Effect of processing techniques on chemical composition and hydrogen cyanide content in rubber seed (Hevea brasiliensis) meal

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Abstract
An experiment was carried out to find out the suitable physical processing method to reduce HCN level of rubber seed meal. Rubber seed was processed and analysed for proximate composition. Chemical composition (g/kg) of raw rubber seed was found to have 11.64% crude protein, 21.58% crude fat and 23.70% crude fibre. The seeds were processed by three physical methods to check the level of principal anti nutritional factor i.e. Hydro cyanic acid (HCN). These physical processing includes overnight soaking, boiling for one hour and oven drying at 100 ± 2°C and stored for 21 days. It was observed that physical processing of rubber seed meal did not have significant effect on chemical composition. HCN level was estimated at different days of storage period i.e. 0, 7th, 14th and 21st day after the treatment. HCN content of the raw rubber seed was found to be 387 mg/kg. After different physical processing methods, the level of HCN was reduced and ranged between 129.6 to 259 mg/kg. Overnight soaking was found to be suitable processing method to reduce the HCN level of Rubber seed meal.

Keywords: Rubber seed meal, HCN, processing method, soaking, boiling, oven-drying

Introduction
It is not possible to overemphasize the value of expanded livestock production in developing countries. In order to ensure that affordable energy and protein is provided for the ever-increasing population of developing countries, livestock breeders, animal nutritionists and poultry farmers have roles to play (Aguije et al., 2017) [1]. However, as a result of high prices and competition between humans and animals, the growing production costs involved have not made this feasible (Taiwo et al., 2005 and Annongue et al., 2006) [2, 3]. Increasing livestock feed costs and the lack of traditional proteins and energy concentrates for feed formulation have forced the animal nutritionists to look for desirable, cheaper and easily accessible protein and energy sources in developing countries.

Rubber seed is an unconventional concentrate source containing substantial amount of proximate components. Rubber seed is the sub-product of natural rubber cultivation (Hevea brasiliensis) which is widely available in certain states of the country (Deng et al., 2015) [4]. While rubber seed provides a significant amount of protein and other nutrients, farmers do not use it in livestock diets due to the lack of scientific information on the use of rubber seed in livestock feed and the existence of an anti-nutritional element, i.e. HCN. According to Aguije et al. (2017) [1] the main constraint in the use of rubber seed is the presence of this hydrocyanic acid. Narahari and Kothandaraman (1983) [5] observed that the rubber seeds kernel contained 749 mg hydrocyanic acid per kg of seed. This hydrocyanic acid can be detoxified by different physical, chemical and biological methods and the processed rubber seed meal can be used in the diet of livestock. Kinh et al. (2006) [6] processed rubber seed into a useful pig feed and observed that the optimal processing method was to de-hull, de-fat, mixing with water (1:5), which eliminated almost the 98% of HCN in rubber seed meal then sundry or dry in drier. Ly et al. (2001) [7] evaluated nutrients of rubber seed meal in Mong Cai pigs and recorded that approximately by 45 days of storage the cyanide content of the rubber seeds decreased from 82.5 to 29.3 mg/kg DM. The findings of the different workers regarding the HCN content of processed rubber seed are inconsistent. Therefore, the present study was conducted to find out the suitable and practically feasible physical processing method to reduce the HCN level in order to utilize it as pig feed.
Materials and Methods
Procurement and preparation of rubber seed meal
The fruit of the rubber tree has a 3-lobed, 3-seeded ellipsoid capsule and 1 seed for each carpel. The seeds are ellipsoidal, colour is mottled brown, 2.5-3 cm long, variable in size, lustrous and weighing 2-4 g each. For our experiment rubber seed was procured from the farmers of the state Tripura. All the procured rubber seeds were hammer-milled prior to experimental processing to produce the respective meals as soaked RSM (SRSM), boiled RSM (BRSM) and oven dried RSM (ORSM).

