

## KAJIAN SENYAWA BIOAKTIF DAN ASAM AMINO GLUKOGENIK BEBERAPA LEGUM PAKAN TERNAK SEBAGAI KANDIDAT GALAKTOGOGUM UNTUK SAPI PERAH PERIODE PERIPARTURIEN

### INTISARI

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Penelitian ini bertujuan untuk mengidentifikasi, mengkarakterisasi, dan mendeterminasi senyawa bioaktif dan asam amino glukogenik beberapa tanaman legum pakan ternak, yang meliputi: *Gliricidia maculata*, *Calliandra calothyrsus*, *Indigofera arrecta*, dan *Desmodium rensonii*. Sampel diambil secara acak dari bagian tanaman yang dapat dimakan (*edible portion*), dari kebun koleksi tanaman pakan Fakultas Peternakan, Universitas Gadjah Mada (UGM). Proses liofilisasi dilakukan pada suhu 55°C selama 3x24 jam terus menerus menggunakan Sanyo MOV-112 *Drying Oven*. Pulverisasi dilakukan dengan Foss Tecator Cyclotec™ 1093 *Sample Mill* dengan derajat halus serbuk 400 *mass*. Senyawa bioaktif tanaman legum diekstraksi menggunakan metode *ultrasonic-assisted extraction* (UAE). Identifikasi dan karakterisasi senyawa bioaktif tanaman legum dikerjakan dengan SHIMADZU GCMS-QP2010S *Instrument*. Reaksi derivatisasi asam amino dilakukan secara pre kolom dengan pereaksi ortho-phtalaldehid (OPA, C<sub>8</sub>H<sub>6</sub>O<sub>2</sub>). Pemisahan dan determinasi asam amino dilakukan dengan metode kromatografi cair kinerja tinggi (KCKT) menggunakan Thermo Scientific™ Dionex™ UltiMate™ 3000 UHPLC Systems. Determinasi senyawa galaktogogum dilakukan dengan analisis pengambilan keputusan multikriteria (*multicriteria decision making* (MCDM) menggunakan metode proses hirarki analitik (*analytical hierarchy process*, AHP) berdasarkan enam golongan senyawa bioaktif (sesuai tujuan penelitian), yaitu amina, asam lemak, ester asam lemak, terpenoid, glukosida, dan alkohol. Total jenis senyawa bioaktif yang teridentifikasi pada masing-masing spesies berturut-turut adalah 32, 29, 23, dan 38 jenis senyawa dengan total senyawa target pada masing-masing spesies adalah 14, 14, 12, dan 18 senyawa target. Hasil analisis menunjukkan bahwa *Calliandra calothyrsus* adalah spesies dengan kandungan senyawa bioaktif terbanyak yang berpotensi sebagai galaktogogum, dengan skor 93,2, diikuti oleh *Gliricidia maculata*, *Desmodium rensonii*, dan *Indigofera arrecta*, dengan skor masing-masing adalah 86,9, 80,2, dan 77,5. Analisis kuantitatif asam amino dilakukan dengan membandingkan hasil pengukuran luas puncak (*peak area*) dari sampel dengan puncak standar referensi pada konsentrasi yang diketahui. Validasi metode dilakukan dengan membuat persamaan linier yang menghubungkan antara konsentrasi asam amino dengan luas puncak sesuai dengan persamaan linier  $y=ax+b$ . Penentuan skala prioritas tanaman legum menggunakan analisis multikriteria (AMK) menggunakan metode AHP. Hasil analisis menunjukkan terdapat empatbelas macam komponen asam amino pada setiap spesies tanaman legum, antara lain: *aspartic acid*, *glutamic acid*, *serine*, *histidine*, *glycine*, *arginine*, *alanine*, *tyrosine*, *methionine*, *valine*, *phenylalanine*, *isoleucine*, *leucine* and *lysine*. Urutan prioritas sumber asam amino glukogenik pada beberapa spesies legum pakan ternak berturut-turut adalah *Desmodium rensonii*, *Gliricidia maculata*,

*Indigofera arrecta*, dan *Calliandra calothyrsus* dengan nilai skor masing-masing adalah 1172.52; 776.80; 731.53; dan 295.73. Evaluasi pakan dengan variasi jenis dan level legum dilakukan pengujian parameter fermentasi rumen secara *in vitro*. Hasil penelitian menunjukkan terdapat efek interaksi yang sangat nyata ( $P < 0,01$ ) antara jenis dengan level legum terhadap produksi gas, kadar protein mikroba dan aktivitas enzim endoglukanase cairan rumen. Jenis dan level legum memberikan efek nyata ( $P < 0,05$ ) terhadap kadar amonia ( $\text{NH}_3$ ) dan kadar total *volatile fatty acids* (VFA), namun tidak terdapat efek interaksi antara jenis dan level legum dalam ransum. Sedangkan nilai pH cairan rumen tidak dipengaruhi ( $P > 0,05$ ) oleh jenis maupun level legum.

