Green Farming Vol. 6 (6) : 1357-1360 ; November-December, 2015 **Research Paper**

Growth and reproductive performance of white mice (*Mus musculus* Linn.) as influenced by pigeonpea (*Cajanus cajan* (L.) Millsp.)

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Received : 04 March 2015 ; Revised accepted : 31 October 2015

ABSTRACT

The research was conducted to investigate the effect of pigeonpea *dal* on the growth and reproductive performance of white mice (*Mus Musculus Linn.)* at the Pampanga State Agricultural University (PSAU), Magalang, Pampanga, Philippines. A total 20 male mice of three weeks old was fed with different level ratio (25%, 35% and 45%) of pigeonpea *dal* to determine the growth performance while another 20 nulliparous female and 20 male of eight week old mice were fed with different levels containing 20%, 30% and 40% of pigeonpea *dal* to investigate the effect on its reproductive performance. The inclusion of 25%, 35%, or 45% pigeonpea *dal* rations on the growth performance on treated mice showed that the body weight; average daily gain (ADG); and feed intake and feed conversion ratio (FCR) efficiency were comparable from each other and the control group (commercial hamster feed). Whereas 100% whelping rate was observed without any recorded abortion to each treatment group of feeding rations containing 20%, 30% and 40% pigeonpea *dal* on the reproductive performance of mice. Litter size (11.6) was highest in mice fed with the control ration but was comparable to the treated groups. Mean birth weight of mice fed with 20% pigeonpea *dal* was significantly heavier as compared to the other treatments. However, after 21 days, the mean weaning weights of all pups was similar for all treatments. This suggests that different levels of pigeonpea *dal* do not affect whelping rate, birth rate and weaning weight. Therefore the inclusion of 20%, 30%, or 40% pigeonpea *dal* in the diet does not affect normal reproduction in mice.

Key words : *Alternative feeds, Growth and reproductive performance, Pigeonpea dal, White mice*

INTRODUCTION

The dramatic increases in feed prices during the past years are remarkable. A number of causes for this crisis have been cited but the massive diversion of feed ingredients to biofuels, reduced crop yields and an increased demand for animal protein in developing countries have all been contributing factors. However, what has occurred over the past several decades is that the abundance and generally favorable pricing of corn and soybean meal have led to a situation in which other ingredients, which may have been widely studied, have been largely overlooked.

Alternative feed ingredients offer the most possible option to combat the inevitable price increases of conventional feedstuffs, particularly protein sources. Protein is one of the most expensive nutrients to supplement in the diet (www.smallstock.info/info/feed/nutrition). Presently, the most preferred source of high quality plant protein belongs to the legume family, referring specifically to soybean. The exploitation of soybean is a classic example of successful development and use of legumes for animal feed and their production (www.fao.org, 2007).

Pigeonpea [*Cajanus cajan* (L.) Millspaugh] is a perennial member of family Leguminosae. This crop ranks only sixth in area and production worldwide but is used in more diverse ways than other legumes (Mula and Saxena, 2010). Pigeonpea forage is useful as a protein supplement when pasture quality is low (www.icrisat.org 1993). The crude protein of boiled pigeonpea seed meal (PSM) has been reported to be in the range of 23.2 to 25.3%, while that of boiled

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and dehulled PSM was 25.5% (Amaefule and Obioha 1998).

The worldwide utilization of legumes and vegetables has paid attention on investigating the possible effects in reproduction of phytoestrogens. Phytoestrogens are chemicals that may have weak estrogenic effects when they are ingested and metabolized (www.cdc.gov). Mice is a useful tool that allows researchers to study human and animal conditions (Alving 2007). Mice and rats exposed before or right after birth to several phytoestrogens, including coumestrol and genistein develop adverse reproductive function which will alter ovarian development, alter estrous cycles, problems with ovulation and subfertility (fewer pregnancies; fewer pups per litter) and infertility (Delcios *et al.,* 2001; Jefferson *et al.,* 2002b, 2005, 2006; Kouki *et al.,* 2003; Nagao *et al.,* 2001).

The positive interaction between nutrition and reproduction is already established in improving animal's genetic potentials. Proper nutrition can limit the chance of delay in puberty, reduce ovulation, lower conception rates and poor lactation (Smith and Somade, 1994).

The availability of pigeonpea in the locality and the presence of a small rodent laboratory compelled the researcher to test the suitability and the effect of incorporating the said ingredient in mice ration to know its effect on growth and reproduction. This study also aimed to produce a ration specifically formulated for gestating and lactating mice that will address the lack of a commercially available feeds in the country. Moreover, this study intends to promote alternative but beneficial crops as feed ingredients for animal feed.

MATERIALS AND METHODS

Twenty (20) male of three weeks old while 20 female and 20 male of eight weeks old white mice were fed with different levels of pigeonpea *dal* ratio to determine the growth performance and reproductive performance, respectively at the Pampanga State Agricultural University (PSAU), Magalang, Pampanga, Philippines in 2013.