Chemical analysis
Rubber seed meal were analyzed (AOAC, 2000) [8] for dry matter (DM; method 934.01), CP (method 968.06; Kelplus, Pelican Equipments, Chennai, India), crude fiber (CF; Fibre plus, Pelican Equipments, Chennai, India) and ether extract (EE; method 920.39; Socplus, Pelican Equipments, Chennai, India). All the samples for the analysis were weighed using an electronic balance (Model No.ABS 220-4, Kern & Sohn GmbH).

Processing of RSM
Soaking: The collected rubber seed was subjected to grinding followed by overnight (18-20 hrs) water soaking, sun drying and stored for 21 days. While in storage the dried rubber seed meal was checked for the HCN level at 0 day, 7th day, 14th day and 21st day.

Boiling: Rubber seed was boiled for 1 hr after grinding followed by sun drying and stored for 3 weeks. Level of HCN was estimated by following standard procedure (AOAC, 1995) [9] at 0 day, 7th day, 14th day and 21st day while in storage.

Heat treatment: In this method, rubber seed was subjected to grinding followed by dry heating at 100±2°C for 60 minutes and stored up to 21 days. Level of HCN was checked at 0 day, 7th day, 14th day and 21st day while in storage.

Results and Discussions
Proximate Composition of Fresh/raw and Processed Rubber Seed Meal
The rubber seed used in the present study was analysed for proximate principles before and after different physical processing method in the laboratory and are presented in Table No.1.

The crude protein value of fresh/raw rubber seed meal in the present study were similar to the observation made by Narahari and Kothandaraman (1984) [10], Ly et al. (2001) [7], Thanet et al. (2002) [11] and Oyewusi et al. (2007) [12]. They found the crude protein content as 11.50, 14.94, 12.19 and 10.30% respectively. However, the crude protein value in the present study contradicts with Sharma et al. (2014) [13], Suprayudi et al. (2014) [14], Hossain et al. (2015) [15], Khatan et al. (2015) [16], Pha-obnga et al. (2016) [17], Aguihe et al. (2017) [17], Deng et al. (2017) [18], Ahaotu (2018) [19], Udo et al. (2018) [20], Kouakou et al. (2018) [21], Oludo et al. (2018) [22], Farr et al. (2019) [23], who reported higher values in their experiments.

The ether extract of RSM was similar to the observation made by Thanet et al. (2002) [11], Samkol et al. (2002) [24], Aguihe et al. (2017) [1], Oludo et al. (2018) [22] and Kouakou et al. (2018) [21] but lesser than the values observed by Chanjula et al. (2011) [25], Sharma et al. (2014) [13], Suprayudi et al. (2014) [14], Pha-obnga et al. (2016) [17] and Udo et al. (2018) [20], and higher than the values observed by Hossain et al. (2015) [13], Khatan et al. (2015) [16], Khatan and Khan (2015) [19], and Deng et al. (2017) [18], Ahaotu (2017) [10], Udo et al. (2018) [20], Ahaotu (2018) [19] and Farr et al. (2019) [23] and lower than the values reported by Hossain et al. (2015) [13]. In the present study, the total ash in RSM was found to be similar to the findings of Sharma et al. (2014) [13], Suprayudi et al. (2014) [14], Hossain et al. (2015) [15], Khatan et al. (2015) [16], Khatan and Khan (2015) [19], Pha-obnga et al. (2016) [17] and Farr et al. (2019) [23] but lesser than the observation made by Chanjula et al. (2011) [25], Deng et al. (2017) [18], Oludo et al. (2018) [22], Udo et al. (2018) [20], Ahaotu et al. (2017) [1] and Ahaotu (2018) [19]. The NFE value was similar to the values reported by Udo et al. (2018) [20] and Deng et al. (2017) [18] and contradicts with Suprayudi et al. (2014) [14], Hossain et al. (2015) [15], Khatan and Khan (2015) [19] and Aguihe et al. (2017) [1].

