Effect of variously-processed rubber seed meals on reproductive performance: The use of Sprague-Dawley laboratory rats as model for pigs

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ABSTRACT: The study aimed to determine the potential adverse effects of variously-processed rubber seed meals on the reproductive performance, postnatal growth and offspring survival rate using the Sprague-Dawley laboratory rat as model for pigs. Eighteen Sprague-Dawley female rats with an average initial body weight of 154.0 g were randomly allotted into 6 groups designated as T1 control (no rubber seed meal), T2 raw rubber seed meal, T3 soaked rubber seed meal, T4 sundried rubber seed meal, T5 boiled rubber seed meal and T6 roasted rubber seed meal. The diets were prepared with various types of rubber seed meals incorporated at the level of 0 g kg⁻¹ (control diet, T1) and 100 g kg⁻¹ of each of the five rubber seed meal-containing diets. The study was conducted for 70 days and parameters studied included: feed intake, number of fertile females, pregnancy index, gestation length (length of pregnancy), number of pups born, number of pups born alive, live birth index and number of still births. Upon parturition (day 1), pups were individually counted, weighed and examined for external malformations. Other parameters evaluated were number of pups weaned and mortality of pups. The inclusion of various types of rubber seed meals in the diets did not alter the general health of all the experimental rats. With the exception of rats on the T6 diet, all other rats recorded successful mating and pregnancies. There were no significant (p>0.05) differences in the gestation length (22 to 23 days) and all the pregnant rats delivered normally. No external malformations were observed in any of the pups delivered. No significant (p>0.05) differences in the maternal body weight (199.8 g), litter size (4.0 to 10), pup birth weight (4.88 to 7.07 g) and number of pups weaned (3.33 to 9.67) were observed. However, dietary treatments significantly (p<0.05) influenced weaning weight (29.60 to 48.25 g) and postnatal mortality of pups, with those on the T1 (control) and the T4 diets having the highest mortalities of 2.67 and 2.33, respectively. Based on the findings of the present study, up to 100 g of the raw and the variously-processed rubber seed meals could be included in 1 kg diets of laboratory rats without compromising on the health and reproductive performance in terms of reproductive toxicity or complications in pregnancy, delivery and other reproductive indices studied.

Keywords: Sprague-Dawley rat, pig, reproductive performance, rubber seed meal.

