cytosine (PSS non-susceptible) by a thymine (PSS susceptible) at nucleotide 1843. This alteration results in the replacement of an arginine at position 615 by a cysteine. As a consequence, in recessive homozygote's (*nn*) the gene *hal* leads to PSS and the major *post-mortem* manifestation of pale, soft and exudative pork (PSE) (Marriott & Schilling 2006). In heterozygosis (*Nn*), the *hal* gene produces lower carcass quality but possibly higher carcass weight. The polymorphism at nucleotide 1843 of the *hal* gene has recently been characterized by a DNA test using blood or a muscle biopsy as the source of genomic DNA (Bastos et al 2000). Fisher et al (2000) reported that the effect of the halothane gene Initial bacon gain in pumped weight was significantly higher (p<0.001) for the NN (15.2%) and Nn (14.9%) compared to the nn pigs (8.9%). Similarly, the total gain in bacon yield was the highest for the Nn (11.5%) and NN pigs (10.0%), and significantly higher (p<0.05) than the bacon from nn pigs (3.4%). Fàbrega et al (2002) reported that for improving meat quality and welfare the halothane gene should be removed from breeding schemes in order to obtain production of piglets that are less susceptible to stress, better performance, and improved meat quality and welfare. De Smet et al (1996) reported that halothane genotype Belgian Landrace and Pietrain pigs were predominant in meat quality traits. Garcia-Macias et al (1996) and Larzul et al (1997) also reported that halothane genotype had a significant effect on meat quality of the pigs. Bidanel et al 2020 reported the differences in crossbreeding for meat quality traits were in favor to Meishan (MS) genes.

Probiotic products for various livestock production systems were introduction mostly as feed supplement and in swine, mostly for piglets (Ho 2004). As with antibiotics, the primary reason for their use is to improve intestinal health by modifying the composition of the resident intestinal microbes (Böhmer et al 2006). It is a well-established fact that the health of the gastrointestinal tract generally affects the health of the animal (Altemueller et al 2009). Several studies have already been conducted to determine the effect of dietary supplementation with probiotics in swine and in other livestock species (Estienne et al 2005). These studies used either the lactobacillus species or the yeast cells (Jacela et al 2010). Simon (2005) used the above microorganisms alone or in combination with other ingredients including probiotics, enzymes or herbal extracts.

This paper reports the effects of supplementation with various levels of a novel product containing probiotic and yeast cells, a feed enzyme (Alpha-Amylase, IUB No. 3.2.1), short-chain oligosaccharides, and herbal extracts and the use of stress gene-free PIC GTC semen on two successive parities in sows in selected farms in Leyte. In this study, probiotic is a preparation of or a product containing viable, defined microorganisms in sufficient numbers, which alter the micro flora (by implantation or colonization) in a compartment of the host and by that exert beneficial health effects in this host (Schrezenmeir & de Vrese 2001).

MATERIALS AND METHODS

Experimental Animals

The Gene Transfer Corporation (GTC) semen used in treatment two (2) and three (3) is halothane free gene or coming from PIC pigs with Stress Free proper name (Ter 2009). Production of piglets includes those that are less susceptible to stress, better performance, and improved meat quality and welfare (Kathirvel & Archibald 2001). Clutter et al (2007) reported that in crossbreeding crossbred pigs is widely accepted and recommended practice in commercial swine production. The enrolled farms had crossbred sows for this study and were chosen purposively using the following criteria:

