# Techniques of removing saponins from Mahua *(Bassia longifolia)* seed cake and its suitability as animal feed

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*Summary.* A new process of removing saponins from Mahua seed cake, a commonly available tree of genus *Bassia,* has been developed. The processed cake when added to the rations of calves to the extent of 50% digestible crude protein replacement, had no adverse effect on growth rate of cross-bred dairy calves.

*Bassia* Linn. a genus of tree belonging to natural order Sapotaceae, is on inhabitant of India and Malaya. *B. latifolia* and *B. longifolia* are the 2 most common varieties available in India. The cake obtained after extraction of oil from the seeds (known as Mahua cake) contain sapoglucosides (saponins) which are toxic to livestock and have an extremly bitter taste. Since there is shortage of protein and energy feeds for ruminants in most part of the world, attempts were made to remove saponins from the cake to make it suitable for animal feeding.

*Materials and methods.* Samples of Mahua cake were analyzed for its chemical composition[2]. Saponins in the Mahua cake were quantitatively estimated by chemical procedures[3,4], modified for the present studies. Defatted Mahua cake was extracted for 6 h in Lab-Con-Co fat extraction apparatus with methyl alcohol. The extract was concentrated and diluted with water. This was further extracted with n-butyl alcohol and concentrated under reduced pressure and dissolved in small amount of methyl alcohol which was poured drop by drop into 3 volumes of solvent ether while stirring. The precipitate of crude saponins was dried under vacuum and weighed. The crude saponins were hydrolyzed with 15% ethanolic sulphuric acid for 15 h and then poured into water. The precipitate of crude sapogenols so obtained was filtered on pre-weighed filter paper which was later dried and weighed.

Laboratory process of removal of saponins from Mahua cake. 4 treatments were tried for removing saponins from Mahua cake. a) Cold water treatment: 5 g of Mahua cake was treated with water (1:4) at room temperature for different time interval and stirred. The foam formed due to stirring on top layer were removed by decantation process. The residual saponins and sapogenols were estimated in treated Mahua cake. b) Hot water treatment: The cake was treated with hot water (70–80 °C) in thermostatically controlled water bath, and after removal of foams, the saponins and sapogenols were estimated in the residual cake. c) Alkali treatment: 5 g of Mahua cake was treated with aqueous NaOH solutions (0.5% and 0.25%) for various time intervals, the estimations were the same as in cold water treatment.

Large scale recommended method of removing saponins of Mahua cake. During our field experiments, a method of removing saponins was developed, where dry matter loss was only 5% and palatability of cake was much improved. 16 kg Mahua cake was soaked in galvanized iron tube in 64.1, (1:4) of water for 10 min, and stirred manually for 1 min. A pause of about 2 min was allowed for developing and settling of the foam on the top layer. Foam were removed by a coarse strainer. The above process was repeated 10 times within 1 h. During the last few stages foam becomes oily and yellowish. During the palatability test studies we estimated the crude residual saponin content, which varied from 12 to 16% depending upon the thickness of the cake and method of oil extraction, but all types of cake become palatable if the above process is repeated 10 times within 1 h when the colour of the foam changes from soapy white to oily yellowish. Simple appearance of the mass is more feasible indication of palatability for farmers & field trials than the estimation of residual saponins in the cake. Saponins from Mahua cake during the whole course of studies were removed by this process.

Animals and feeding regimes: 16 g growing dairy calves weighing between 150 and 200 kg were randomly divided into 4 groups of 4 animals each. The animals were fed rations as per National Research Council recommendation[5]. The concentrate mixture was composed of groundnut cake, maize and wheat bran (equal parts). Common salt, mineral mixture and vitamins A and $D_3$ were supplemented to meet their requirements. Wheat straw was fed as the basal roughage. The feeding period was 130 days.

The treated Mahua cake was sprinkled over concentrate mixture and wheat straw and after a thorough mixing the

Table 1. Chemical composition of Mahua cake (% dry matter)

| Chemical constituents | Quantity |
|---|---|
| Crude protein | 19.1 |
| Ether extract | 10.5 |
| Crude fibre | 7.8 |
| Ash | 9.2 |
| Nitrogen free extract | 53.4 |
| Organic matter | 90.8 |
| Saponins | 5.1 |
| Sapogenols | 1.6 |

Table 2. Estimation of crude saponins from Mahua cake by various treatments

| Treatments | Period of treatment (min) | Crude saponins residue (%)* | Sapogenol residue (%)* |
|---|---|---|---|
| Cold water treatment | 15 | 36.1 | 1.0 |
| | 60 | 20.4 | 0.8 |
| | 240 | 19.5 | 0.6 |
| | 360 | 14.8 | 0.6 |
| | 1040 | 12.9 | 0.6 |
| Hot water treatment | 15 | 40.0 | 2.1 |
| | 60 | 26.0 | 1.6 |
| | 240 | 24.2 | 1.8 |
| | 360 | 23.6 | 1.6 |
| | 1040 | 14.3 | 1.4 |
| 0.25% NaOH treatment | 60 | 38.1 | 1.5 |
| | 120 | 51.2 | 3.0 |
| | 240 | 48.1 | 2.5 |
| 0.5% NaOH treatment | 60 | 45.4 | 2.0 |
| | 120 | 48.5 | 1.7 |
| | 240 | 61.1 | 2.0 |

* Diminution of the saponin and sapogenol residue compared to the untreated Mahua cake is shown.