INTRODUCTION

The role and effects of nutrition on reproductive events in farm animals is well documented. Kamalzadeh et al. (2009) reported that the nutrition characteristic and toxicity index of a diet alters the time course of development, reproductive ability and survival of organisms. Hajalizadeh et al. (2019) reported that the addition of fennel (Foeniculum vulgare) seed powder (FSP) at a level of 1.5% in the diet of fattening lambs increased blood testosterone hormone concentration, along with improvement in feed conversion. However, Myreseyed et
al. (2008) reported that using 280 mg kg\(^{-1}\) of FSP ethanol extract in male rats decreased blood testosterone concentration in male reproductive activity. This difference was attributed to the type of animal and dosage used. Reproductive performance of domestic animals, in general, is determined by four factors, namely: genetic merit, physical environment, nutrition and management. Khodabakhshzadeh et al. (2016) observed that reproductive activity is a multi-functional process and numerous genes, proteins, growth factors and hormones are involved in this activity. Smith and Akinbamijo (2000) reported that nutritional factors are perhaps the most crucial, in terms of their direct effects on the reproductive phenomenon, and the potential to moderate the effects of other factors. Thus, adequate nutrition could influence low quality biological types to reach their genetic potential, alleviate the negative effects of adverse physical environment, and minimise the effects of poor management practices. Poor nutrition on the other hand, will not only reduce performance below genetic potential, but also worsen the adverse environmental effects. Moreover, nutritional factors, in comparison with the others, are readily manipulated to achieve positive results. Hence, further studies need to be conducted with respect to interactions between nutrition and reproduction particularly in most developing countries, where, for various reasons, nutritional inadequacies in terms of quantitative feed intake and qualitative nutrient imbalances prevail. Several reports (Smith and Somade, 1994; Robinson et al., 2006; Roche et al., 2011; Bindari et al., 2013; Goodband et al., 2013) have examined the effects of quantitative feed and energy, as well as qualitative protein and macronutrients intake on animal reproductive performance. In general, the results of such studies suggest that poor nutrition caused by inadequate, excess or imbalanced nutrient intake may adversely affect the various stages of the reproductive process, starting from delayed puberty, reduced ovulation and lower conception rates, through high embryonic and foetal losses to excessively long post-partum anoestrous, poor lactation, high peri-natal mortality and poor neonatal performance. According to their chemical composition and nutritive value, rubber seeds can be considered as a very good feedstuff for animals. However, they contain a toxic factor, cyanogenic glycoside and other bioactive chemical compounds, such as phytates, oxalates, tannins and trypsin inhibitors (Eka et al., 2010; Udo et al., 2016; Aguihe et al., 2017; Farr et al., 2019) that pose a problem in their use as animal feed. The cyanogen compound is more prominent in rubber seeds. Udo et al. (2016) and Aguihe et al. (2017) reported cyanogen contents of 249 mg kg\(^{-1}\) and 315 mg kg\(^{-1}\), respectively for raw rubber seed meals, which are lower than the values of 391.6 mg kg\(^{-1}\), 410 mg kg\(^{-1}\) and 609.5 mg kg\(^{-1}\) reported by Okafor et al. (2006), Sharma et al. (2014) and Farr et al. (2019), respectively. Narahari and Kothandaraman (1983) found that the kernels of rubber seeds contained 749 mg HCN kg\(^{-1}\). The toxic factor of rubber seeds is a cyanogenic glycoside that decomposes either as a result of enzyme action or from being in a very slightly acidic medium (Stosic and Kaykay, 1981). Cyanogenic glycosides on hydrolysis yield toxic hydrocyanic acid (HCN). The presence of the cyanogen compound becomes harmful if its content is above the safe margin for consumption. The cyanide ions inhibit several enzyme systems, depress growth through interference with certain essential amino acids and utilization of associated nutrients (Uluodo et al., 2018). They also cause acute toxicity, neuropathy and death (Fernando, 1987). There are a wide variety of different methods of processing the rubber seeds to reduce their content of cyanogenic glycosides and the other biochemical active compounds and hence their toxicity (Udo et al., 2016; Farr et al., 2019).

The study therefore aimed to determine the effects of unprocessed rubber seed meal (RRSM) and variously processed rubber seed meals (soaked, SRSM; sundried, SDRSM; boiled, BRSM; and roasted, RoRSM) supplementation on reproductive performance using the Sprague-Dawley rat as an experimental model for the pig. The laboratory rat has proved a valuable experimental model for investigations into basic processes of reproduction (Dey et al., 2004; Lee and DeMay, 2004; Iannaconne and Jacob, 2007; Hamid and Zuki, 2013; Auger et al., 2014; Schumacher et al., 2015), and tests with laboratory rats have been used in the development of methods designed to replace the use of other live animals, such as the growing pig.

MATERIALS AND METHODS

Source of rubber seeds, processing methods and dietary treatments

The rubber seeds used in the study were obtained from Rubber Plantations Section of the Department of Crop and Soil Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. The seeds were de-hulled, partially sun-dried for 24 hours at an ambient temperature of about 30°C, divided into five lots, with each lot receiving one of five processing methods:

Raw rubber seed meal: this lot was not subjected to any processing method but ground in a hammer mill and stored in polythene sacs.

Soaking in water: the partially dried, de-hulled rubber seeds were soaked in water for 3 days with a rubber seed to water ratio of 1:3. The water was replaced daily. After 3 days, the water was decanted and seeds sun-dried for 3 days at ambient temperatures ranging from 30 to 35°C.

Sun-drying: this treatment consisted of spreading out a portion of the partially dried raw rubber seeds in the open and sun-drying for 3 days at ambient temperatures ranging from 30 to 35°C.
Boiling in water: the partially dried, de-hulled rubber seeds were placed in a 20 litres aluminum bowl containing water and subjected to heating at a temperature of about 100°C for 30 minutes with a rubber seed to water ratio of 1:3. After heating for about 30 minutes, the water was decanted and seeds sun-dried for 3 days at ambient temperatures ranging from 30 to 35°C.

Roasting: the fifth lot was subjected to dry heat for 30 minutes in an open pan at a temperature of about 80°C. The seeds were stirred constantly to avoid burning.

Each processed lot was ground using a hammer mill and stored in polythene sacs.