First, the three farms had at least eighteen heads sows served as experimental animal around 2-3 years old and into their second parity with eighteen sows to be bred around January and March 2009 (Batch 1) and the same eighteen sows (Batch 2) in June and August 2009. The sow was not a repeat breeder in its first production cycle and the farm owner agreed to use the halothane free gene semen for artificial insemination in the T_1 and T_2 sows and the probiotic supplement treatments, for two reproductive cycles starting on the day of artificial insemination until weaning day of the third cycle. A total of 54 heads of sows were used in this study to complete the batch 1 and batch 2 farrowings. All the semen samples from GTC were evaluated upon arrival and prior to insemination. Only semen that had met the minimum criteria of 70% motility and 80% morphology were used including farm's boar semen. The piggery farm at DAS-CAFS Visayas State University, Gaas piggery farm at Barangay Gaas, Baybay, Leyte and Tabgas piggery farm at Barangay Tabgas, Albuera, Leyte had never used halothane free gene semen in the past. The AI was done by only one person across three treatments and all parameters such as Littersize at Birth Born Alive (LSBBA) and Littersize at Weaning (LSW) within and among farms were properly counted on the number of piglet heads per sow. The Litter Weight at Birth (LWB) and Litter Weight at Weaning (LWW) within farms were accounted per head using calibrated hog weighing scale in kilogram. Pre-weaning Mortality (PWM) was by counting the number of pre-weaning deaths of the piglets among the litters in three farms. Lactational Weight Loss (LWL) was accomplished by getting the sow's weight after farrowing minus the actual body weight after the successful lactation and weaning the piglets. Weaning-to-Conception Interval (WCI) was the post-partum successful mating of the sows and Number of Service per Litter (SPL) was counted on the mating and the conduct of AI services per sow. Breeding to Breeding Interval (BBI) was accompanied by the period of first mating or AI plus the number of days of gestation period, lactation period, post-partum days

until the next successful mating or breeding of the sow. Litters produced per sow per year (LPPSY) was the sow index or the number of parity of the per year and Pigs Produced per Sow per Year (PPSY) was the number of piglets produced in the sow index.

Second, the farm was using gestating and lactating commercial feeds for its sows and pre-starter feed for the litter and no changes would be made on feeding management during the experimental period. Sow diet was pelleted but piglet diet was in crumbled form; gestating sows and lactating sows were fed restrictively according to their body mass and piglets had *ad libitum* access to pre-starter feed from day 15 to 30. The sow and litter were kept in individual farrowing pens provided with a crate for the sow and a creep area for the litter with partition in between. The farm followed a thirty (30) day lactation period; and the research was allowed access to farm records on the experimental animals.

Experimental Design

A total of fifty-four 2-3 years old lactating sows were used in the study for three experimental farms (Table 1). From each of these farrow-to-finish farms, fifty-four sows from each farm were into their second parity and expected to be in-heat each within the months of January and March 2009 (Batch 1), and June and August (Batch 2) 2009. The fifty-four sows in three farms were randomly distributed to the three treatment groups and were observed during their second and third parities. The sows in DAS-CAFS, Ga-as, and Tabgas piggery farm were crossbreds of unknown percentage of mixed breed of Landrace, Large White, Pietrain, and Poland China.

Table 1. Experimental layout in three farms showing the block allocation

Treatments	Second Heat/Parity (January and March 2009)			Third Heat/Parity (June and August 2009)		
T_0 - (in-feed antibiotics: (CSP 500 premix at 2g kg^{-1} feed, Dynamutilin 10% feed premix at 3g kg^{-1} feed & Lincomycin 4.4% feed premix at 1g kg^{-1} feed); 0g probiotic/kilogram feed and AI using semen from farm's boar)	S1	S4	S7	S10	S13	S16
T_1 - (0 antibiotics; 2g probiotic/kilogram feed and AI with halothane free gene semen)	S2	S5	S8	S11	S14	S17
T_2 - (0 antibiotics; 3g probiotic/kilogram feed and AI with halothane free gene semen)	S3	S6	S9	S12	S15	S18

Note: Both these boar/semen sources have met the minimum standard by the author.

The fifty-four sows in three farms were randomly distributed to the three treatment groups as follows: T_0–farm's practice (in-feed antibiotics, 0g probiotic/kilogram feed and AI using semen from farm's boar); T_1–0 antibiotics, 2g probiotic/kilogram feed and AI with halothane free gene; and T_2–0 antibiotics, 3g probiotic/kilogram feed and AI with halothane free gene semen.

