Raman Microspectroscopy (RMMS) for Exploration of Feed Structure and Nutrition Interaction: Using Non-Invasive Techniques in Animal Nutrition

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Raman microspectroscopy is a non-invasive bioanalytical technique for studying and imaging molecular vibrations and structure. In this article, recently obtained information on applications of Raman microspectroscopy in exploration of animal feed and animal nutrition interaction was introduced. The emphasis of this review focused on principles and advantage of Raman microspectroscopy techniques to feeds and nutrition research and recent applications and current studies in our team by using Raman microspectroscopy technique for feed chemistry, feed structure, and feed processing studies. The information described in this article may provide an insight in how feed structure, chemistry and nutrition revealed by Raman microspectroscopy.

Keywords: Raman microspectroscopy; Feed Structure and Nutrition Interaction; Feed chemistry; Feed Processing; Animal nutrition; Non-Invasive Techniques

1. Introduction

1.1 Feed and nutrition study with conventional methods

Accurate prediction of livestock performance in modern farms requires accurate feed and diet evaluation. In feed and diet evaluation, there are many methods including conventional chemical composition analysis, in situ, in vitro and in vivo trials [1]. The chemical analyses provide feed composition, such as total crude protein, total starch, total fibers, total ash, total carbohydrate etc. The in situ trials provide feed degradation kinetics and bypassed fractions to small intestine [2,3]. The in vitro methods provide information about digestions of various nutrients [4,5]. The in vivo trails provide livestock production performance [6]. The modeling methods [7,8] are able to provide information about truly nutrient supply [9] and/or animal performance based on chemical composition, in situ and in vitro results..

1.2 Current issues in conventional methods for feed analysis.

A big issue in conventional feed analysis than employed by NRC for livestock is that these methods fail to provide information about feed inherent structure and fail to link feed inherent structure in relation to nutritional value in animals [10,11]. This is mainly due to the fact that conventional methods destroy inherent structure in processing for chemical digestion during lab analysis [12]. It is often observed that the same chemical composition in two feeds, but dramatic differences in digestion and degradation [13]. The conventional NRC methods [14] to evaluate a feed are usually based on total feed chemical composition. Therefore this method is not accurate because it doesn't consider feed inherent structure, or structure and nutrition interaction. Feeds that have the same composition may have different chemical make-up and conformation [13,15]. The non-invasive techniques in feeds and animal nutrition are required. In our previous published book chapter, we discussed the advantages and limitation of synchrotron-based Infrared microspectroscopy as a non-invasive technique for feed intrinsic structure studies.

1.3 Objective of this review article

The article aims to introduce another non-invasive technique for animal feed and nutrition interaction evaluation using molecular microspectroscopy - Raman microspectroscopy (RMMS). The emphasis of this article focused on introduction of principles, advantages and limitation of Raman (micro)spectroscopy to feeds and nutrition research and recent applications and current studies in our team by using Raman microspectroscopy technique for feed chemistry, feed structure, and feed processing studies. The information may provide an insight in feed structure, feed chemistry and animal nutrition interaction which could be revealed by Raman microspectroscopy beside other spectroscopy techniques such as FT-IR, DRIFT, SR-IMS.

2. Non-Invasive Technique - Raman microspectroscopy (RMMS)

2.1 Different types of molecular spectroscopy

In feed evaluation and characterization, various molecular spectroscopy have been applied. These methods include near-infrared (NIR) [12,16], middle-infrared (MIR) spectroscopy (DRIFT) [17,18,19] and FTIR microspectroscopy [12,17]. Recently more cutting-edge synchrotron radiation based FTIR microspectroscopy (SR-IMS) and synchrotron radiation-based soft x-ray (SR-XMS) and near edge X-ray absorption fine structure (NEXAFS) [21] have been introduced for feed and nutrition evaluation [20,22].

2.2 Brief principle of Raman microspectroscopy

Wikipedia gives a very clear introduction of Raman spectroscopy and it states that "Raman spectroscopy is a spectroscopic technique used to observe vibrational, rotational, and other low-frequency modes in a system. It relies on inelastic scattering, or Raman scattering, of monochromatic light, usually from a laser in the visible, near infrared, or near ultraviolet range. The laser light interacts with molecular vibrations, phonons or other excitations in the system, resulting in the energy of the laser photons being shifted up or down. The shift in energy gives information about the vibrational modes in the system" [23].

   "Raman Microspectroscopy is a spectroscopic technique that uses a specialized Raman spectrometer to measure the spectra of microscopic samples. In general terms, a Raman spectrometer is integrated with a Raman microscope. Different exciting lasers may be used to excite a microscopic sample at different wavelengths so that the Raman microspectrometer can collect and analyze the vibrational spectra." [24]

2.3 Advantages and limitation of Raman microspectroscopy

Raman microspectroscopy (RMSS) is able to study biological tissue at ultra-spectral resolution (1 micron by 1 micron). It is able to link structural and chemical information within intact tissue. It is able to generate detailed chemical image based on sample spectrum at every pixel of mapping area. It is able to interrogate all pixel to generate false color images based on material composition and structure [25]. In general, Raman microspectroscopy, like FT-IR microspectroscopy with Globar source or synchrotron light source, enable to provide four kind of information: tissue chemistry, tissue environment, tissue composition and tissue structure. The limitation of Raman microspectroscopy could be relatively lower signal to noise ratio and the potential feed damage by the laser [26]

2.4 Raman spectral analysis

In Raman spectral analysis, there are two common different methods that are being used. The first common method is univariate spectral analysis, which includes Raman peak area or height intensity measurements. In the imaging analysis, Raman peak intensity yields images of material concentration and distribution. Raman peak position yields structure images of tissues [12]. So Ramen images provide tissue or material in chemical and structural sense, well beyond what the researcher's eye can see.