Three (3) isocaloric and isonitrogenous rations were formulated to meet the requirement of the National Research Council for reproduction (gestation and lactation) of mice with protein ranges from 18-24% in the diet, fat 5-12%, fiber 2.5%, carbohydrates which account for about 45-60%, 1.23% calcium and 0.99% phosphorus (National Research Council, 1995). While commercially available rodent diet was fed in the experimental animals in the control group.

The percent inclusion of pigeonpea *dal* in rations is presented in **Table 1**. Two experimental set-ups were conducted: one for growth performance (**Table 2**) and the other for reproductive performance (**Table 3**). The experimental rations were pelletized manually using hard plastic straw as molder where well-mixed ingredients were loaded in and compacted then oven-dried for five hours at 40°C. The two formulated rations were fed *ad libitum* to experimental animals for 30 and 60 days for growth and reproduction (covering 2 parities only) performance, respectively. Mice were randomly distributed to four (4) treatments and each treatment was replicated five times following the Completely Randomized Design (CRD).

Table 1. Percent inclusion of pigeonpea dal rations.

Treatment	Growth performance for 3 weeks old mice (Pigeonpea % Inclusion Rate)	Reproductive performance for 8 weeks old mice (Pigeonpea % Inclusion Rate)
T_0	Commercial	Commercial
T_1	25	20
T_2	35	30
T_3	45	40

Table 2. Pigeonpea feed formulation composition for growth performance

Ingredients	Treatments		
	T_1	T_2	T_3
Pigeonpea	25.00	35.00	45.00
Corn yellow	28.50	26.00	20.00
Wheat flour, feed grade	10.00	10.00	10.00
Rice bran (D1)	6.25	2.00	1.25
Soybean meal	8.50	5.50	2.00
Fishmeal analog	8.50	8.25	8.00
Sugar, brown	1.50	1.50	1.50
Skim milk powder, dried	2.00	2.00	2.00
Salt	0.25	0.25	0.25
Dicalcium phosphate	2.00	2.00	2.00
Vitamin and mineral premix	1.00	1.00	1.00
Corn oil	6.25	7.00	7.00
Total	100.00	100.00	100.00
Calculated analysis ME, kcal/kg	3087.75	3091.65	3048.75
Crude protein (%)	18.11003	18.17458	18.12458
Calcium (%)	1.097175	1.12885	1.157875
Phosphorus (%)	0.744275	0.74415	0.748675

Data gathered for growth performance (body weight; average daily gain (ADG); feed conversion ratio (FCR); and acceptability and palatability) and reproductive performance (whelping rate (%); abortion rate (%); litter size; birth weight (g); weaning weight (g); and pre-weaning mortality) were recorded, tabulated and analyzed using the analysis of variance (ANOVA) using the Least Significant Difference at 1% and 5% level of significance.

RESULTS AND DISCUSSIONS

Growth performance

Effect on body weight and average daily gain (ADG) :

Table 3. Pigeonpea feed formulation composition for reproductive performance

Ingredients	Dietary Treatments		
	T_1	T_2	T_3
Pigeonpea	20.00	30.00	40.00
Corn yellow	18.00	15.00	11.72
Soy bean (Argentina)	11.00	9.00	5.00
Rice bran (D1)	10.00	5.00	4.00
Fish meal (analog)	12.50	11.60	11.70
Wheat flour	15.15	16.40	14.00
Sugar brown	2.00	2.00	2.00
Skim milk	2.00	1.50	2.00
Vitamin and Mineral Premix (Vionate®)	1.00	1.00	1.00
Dicalcium phosphate	1.85	2.00	1.58
Salt	0.50	0.50	0.50
Corn oil	6.00	6.00	6.50
Total	100.00	100.00	100.00
Nutritional Analysis			
ME kcal/kg	3020.00	3002.00	2999.00
Crude Protein %	22.23	20.79	20.72
Crude Fat %	19.86	19.88	9.94
Crude fiber %	2.99	2.48	2.53
Ash %	9.97	9.98	4.98
Moisture %	55.55	37.5	37.5
Calcium %	1.20	1.21	1.20
Phosphorus %	0.82	0.82	0.77

*Vitamins; 220,000 I.U. Vitamin A, 22,000 I.U. Vitamin D3, 39.6 mg. Vitamin B1 (Thiamine Mononitrate), 79.2 mg. Vitamin B2 (Riboflavin), 9.98 mg. Vitamin B6 (Pyridoxine Hydrochloride), 0.15 mg. Vitamin B12, 110 mg Calcium pantothenate., 275 mg. Niacin, 2.2.mg. Folic Acid, 5,720 mg Choline Chloride, 2,494.8 mg. Ascorbic Acid, 119.9 I.U. Vitamin E.