For the sows in T_0 (in-feed antibiotics, 0g probiotic/kilogram feed and AI using semen from farm's boar, table 1), the brand of the gestating or lactating feed was the choice of the farm. This feed was mixed with the antibiotic additives and without the probiotic (Taras & Simon 2007), while T_1 and T_2 sows had the antibiotic additive replaced with the probiotic supplement.

Aside from the AI and probiotic supplementation, the gestating and lactating feeding and other management practices of the enrolled farms were not modified except for the administration of Vitamin ADE on the 70th, 95th and 110th day of gestation, vaccination against hog cholera on the 90th day of gestation (at 2mL per head regardless of body weight) and deworming on the 97th day using Ivermectin® at 1mL per 33kg body weight.

Frequency counts and relative frequency, percentages and means were used for descriptive statistics. In addition, data obtained from the two successive parities or in this case, the second and third reproductive cycles of the two batches of sows each in three treatment groups were subjected to univariate ANOVA for a randomized complete block design while Tukey's HSD was used for treatment means comparison.

RESULTS AND DISCUSSION

Litter Performance

Litter Size at Birth Born Alive (LSBBA) and Litter Size at Weaning (LSW)

The univariate ANOVA showed that at (p<.05) the treatments caused significant differences in litter size at birth born alive (LSBBA) in the two farms. Tukey's HSD analysis revealed that T_1 and T_2 were significantly higher than those in T_0 but not from each other.

Table 2 and table 3 shows the mean of LSBBA and LSW of the piglets in the three treatment groups in the three farms. In the three farms, T_1 and T_2 LSBBA and LSW were significantly higher than those of T_0 at p<0.05 significant level.

Thus, in three farms which crossbred sows did not benefit much from heterosis since the semen used in them including those in T_1 and T_2. Conversely, the T_1 and T_2 groups in farms 2 and 3 benefited the most with the use of the halothane free gene semen. In addition, the results obtained on LSBBA and LSW in this study could be due to the effect of the probiotic in the feed of the sows which could have resulted in probiotic being vertically transferred from the sows to the piglets thus increasing the number of piglets that survived up to birth and up to weaning age (Taveros & More 2005, Noguera et al 2002).

Table 2. Mean Litter Size at Birth Born Alive & Litter Size at Weaning of the piglets in the three treatment groups in the farms

Parameters and Treatments	Farm 1	Farm 2	Farm 3
Mean Litter Size at Birth Born Alive LSBBA (Number of heads)			
T_0	9.5	9.42	9.42
T_1	9.5	9.42	9.33
T_2	9.5	9.42	9.17

Table 2. continued

Parameters and Treatments	Farm 1	Farm 2	Farm 3
Mean Litter Size at Weaning LSW (Number of heads)			
T_0	9.42	8.57	9.25
T_1	9.50	9.25	9.33
T_2	9.50	9.42	9.17

Table 3. Mean Litter Size at Birth Born Alive & Litter Size at Weaning of the piglets in three farms

Parameters and Treatments	DAS-CAFS, Gaas and Tabgas Piggery Farm
LSBBA (count, number of heads)	
T_0	9.67^b
T_1	10.67^a
T_2	10.92^a
LSW (count, number of heads)	
T_0	9.25^b
T_1	10.67^a
T_2	10.92^a

Means with the same letters are not significantly different from each other at $p<0.05$ significant level

Litter Weight at Birth (LWB) and Litter Weight at Weaning (LWW)

The univariate analysis of variance showed that the treatments caused significant differences ($p<.05$) in LWB (kg). Tukey HSD analysis revealed that the mean LWB (kg) of T_2 in the three Farms – (1.46) and T_1 (1.43) were significantly higher than T_0(1.33) (Table 4). This trend was the same for the three farms, except in Farm 1 that the mean LWB (kg) of T_2 was significantly higher both T_1 and T_0.