After processing the seeds, there was no such change in the crude protein content of soaked, boiled and oven-dried rubber seed meal. Ether extract and crude fibre values have been lower in processed rubber seed meals than in fresh/raw seed meal, suggesting that during the processing of rubber seeds, some of these nutrients may have been lost. However, nitrogen free extract was higher in all the processed RSM than the fresh/raw RSM. The total ash amount was higher in ORSM and BRSM than the values obtained in fresh/raw rubber seed meal and SRSM.

Table 1: Proximate constituents (g/kg DM) of RSM after physical treatment

| Attributes | Fresh/raw RSM | SRSM | BRSM | ORSM |
|------------|---------------|------|------|------|
| Moisture   | 63.88±0.45    | 88.66±0.38 | 95.20±0.36 | 95.31±0.26 |
| DM         | 936.12±0.45   | 911.34±0.38 | 904.80±0.36 | 904.69±0.26 |
| Crude protein | 114.60±2.55 | 13.80±2.58 | 117.16±0.66 |
| CP         | 976.09±0.48   | 982.00±0.19 | 971.70±0.63 | 971.27±0.43 |
| Ether extract | 215.80±1.35  | 187.60±1.34 | 59.80±1.64 | 200.60±2.49 |
| Crude fibre | 237.06±2.09   | 175.60±1.34 | 59.80±1.26 | 229.00±1.21 |
| Total ash  | 23.91±0.48    | 18.00±0.19  | 28.23±0.63 | 28.73±0.43 |
| Nitrogen free extract | 406.84±1.67 | 504.20±4.5 | 338.57±3.76 | 424.51±2.63 |

HCN content of the fresh/raw RSM and processed RSM
The HCN levels of RSM were determined after different processing method and are presented in Table No.2. The HCN level was checked after soaking, boiling and dry heating at different day of storage period after treatment i.e. 7th, 14th and 21st day and was found to be in the range of 129.60±1.72 to 187.00±2.97 for soaked, 144.00±4.40 to 198.00±3.27 for boiled and 201.60±1.78 to 259.00±3.36 for oven dried. However, the storage of fresh / raw rubber seeds can also reduce the hydrogen cyanide content. In the present study, the hydrogen cyanide content of the fresh/raw RSM was 387 mg/kg, which was similar with the observation of Sharma et al. (2014) [13] and contradict the value reported by Thuy and Ly (2002) [22], Tean et al. (2002) [11], Suprayudi et al. (2014) [14], Eka et al. (2010) [28], Kouakou et al. (2018) [21], Pha-obnga et al. (2016) [17], Ly et al. (2001) [7], Farr et al. (2019) [23] and Aguihe et al. (2017) [1].
Because of genetic and environmental factor, season, location and soil conditions, cyanogenic glycosides can vary widely (Ernanzs et al., 1980) [20]. Moreover, reductions in hydrogen cyanide (HCN) levels were effective with the implemented treatment approaches and this revealed that on 0 day cyanide level in the fresh/raw seed reduced by 51.68%, 48.84% and 33.07% for soaking, boiling and oven-drying respectively. The higher concentration of hydrogen cyanide reduced in the SRSM and BRSM were in agreement with the reports from previous researchers (Udo et al., 2016, Farr et al., 2019 and Nambliso. 1994) [20, 23, 30] that soaking and boiling tends to reduce the RSM HCN concentration.

| Period  | Fresh/raw RSM | SRSM | BRSM | ORSM |
|---------|----------------|------|------|------|
| Day-0   | 387.00±2.92    | 187.00±2.97 | 198.00±3.27 | 259.00±3.36 |
| Day-7   | 376.00±2.85    | 169.20±1.59 | 194.40±4.16 | 252.00±2.5  |
| Day-14  | 359.00±2.93    | 143.60±2.32 | 176.40±2.74 | 244.80±1.37 |
| Day-21  | 344.00±1.96    | 129.60±1.72 | 144.00±4.40 | 201.60±1.78 |

Conclusion
The findings of this experiment revealed that water soaking caused substantial reduction in hydrogen cyanide content in the rubber seeds without change of chemical composition and overnight soaked and dried rubber seed meal may be utilized as pig feed.

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