   The 2nd common method is multivariate methods [27]. These methods are used to classify spectral groups by applying the whole spectral information in different regions. The multivariate analysis in our feed and nutrition studies included agglomerative hierarchical cluster analysis, using Wards' algorithm method without prior to parameterization, and principal component analysis [1,27]. The detailed methodology applied for feed and nutrition interaction have been reported in synchrotron-based FTIR microspectroscopy study [27,28].

3. Application of Raman Microspectroscopy in Feed Structure and Animal Nutrition Studies

3.1 Recent applications of Raman microspectroscopy in feed structure and chemistry

The study by Khan and Yu [25] aimed to use Raman spectroscopy to explore structural difference in different types of cereal grains and reveal the chemistry of the protein structures of cereal grain tissues affected by types and treatments, and to quantify the protein secondary structures using multicomponent peak modeling Gaussian and Lorentzian methods.

   Agglomerative hierarchical cluster analysis (AHCA) and principal component analysis (PCA) have been used to identify differences in the Raman spectra between different types of cereal grain and different treatments. It showed the difference between different types of cereal grain and different treatments based on univariate and multivariate spectral analysis.

3.2 Recent applications of Raman microspectroscopy in heat-related feed processing

In our study [25], we combined Raman microspectroscopy with Differential Scanning calorimeter "to reveal molecular thermal stability and thermal degradation behavior of heat-induced cereal grains. The modeled cereal grains include wheat, triticale, and corn which were heated using autoclaving (moist heating) and dry heated (roasted), respectively. The results showed that the protein secondary structures are significantly different among the heated treatments and among the different types of cereal grain." [25],

   We found that: "by using Raman microspectroscopy, the sensitivity of cereals to moist heating was much higher than the sensitivity to dry heating. The multivariate analyses showed that heat treatments were significantly isolated between the different Raman raw spectra. The DSC study revealed that the thermal degradation behavior of cereals was significantly changed after moist and dry heat treatments. The position of the major endothermic peak of dry heated cereals shifted toward higher temperature, from 131.7 to 134.0°C, suggesting the high thermal stability of dry heated cereals. In contrast, the endothermic peak position was slightly decreased to 132.1°C in case of moist autoclaved heating. The digestive behavior and nutritive value of rumen-undegradable protein in animals may be related to the changes of the protein secondary molecular structure and thermal stability of the cereal grain materials which is attributed by Raman microspectroscopy and DSC endotherm profiles." [25]. This is a typical example employing Raman (micro)spectroscopy to explore feed and nutrition interaction, feed inherent structure, feed processing and feed architecture.

3.3 Recent applications of Raman microspectroscopy in feed structure and animal nutrition interaction and relationship

Recently, we are studying the spectral features and profiles detected by different techniques: Raman microspectroscopy, synchrotron-based FT-IR microspectroscopy and FT-IR/ATR spectroscopy in relation to nutrient availability and utilization in ruminants. First we employed univariate technique to determine various functional group intensity that related to lipid, carbohydrate (both structural and non-structural) and protein biopolymers in raw and processed cereal grains as ruminant feeds. Then we did in vitro and in situ studies to determine the degradation and digestion of various nutrients in the feeds such as fibers (NDF, ADF), protein, starch. We also fractioned the protein and carbohydrate into different fractions using CNCPS system that are related to different degradation rate and extent in rumen and nutrient supply in the small intestine. The following is that we are studying: (1) detecting the processing induced changes on a molecular basis of the processed seed designated as animal feeds; (2) detecting response and sensitivity of different chemical functional groups to each different types of heating processing; (3) quantifying the protein inherent structure based on multi-peak fitting and modeling; (4) detecting correlationship between feed molecular structure and nutrient availability; (5) Building a prediction model based on both chemical composition and structure functional group intensity [29]

4. Conclusions and future research

The current feed quality and nutrition models are based on total chemical composition. This method is not accurate mainly becuase feed quality is not only related to chemical composition but also related to feed structure and biological component matrix. The non-invasive technique that could be used to provide us feed composition and feed structure is urgently needed. Raman microspectroscopy is one of non-invasive techniques, which is able to chemically and structurally characterize feeds at the cellular and molecular levels and can be used detect the structural change after various feed processing in relation to nutrient availability in animals, Just like synchrotron-radiation based FTIR microspectroscopy and synchrotron radiation-based soft x-ray. The spectroscopic techniques can link feed structure information to chemical information within intact tissue. It is able to image feed tissue chemistry and can provide four kind information, chemistry, structure, environment and composition, simultaneously. The limitation of Raman microspectroscopy could be relatively lower signal to noise ratio and the potential feed damage by the laser

   Future study is needed to compare Raman microspectroscopy and synchrotron-based FTIT microspectroscopy in systematic feed evaluation and develop rapidly diagnostic method to evaluate and predict feed nutritive value based on both composition and structure.

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