In terms of the frequency and relative frequency of the individual birth weight of the piglets in each treatment, in general, the lowest and highest birth weights obtained ranged 0.5-1.00kg for the T_0 and 1.51-2.00kg range for the T_1 to T_2. While, T_0 to T_1 and T_1 to T_2 the number of piglets in the lowest birth weight range decreased while those in the highest range increased (Table 4). This trend was similar in the three farms.

Table 4. Mean Litter Weight at Birth and Litter Weight at Weaning of the three treatments in the three farms

Parameters and Treatments	Farm 1	Farm 2	Farm 3
Mean Litter Weight at Birth (LWB in kg)			
T_0	1.43^b	1.31^b	1.26^b
T_1	1.46^b	1.42^a	1.41^a
T_2	1.50^a	1.44^a	1.45^a
Mean Litter Weight at Weaning (LWW in kg)			
T_0	7.19^c	6.16^c	6.16^c
T_1	7.81^b	7.12^b	6.98^b
T_2	9.92^a	7.72^a	7.78^a

Means with the same letters are not significantly different from each other

Pre-weaning Mortality (PWM)

Extending the results of LSBBA and LSW, the PWM (%) was computed from the means of the two parameters (Arango et al 2005). PWM showed a decreasing trend in T_0 to T_1 and T_2. Specifically, T_0, T_1 and T_2 in Farm 1, had 0.84%, 0% and 0%, respectively while Farm 2, had 7.97%, 2.40% and 0%; and Farm 3, had 10.67%, 0% and 0%. In Farms 1 and 3, T_1 and T_2 had no mortality while T_1 in Farm 2 had a PWM of 2.40% indicating pre-weaning deaths in this group.

Sow Performance

Lactational Weight Loss (LWL)

The mean lactational weight loss of sows in the treatment groups in the three farms decreased from T_0 to T_1 and from T_1 to T_2 with the biggest weight loss in the T_0 sows and the lowest, in T_2 sows. In the T_0 sows, mean weight loss was 23kg, 23.50kg and 23.54kg. In T_1 sows, weight loss was 21.21kg, 21.54kg and 21.79kg, respectively; while in the T_2 sows, weight loss was 18.50kg, 20.46kg and 20.08kg, respectively (Table 5).

The univariate ANOVA showed differences in terms of the mean weight loss during lactation. The results of the Tukey's HSD showed the same trend in the three farms in that the mean weight loss of the T_0 sows was significantly higher than that in the T_1 sows which was also significantly higher than that in the T_2 sows.

Table 5. Mean Lactational Weight Loss, Service per Litter, Weaning-to-Conception Interval and Breeding to Breeding Interval of the treatment sows in the three farms

Parameters and Treatments	DAS-CAFS, Gaas and Tabgas Piggery Farms
LWL (kg)	Mean
T_0	23.54^c
T_1	21.79^b
T_2	20.08^a
SPL (count)	Mean
T_0	3.33^b
T_1	1.00^a
T_2	1.00^a
WCI (days)	Mean
T_0	22.42^b
T_1	11.25^a
T_2	9.42^a
BBI (days)	Mean
T_0	169.17^b
T_1	154.33^a
T_2	152.33^a
Predominance of *E. coli* in sow's pooled milk sample	Mean
T_0	8^b
T_1	3^a
T_2	4^a

Means with the same letters are not significantly different from each other

Weaning-to-Conception Interval (WCI) and Number of Service per Litter (SPL)

It is worth mentioning that around 50% of the sows in T_1 were successfully mated in less than 10 days. This could mean that while 2g of probiotic/kilogram feed was able to prevent repeated service in the sows, this level was able to contribute in bringing the sows into heat <10 days post weaning.