A control diet and five other treatment diets (produced by adding 100 g of one of the five rubber seed meals, i.e. the raw and 4 processed RSMs to the control to replace portions of maize and soyabean meal) were formulated (Table 1). The diets were designated as T₁ (control with no rubber seed meal), T₂ raw rubber seed meal, T₃ soaked rubber seed meal, T₄ sun-dried rubber seed meal, T₅ boiled rubber seed meal and T₆ roasted rubber seed meal. The experimental diets were formulated to be iso-nitrogenous and iso-caloric.

Table 1. Ingredient composition of the experimental diets fed to rats.

<table>
<thead>
<tr>
<th>Ingredients (g kg⁻¹)</th>
<th>Control</th>
<th>RRSM</th>
<th>SRSM</th>
<th>SDRSM</th>
<th>BRSM</th>
<th>RoRSM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>550</td>
<td>400</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>Fishmeal</td>
<td>120</td>
<td>120</td>
<td>120</td>
<td>120</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>Soya bean meal</td>
<td>160</td>
<td>110</td>
<td>110</td>
<td>110</td>
<td>110</td>
<td>110</td>
</tr>
<tr>
<td>RSM</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>Vitamin/mineral premix</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Salt (NaCl)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Oyster shell</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

Chemical analysis (g kg⁻1 DM)

- Crude protein: 216.8, 206.9, 208.8, 208.9, 209.2, 209.7
- Crude fibre: 37.83, 47.21, 42.12, 46.26, 42.43, 43.63
- Ether extract: 39.35, 53.15, 56.65, 50.15, 54.15, 53.65
- Calcium: 8.75, 8.82, 8.77, 8.81, 8.80, 8.76
- Phosphorus: 8.34, 8.13, 8.16, 8.30, 8.30, 8.27
- ME (MJ kg⁻¹): 12.78, 12.58, 12.70, 12.58, 12.66, 12.69

*Vitamin/mineral premix specified to provide the following kg⁻¹ diet: vitamin A 10,000 IU; D 2000 IU; K 3 mg; riboflavin 2.5 mg; niacin 12.5 mg; cobalamin 0.05 mg; pantothenic acid 5 mg; choline 175 mg; folic acid 0.5 mg; zinc 25 mg; iron 0.5 mg; copper 50 mg; cobalt 625 mg; iodine 0.5 mg; selenium 0.3 mg; chlorine 1.3 g; sodium 1.3 g; sulphur 0.4 g; potassium 3.0 g.

*Calculated from data of NRC (1998) and the estimated metabolizable energy values of the various types of rubber seed meals.

Experimental animals and management

Eighteen Sprague-Dawley growing female rats (154 g body weight) were kept individually in raised stainless steel cages with intact floors, in a room with a 12 hours light/dark cycle. The rats were randomly allocated to the six experimental diets such there were 3 female rats per treatment. The rats were dewormed using Ivermectin before the start of the trial. Each rat had access to its respective diet for a 28-day adaptive period. Water was available ad libitum.

Parameters measured

Rat growth performance was assessed weekly by measuring feed intake and body weight. Female rats for the reproductive trial were weighed prior to mating. The mating ratio was three females to one male. For each treatment, the male rat was placed with the females for 3 weeks and consumed the same diets as the females, after which the females were individually housed. Starting the day before expected parturition, the presence of pups in each cage was checked twice daily. Upon parturition, the litters were individually counted, weighed and examined for external malformations and again weighed at weaning at 4 weeks of age. Other parameters studied included: number of fertile females, pregnancy index (no. of females delivering young offspring/no. of females examined X 100), gestation length (length of pregnancy), number of pups born, number of pups born alive, number of still births and live birth index (no. of live offspring/no. of offspring delivered X 100).
significant difference procedure.

All the number of fertile females, gestation that the inclusion of the various types of rubber rubber seed meals in diets did not alter the general health of animals. The results indicate that rats will consume diets containing both the raw and processed rubber seed meals. A major factor affecting feed consumption in animals is the dietary energy content because animals eat to satisfy their inner metabolic need for energy. In this study, the experimental diets were formulated to be isocaloric. The results indicated that the inclusion of the various types of rubber seed meals in diets did not alter the general health of the experimental rats. Also, there were no changes in the maternal body weights of rats, which averaged 199.8 g

Conception occurred in all the females fed the various dietary treatments, except those on the roasted rubber seed meal (T₆ RoRSM)-containing diet. In spite of their ability to consume the diet which contained 100 g RoRSM kg⁻¹, no conception occurred in two of the three female rats fed this diet. In this study the males were placed with the females for natural mating to occur. The reasons for this observation are not clear, considering the fact that in a previous study, Farr et al. (2019) observed that the imposition of the various processing methods significantly reduced the toxic factor, hydrogen cyanide, and the other anti-nutritional factor contents in the resultant meals in comparison with the raw seeds and also non-significant effects of dietary treatments on feed intake of rats was observed in this study.