*Minerals;(max) 11.4% 113,762 mg. Calcium, 4.79% 47,828 mg. Phosphorous (P), (NaCl) (min) 0.5% 4,994 mg. (max) 1.5% 14,982 mg. per kg. Salt,) 0.0022% 22 mg. Iodine, 0.055% 550 mg. Iron (Fe), 0.00055% 5.5 mg. Cobalt, 0.0055% 55 mg. Copper (Cu), 0.0424% 423.06 mg. Magnesium (Mg), 0.0076% 75.68 mg Manganese (Mn).

*Other than ME and crude fat obtained by calculation, the remaining values were based on the actual results of analysis done by PAC Feed Testing Center.

Table 4 shows that body weights of mice from the pigeonpea treated groups were heavier as compared to the control group. This shows that the inclusion of 25%, 35% and 45% pigeonpea *dal* in the ration had comparable effects to the commercial rodent diet. The ADG of mice from the treated groups were comparable with those from the control group.

Effect on feed intake and feed conversion ratio (FCR) efficiency : Feed intake (g) and FCR from treated groups were comparable from each other and the control as shown in Table 4. On the average, it would take about 13.1 g of feed to produce 1 g of mouse meat. Water intake was not affected by the different levels of pigeonpea *dal* in the diet.

Table 4. Growth performance of mice

Treat-ment	Fed ratio	Body weight (g)	ADG (g)	Feed intake	FCR	Water intake (ml)
T_0	Commercial ration	14.0^{ns}	0.55^{ns}	3.9^{ns}	13.4^{ns}	7.2^{ns}
T_1	25% pigeonpea dal	16.1	0.57	4.4	17.7	8.5
T_2	35% pigeonpea dal	15.5	0.62	4.4	11.2	9.5
T_3	45% pigeonpea dal	16.0	0.67	4.3	10.0	11.1
	Total	15.4	0.60	4.3	13.1	9.1

Note : ns - not significant

Palatability and acceptance : Mice fed with different levels of pigeonpea *dal* consumed the same amount eaten by mice from the control group receiving the commercial ration. The presence of brown sugar and skim milk in the ration have contributed to the acceptability and palatability. Pigeonpea *dal* when included in the ration did not manifest off-taste and odor. The ADG of mice fed with pigeonpea *dal* were higher compared to the control group. This means that their body requirements for essential nutrients are met with said rations.

Reproductive performance

Whelping rate : All the experimental does assigned to the different treatments successfully completed two parities. A 100% whelping rate was observed for all the treatment groups.

Abortion rate : There was no incidence of abortion throughout the duration of the study. Dates of expected mating with the aid of a vaginal plug was monitored and used as a basis for the parturition dates of female mice.

Litter size : The average litter size of mice from treatment groups was 10.85 pups for the two consecutive whelpings. The inclusion of pigeonpea in the diet of mice did not affect litter size as revealed in **Table 5**.

Birth weight and weaning weight : There were no significant differences on the weaning weight and total weight gain of pups among the treatments but the result of the birth weight of pups from T_1 was significantly lower than T_2 as shown in Table 5.

Birth weight of T_2 (1.47 g) was significantly different from T_1 (1.20 g), which also had the least birth weight among treatments. Moreover, birth weight of T_2 was comparable to the birth weights of T_3 and T_4, which were all fed with diets containing pigeonpea *dal*. It is therefore concluded that mice fed with pigeon pea rations performed well on their birth weights as compared to those fed with commercial ration.

Mice from T_4 produced the highest weaning weight of 7.23 g followed by T_2 (6.71 g) and T_1 (6.13 g) while T_3 had the least weaning weight of 6.01 g. However, the total gain weight of mice is recorded higher in T_4 (5.87 g) with the least coming from T_1 (4.93 g).

Table 5. Reproductive performance of mice

Treat-ment	Fed ratio	Litter size	Birth weight (g)	Weaning weight (g)	Total weight gain(g)	Pre-weaning mortality
T_0	Commercial ration	11.6^{ns}	1.20^{a}	6.13	4.93	2.70
T_1	25% pigeonpea dal	10.50	1.47^{b}	6.71	5.24	2.10
T_2	35% pigeonpea dal	10.87	1.33^{a}	6.01	4.68	0.80
T_3	45% pigeonpea dal	10.30	1.37^{a}	7.23	5.87	0.90

Note : ns – not significant; Means with different superscripts are significantly different (P<0.05)

Pre-weaning mortalities. Table 5 showed that there were no significant differences among treatments. However, the pre-weaning mortalities of T_1 was the highest with 2.7 as compared to T_2, T_3 and T_4 with 2.1, 0.85 and 0.89, respectively. The common cause of death was unnecessary movement from the doe during lactation leading to suffocation and death of pups. Maternal behavior is a key-factor in offspring survival, especially in species with altricial young such as rodents that give birth to large litters of relatively undeveloped young totally dependent on their mother for nutrition and thermoregulation (Weber *et al.,* 2007).