Conception Rate at First Service

In three piggery farms, 33.33% or 4 of the 12 T_0 sows conceived at first AI while 8 did not. However, in the T_1 and T_2 sows, all sows conceived at first AI 100%, meaning all the 12 sows conceived at first AI.

Breeding to Breeding Interval (BBI), Litters Produced and Pigs Produced per Sow per Year

As expected, BBI in this study followed the same trend with the WCI. The univariate ANOVA showed treatment differences in the BBI and using Tukey's HSD for treatment comparisons, it was revealed that in the three farms, BBI of the T_0 sows was significantly different from that of T_1 and T_2 however, between T_1 and T_2, there was none.

In terms of the frequency distribution of the BBI of individual sows, majority of the T_0 sows had BBI in the range of 171-175 days; majority of the T_1 sows were in the range of 146-150 days, and finally, majority of T_2 (10 or 83.33%) had 151-155 days.

In terms of pigs produced per sow per year (PPSY), the univariate ANOVA showed differences and the results of Tukey's HSD revealed differences between T_0 and T_1 and T_0 and T_2 but not between T_1 and T_2. The difference between and among treatments in terms of PPSY was significant (Table 6). Compared to what was reported by Lapus (2009), these findings suggest that even at the rate of 2g probiotic/kilogram feed combined with halothane free gene semen for AI is effective in producing stress-tolerant piglets.

Table 6. Mean litters and pigs produced per sow per year

Litters produced per sow per year (Number of Parity)	
Treatment	DAS-CAFS, Gaas and Tabgas Piggery Farms
T_0	2.16
T_1	2.37
T_2	2.40
Pigs produced per sow per year (Number of heads)	
T_0	19.96^b
T_1	25.23^a
T_2	26.16^a

Cost of AI Using Farm Boar Semen or halothane free gene semen and Probiotic or In-feed Antibiotics

Cost of AI

The cost of AI per conception of the T_0, T_1 and T_2 sows, the difference in the cost of AI per conception of each sow was PHP1,500.00 (single dose farm practice) and PHP1,418.74 (two doses halothane free gene semen).

Cost of Probiotic or In-Feed Antibiotics

The computed cost of feed and antibiotics or probiotics in the treatment groups in the three farms were the same amounting to PHP282,397.24.

CONCLUSIONS

In view of the results obtained in this study, the following conclusions are drawn:

1.The probiotic, both at 2g and 3g kg^{-1} feed is effective in improving both litter and sow performance.

2.Cost of using halothane free gene semen for AI is relatively cheaper than using the farm's boar. Likewise, the cost of using probiotic at the two levels as feed additive is relatively cheaper than using antibiotics based on the pre-weaning mortality, litter size and litter weight at weaning records.

3.The offsprings of halothane free gene semen are highly resistant to diarrhea and produce very fast-growing weanlings, hence, requiring less utilization of veterinary drugs or antibiotics.

RECOMMENDATIONS

Further research need to be conducted to provide us deeper information about the effect of halothane free gene semen and the use of probiotics. Some of these studies include:

1. Study to be conducted on Gene Transfer Corporation piglets in terms of disease resistance and productivity performance.

2. Study on GTC piglets is subjected to test for meat tenderness, back fat thickness and gene mapping.

3. Separate study of sow's gene frequency of the *n* allele of the *hal* gene should also be used as basis of the grouping to get more specific and reliable results.

4. Litter production performance test must extend until marketing to determine the effect of using halothane free gene semen or stress gene-positive boar on other parameters affected by stress tolerance of pigs. In addition, because the Philippines is a tropical country and most pig farms are predisposed to various diseases, future studies should include effect of using semen of halothane free gene boar on disease resistance of the litters of sows.

ETHICAL CONSIDERATION

All animals were treated humanely according to the principles of Animal Welfare Act. of the Philippines (RA 8485) and guidelines set by the Bureau of Animal Industry of the Philippines. This study was approved by the approving body the Department Research Committee of the Department of Animal Science, College of Agriculture, Visayas State University.

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