The dietary treatments did not have any significant (p>0.05) influence on the gestation lengths or length of pregnancy of the animals, the average observed in this study being 22.45 days. This observation is in agreement with reports that gestation in rats takes 21 to 23 days from copulation to parturition (Hamid and Zuki, 2013). All pregnant rats delivered normally with no evidence of prematurity or abortion suggesting that the various rubber seed meals were not abortifacient when consumed. No external malformations were observed in any of the pups delivered.

The average litter size and the number of pups born alive both varied from 4.01 for those on RoRSM-containing diet (T₀) to 10.0 for the soaked rubber seed meal (T₅), whiles the number of still births varied from 0 for animals on the control (T₁), the SRSM (T₃) and SDRSM (T₄)-containing diets to 0.33 for the RRSM (T₆) and BRSM (T₇) diets but the values obtained were not statistically different (p>0.05). It has been observed that birth usually occurs at night with 10 to 12 pups being born (The Animal Care and Use Committee, 2018)

Birth weights of the pups were not affected by the

### Statistical analysis

Data collected were subjected to Analysis of Variance (ANOVA) using the GenStat Statistical Software (2007) to identify significance of main effects. Where significant differences were found among treatments, specific effects were tested by the least significant difference procedure. All tests for significance were based on the 5% probability level.

### RESULTS AND DISCUSSION

Feed intake, the number of fertile females, gestation length, number of pubs born, number of pups born alive, still births, weight at birth, number weaned and weaning weight of pups as determined in this study are presented in Table 2.

Feed intake by rats was not significantly (p>0.05) affected by the various dietary treatments. The daily feed intake varied from 10.9 g (T₀ RoRSM diet) to 12.83 g (T₁ control and T₅ BRSM diets). The non-significant effect of the various rubber seed meals inclusion in diets on feed intake suggests that rats will consume diets containing both the raw and processed rubber seed meals. A major factor affecting feed consumption in animals is the dietary energy content because animals eat to satisfy their inner metabolic need for energy. In this study, the experimental diets were formulated to be isocaloric.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>RRSM</th>
<th>SRSM</th>
<th>SDRSM</th>
<th>BRSM</th>
<th>R₆RSM</th>
<th>P-value and level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats examined</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Daily feed intake, g</td>
<td>12.83</td>
<td>12.14</td>
<td>12.63</td>
<td>11.87</td>
<td>12.84</td>
<td>10.90</td>
<td>0.178NS</td>
</tr>
<tr>
<td>No. of fertile females</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>0.05**</td>
</tr>
<tr>
<td>Pregnancy index, %</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>33.3b</td>
<td>0.05†</td>
</tr>
<tr>
<td>Gestation length, days</td>
<td>22.67</td>
<td>22.67</td>
<td>23.0a</td>
<td>22.33a</td>
<td>22.0a</td>
<td>22.0a</td>
<td>0.108NS</td>
</tr>
<tr>
<td>No. of pups born</td>
<td>8.67</td>
<td>7.33</td>
<td>10.0a</td>
<td>5.67a</td>
<td>9.67</td>
<td>4.01a</td>
<td>0.036NS</td>
</tr>
<tr>
<td>No. of pups born alive</td>
<td>8.67</td>
<td>7.0a</td>
<td>10.0a</td>
<td>5.67a</td>
<td>9.33a</td>
<td>4.01a</td>
<td>0.061NS</td>
</tr>
<tr>
<td>Live birth index, %</td>
<td>100</td>
<td>95.5</td>
<td>100</td>
<td>100</td>
<td>96.5</td>
<td>100</td>
<td>0.68NS</td>
</tr>
<tr>
<td>No. of still birth</td>
<td>0</td>
<td>0.333</td>
<td>0</td>
<td>0</td>
<td>0.333</td>
<td>0</td>
<td>0.658NS</td>
</tr>
<tr>
<td>Weight of pups at birth, g</td>
<td>4.88</td>
<td>6.00</td>
<td>5.59</td>
<td>7.07a</td>
<td>5.68</td>
<td>6.25a</td>
<td>0.062NS</td>
</tr>
<tr>
<td>Weaning weight, g</td>
<td>34.06</td>
<td>33.73</td>
<td>34.68</td>
<td>29.60c</td>
<td>32.33</td>
<td>48.25a</td>
<td>0.001***</td>
</tr>
<tr>
<td>No. of pups weaned</td>
<td>6.0</td>
<td>7.0</td>
<td>9.67</td>
<td>3.33a</td>
<td>8.67</td>
<td>4.01</td>
<td>0.015NS</td>
</tr>
<tr>
<td>Mortality of pups</td>
<td>2.67</td>
<td>0.00</td>
<td>0.33</td>
<td>2.33a</td>
<td>0.67</td>
<td>0.00b</td>
<td>0.001***</td>
</tr>
<tr>
<td>Malformations</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
<td>-</td>
</tr>
</tbody>
</table>