CONCLUSIONS

From the study, the feeding of different levels (25%, 35%, 45%) of pigeonpea *dal* in growing mice had similar effect on body weight. However, the ADG of mice fed with different levels of pigeonpea *dal* was statistically higher than those from the control group. Moreover, the FCR was highest in mice treated with 25% pigeonpea *dal*.

In addition, the reproductive performance and some productive parameters of mice fed with different levels of pigeonpea *dal* were comparable to those of the control group. The birth weight of T_2 (1.47 g) was significantly different to that of T_1 (1.27 g) while birth weights of mice fed with pigeonpea rations weighed more than the mice fed with the commercial ration. Therefore, it is concluded that pigeonpea *dal* is a potential source of protein in the gestating and lactating rations of mice without any threat in the reproduction.

REFERENCES

Alving B. 2007. (in) *Increased Availability of Genetically Altered Mice Will Aid Studies of Human Diseases:* National Institutes of Health (NIH) News, US Deptt. of Health & Human Services.

Amaefule K U and Obioha F C. 1998. The substitution of pigeonpea (*Cajanus cajan*) seed for groundnut cake and maize in broiler finisher ration. *Nig. J. Anim. Prod.* **25 :** 9-12.

Delclos K B, Bucci T J, Lomax L G, Latendresse J R, Warbritton A, Weis C C and Newbold RR. 2001. Effects of dietary genistein exposure during development on male and female CD (Sprague-Dawley) rats. *Reproductive Toxicology* **15** (6) **:** 647-63.

Jefferson W N, Couse J F, Padilla-Banks E, Korach K S and Newbold R R. 2002b. Neonatal exposure to genistein induces estrogen receptor (ER) alpha expression and multioocyte follicles in the maturing mouse ovary: Evidence for ERbeta-mediated and nonestrogenic actions. *Biology of Reproduction.* **67** (4) **:** 1285-1296.

Jefferson W N, Padilla-Banks E and Newbold R. 2005. Adverse effects on female development and reproduction in CD-1 mice following neonatal exposure to the phytoestrogen genistein at environmentally relevant doses. *Biology of Reproduction.* **73** (4) **:** 798-806.

Jefferson W N, Newbold R, Padilla-Banks E and Pepling M. 2006. Neonatal genistein treatment alters ovarian differentiation in the mouse: Inhibition of oocyte nest breakdown and increased oocyte survival. *Biology of Reproduction.* **74** (1) **:** 161-168.

Kouki T, Kishitake M, Okamoto M, Oosuka I, Takebe M, Yamanouchi K. 2003. Effects of neonatal treatment with phytoestrogens, genistein and daidzein, on sex difference in female rat brain function : Estrous cycle and lordosis. *Hormones and Behavior.* **44** (2) **:** 140-145.

Mula M G and Saxena K B. 2010. *Lifting the Level of Awareness on Pigeonpea* – A Global Perspective. Patancheru 502 324, Andhra Pradesh, India. International Crops Research Institute for the Semi-Arid Tropics. pp. 540.

Nagao T, Yoshimura S, Saito Y, Nakagomi M, Usumi K, Ono H. 2001. Reproductive effects in male and female rats of neonatal exposure to genistein. *Reproductive Toxicology.* **15** (4) **:** 399-411.

Nan Wu, Kuang Fu, Yu-Jie Fu, Yuan-Gang Zu, Fang-Rong Chang, Yung-Husan Chen, Xiao-Let Liu, Yu Kong, Wei Liu and Cheng-Bo Gu. 2009. Antioxidant activities of extracts and main components of pigeonpea [*Cajanus cajan* (L.) Millsp.] leaves. *Molecules.* **14 :** 1032-1043.

National Research Council. 1995. *Nutrient Requirements of Laboratory Animals.* Committee on Animal Nutrition, Board on Agriculture. National Academy Press., Washington, D.C. Fourth Revised Edition.

Smith O S and Somade B. 1994. Interaction between nutrition and reproduction in farm animals. International Foundation for Science (IFS) *Proceedings Of A Regional Seminar On Animals' Reproduction.* Jan 17-21, Niamey, Nigeria.

Weber E M, Olsson A S and Alger B. 2007. High mortality rates among newborn laboratory mice – is it natural and which are the causes. *Acta Veterinaria Scandinavica.* **40** (1) **:** S8

www.icrisat.org 1993. Nutritive value and uses of pigeonpea and groundnut.

www.cdc.gov/.../Phytoestrogens_BiomonitoringSummary.html

www.fao.org, 2007. Protein sources for the animal feed industry.

www.smallstock.info/info/feed/nutrition