Table 3. Growth data of cross bred dairy calves on the control and Mahua cake ration

| Group | Treatment | Daily gain (g) |
|---|---|---|
| 1 | Control | $663.3 \pm 42.0879$ |
| 2 | 25% Mahua cake | $610.3 \pm 31.2641$ |
| 3 | 50% Mahua cake | $642.0 \pm 42.6790$ |
| 4 | 75% Mahua cake | $566.3 \pm 14.3100$ |

ration was offered to the animals. Animals of group 1 received the conventional concentrate mixture, whereas in group II 25% of the DCP requirement was met by water-treated Mahua cake. Animals of group III and IV received 50% and 75% of their DCP requirement through Mahua cake, respectively. No group with untreated Mahua cake was included in the experiment as animals refuse to accept it due to its extremely bitter taste.

*Results and discussion:* The chemical composition of Mahua cake is shown in table 1. Within 1 h of the treatment of Mahua cake with ordinary water at room temperature, about 80% of the saponins were removed (table 2). No additional advantage was observed by treating the cake either by hot water or alkali. The foamy material developed after aqueous sodium hydroxide treatment adhered more intimately with solubilized cake and with gradual lapse of time it was more difficult to remove saponins; which explains the peculiar nature of NaOH treatment (table 2).

The cake was palatable to the animals after treatment with water. The gain in the body weights of the animals in all the 4 treatments did not differ significantly (table 3). The daily growth rate in the experimental animals were comparable to earlier reports[5,6]. It is concluded that Mahua cake after proper treatment for removing saponins may possibly be used as a cattle feed in limited quantity.

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## Transdermal uptake of a peptide hormone: Inhibition by calcitonin eardrops of induced osteolysis in guinea-pig ossicles

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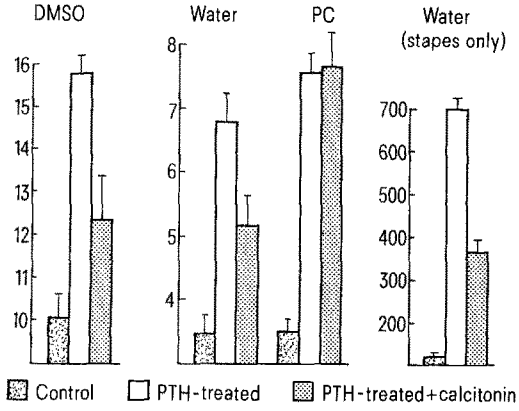
*Summary.* In view of a recent proposal that calcitonin injections may arrest the bony pathology of otosclerosis, we have tested the possibility of obtaining locally effective concentrations by giving salmon calcitonin in eardrops. Osteolysis of guinea-pig ossicles induced by injecting parathyroid hormone shortly before explantation was markedly inhibited by 3 days prior instillation of the calcitonin in an aqueous vehicle or in dimethyl sulphoxide, but not by a solution in propylene carbonate.

The pathological changes of otosclerosis resemble in some respects the appearance of the earbones in patients where Paget's disease has affected the hearing. Since deafness is sometimes relieved during treatment of the latter by injection of calcitonin[2-5], Diamond[6] has suggested a therapeutic trial of calcitonin in the much commoner condition of otosclerotic deafness. (Otosclerosis is common throughout Europe, the Balkans and the Middle East and among Americans of Caucasian origin, causing clinical deafness in 0.3-0.5% of the adult population[7]).

In view of the disadvantages of treating localized disease of the earbones by systemic hormone injection, we have tested the possibility that effective quantities of calcitonin might be absorbed from suitably formulated eardrops. Guinea-pigs injected with radiocalcium were treated with eardrops of solvent alone or containing calcitonin (250 µg/ml) for 3 days and sacrificed 1 h after i.v. injection of 100 units of bovine PTH (2000 units/mg). Ossicles were removed by opening the temporal bone from its lower surface, remote from the external ear to which the calcitonin had been applied. Ossicles were explanted on to filter discs and cultured in the Fitton Jackson modification of BGJ medium (Gibco-Biocult Ltd), suitably gassed.

Because of the expected low permeability of skin to a peptide and its likely destruction by tissue proteases, we chose the moderately hydrophobic[8] and relatively stable[9] salmon calcitonin, and carried out initial experiments with the aprotic polar solvent dimethyl sulphoxide (DMSO). Other solvents tested were water (containing 0.1% dioctyl sodium sulpho-succinate) and propylene carbonate (PC).




Bar graph illustrating the effect of in vivo parathyroid hormone (PTH) and eardrops with and without calcitonin on the in vitro release of calcium$^{45}$ from guinea-pig ossicles. The ordinates show radioactivity (cpm $\times 10^{-3}$) in 1 ml of bathing medium withdrawn from explants at the end of 20 h incubation in 3 separate experiments (mean + SEM, n = 8).

As shown in the key, the 2nd and 3rd of each set of 3 bars compare the PTH-stimulated release from ossicles of animals which received eardrops of vehicle alone on one side and added calcitonin on the other, while the 1st bar of each set shows the release from ossicles of animals given eardrops of vehicle alone, without injection of PTH. Except when the vehicle was propylene carbonate, the antagonism by calcitonin of the PTH-stimulated resorption was highly significant ($p < 0.01$ in experiments 1 and 2 and $p < 0.005$ in the 3rd experiment, which was carried out with the explanted stapes alone).