*abc* Means within a row with different superscripts are significantly different. NS, non-significant (p≥0.05); *p≤0.05; **p≤0.01; ***p≤0.001.
various dietary treatments. Weight of pups at birth were 4.88, 6.00, 5.59, 7.07, 5.68 and 6.25 g for the control (T₁), RRSM (T₂), SRSM (T₃), SDRSM (T₄), BRSM (T₅) and R₂RSM (T₆) diets, respectively. The weaning weights of pups were significantly (p<0.05) influenced by the dietary treatments and the values varied from 29.6 g for SDRSM (T₄) diet to 48.25 g for RoRSM (T₆) diet. However, the results obtained in this study is in agreement with observation of Yang and Mickelsen (1974) who reported that the rat, at birth, weighs about 6 g and weighs about 7 times its birth weight when weaned at 3 weeks.

Post-natal mortalities were significantly (p<0.05) affected by the dietary treatments. The values recorded varied from 0 for the RRSM (T₂) and RoRSM (T₆) diets to 2.67 for those on the control (T₁) devoid of any of the rubber seed meals.

Svinen et al. (2018) reported that fetal programming, also known as prenatal programming, is a theory which suggests that the environment surrounding the fetus during its developmental phase, plays a seminal role in determining its health risk during the later stage. It is a tightly, highly complex process that ensures the development of a viable offspring. It largely depends on in-nate programme inherited from parents at conception, but is also susceptible to influences from the external environment. Apart from essential nutrients and oxygen provided by the pregnant female, certain chemical compounds, such as the anti-nutritional factors reported to be contained in rubber seeds, can find their way to the fetus and interfere with molecular and cellular signalling events and thereby disturbing the fetal programming and causing permanent alterations with long lasting consequences. The results of the present might, however, suggest that the presence of the anti-nutritional factors in the various rubber seed meals might not have been at levels or concentrations capable of negatively affecting the reproductive performance of the rats, with the exception of rats on the T₆ (RoRSM) diet where only one conception out of the three occurred. Studies conducted by Tewe and Maner (1981) indicated that reproductive performance of rats fed diets containing 500 mg CN/kg diet throughout gestation and lactation was unaffected. Furthermore, litter size, weight of the pups at birth and feed consumption and growth rate of pups after birth were not significantly different from the control.

**Conclusion**

Animal nutritionists, worldwide, have intensified the search for non-conventional feedstuffs as a result of the competition between humans and animals for the same conventional feedstuffs. From the perspective of the nutrient compositions of rubber seed meals reported by several researchers, it could be an optional for conventional feedstuff in animal feeding systems. Rubber seed meal reportedly contains 20 to 30% crude protein and adequate amino acids that could support growth, development and reproduction in animals. Rubber seed meal contains some anti-nutritional factors, such as cyanogens, that inhibit the availability of nutrients for domestic animal utilization, which could be addressed by the application of appropriate processing methods. The present findings indicated that the addition of 100 g of the variously-processed rubber seed meals to 1 kg diet, with the exception of the roasted rubber seed meal diets, do not pose any significant health hazard, reproductive toxicity or complications in pregnancy, delivery and early pup growth in rats.

**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest

**REFERENCES**