Kata kunci: Ekstrak etanol, Legum, Senyawa Bioaktif, Kromatografi gas-spektrometri massa, Galaktogogum, Kromatografi cair kinerja tinggi, Asam amino glukogenik

## STUDY OF LEGUMES BIOACTIVE COMPOUNDS AND GLUCOGENIC AMINO ACIDS AS A GALACTOGOGUE CANDIDATES FOR DAIRY COW PERIPARTURIEN PERIOD

### ABSTRACT

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This study aims to identify, characterize, and determine the bioactive compounds and glucogenic amino acids of several legumes for animal feed, *i.e.*: *Gliricidia maculata*, *Calliandra calothyrsus*, *Indigofera arrecta*, and *Desmodium rensonii*. Samples were taken randomly from the edible portion of the plant, from the forage collection garden of the Faculty of Animal Science, Universitas Gadjah Mada (UGM). Lyophilization was done by heating the sample at 55°C for 3x24 hours continuously using the Sanyo MOV-12 Drying Oven. Reducing the sample size (pulverization) was performed with a Foss Tecator Cyclotec™ 1093 Sample Mill with 400 mass powder. Legume plant bioactive compounds were extracted using the ultrasonic-assisted extraction (UAE) method. Identification and characterization of the bioactive compounds of legumes were carried out by the SHIMADZU GCMS-QP2010S Instrument. Amino acid derivatization reactions were carried out pre-column by  $\mu\text{L}$  orthophthalaldehyde reagent (OPA,  $\text{C}_8\text{H}_6\text{O}_2$ ). A High-Performance Liquid Chromatography (HPLC) method was used to separate and determine the amino acids using the Thermo Scientific™ Dionex™ UltiMate™ 3000 UHPLC Systems. Determination of galactogogum compounds was done by analysis of multicriteria decision making (MCDM) in the analytical hierarchy process (AHP) method based on six classes of bioactive compounds (according to research objectives), *i.e.*: amines, fatty acids, esters of fatty acid, terpenoids, glucosides, and alcohols. The total types of bioactive compounds identified in each species were 32, 29, 23, and 38 types of compounds respectively and the total target compounds in each species were 14, 14, 12, and 18 respectively. The result showed that *Calliandra calothyrsus* is the species with the most bioactive compounds that have the potential as galactogogums, followed by *Gliricidia maculata*, *Desmodium rensonii*, and *Indigofera arrecta*. Quantitative analysis of amino acids was carried out by comparing the measurements results of the peak area of the sample with the peaks of reference standards at known concentrations. Method validation was carried out by constructing a linear equation according to the linear equation  $y = ax + b$ . Prioritization of the legumes was determined using multicriteria analysis (MCA) in the AHP method. The results showed that there were fourteen kinds of amino acid components in each legume species, including: aspartic acid, glutamic acid, serine, histidine, glycine, arginine, alanine, tyrosine, methionine, valine, phenylalanine, isoleucine, leucine, and lysine. The priority sequence of glucogenic amino acid sources in several species of forage legumes is *Desmodium rensonii*, *Gliricidia maculata*, *Indigofera arrecta*, and *Calliandra calothyrsus* with a score of 1172.52; 776.80; 731.53; and 295.73, respectively. Evaluation of feed with various types and levels of legumes was carried out by *in vitro* rumen parameter stripping. The results showed that there was a highly significant interaction effect ( $P < 0.01$ ) between legume species and levels on gas production, microbial protein content and rumen fluid endoglucanase enzyme activity. The types and levels of legumes had a significant effect ( $P < 0.05$ ) on

ammonia ( $\text{NH}_3$ ) and total volatile fatty acid (VFA) levels, but there was no interaction effect between types and levels of legumes in the ration. Meanwhile, the pH value of rumen fluid was not affected ( $P>0.05$ ) by the type or grade of legumes.

**Keywords:** Galactogogues, Gas chromatography-mass spectrometry, Glucogenic amino acids, Gluconeogenesis, Parameters of rumen fermentation