Evaluation Indicators and Measurement Methods for Chyme Properties: Postprint

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Abstract

Chyme characteristics not only affect feed digestibility, but also play a significant role in animal intestinal health. This paper summarizes the evaluation indices and determination methods for chyme characteristics, analyzes these methods, and provides a reference for research and application of chyme characteristics.

Full Text

Evaluation Indexes and Determination Methods of Chyme Characteristics

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Abstract: Chyme characteristics not only affect feed digestibility but also play important roles in animal intestinal health. This paper reviews the evaluation indexes and determination methods of chyme characteristics and analyzes these methods, providing a reference for research and application in this field.

Keywords: chyme; viscosity; residence time; digestive enzyme; microbial flora

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Chyme is the general term for the contents of the gastrointestinal tract, representing a semi-fluid or fluid mixture formed when food is mechanically broken down into fine particles in the digestive tract and mixed with digestive juices, microorganisms, and their fermentation products. The main evaluation indexes for chyme characteristics currently include viscosity, pH, particle size, flow rate and residence time, water-holding capacity, digestive enzyme activity, and microbial flora.

Viscosity

Chyme viscosity is a crucial index affecting feed digestibility in animals. Increased viscosity reduces solute diffusion rate, slows chyme digestion, decreases the rate at which nutrients are released from feed, and ultimately lowers feed digestibility [1]. In animal nutrition research, the viscosity studied is dynamic viscosity, also known as relative viscosity, typically measured using a viscometer. The method involves separating the liquid and solid phases of chyme through some separation technique, then measuring the viscosity of the liquid phase. The final viscosity value is expressed as the ratio of the flow time of the chyme sample to that of an equal volume of distilled water.

In practical applications, researchers employ different sample pretreatment methods. For instance, Tang Haiou et al. [2] added 10 mL of distilled water to chyme samples, Huo Wenying et al. [3] diluted chyme six-fold, while Lázaro et al. [4] performed no dilution. Additionally, the centrifugation speeds used for separating liquid and solid phases vary considerably: Tang Haiou et al. [2] and Huo Wenying et al. [3] used 5,000 r/min, Lü Qiufeng et al. [5] used 3,000 r/min, and Liu Changzhong et al. [6] used 12,000 r/min. These substantial variations in the application of the same measurement method have not been reported in terms of how they affect results.

pH

Chyme pH is one of the important factors affecting animal digestion and absorption, representing a key characteristic of chyme. pH significantly influences digestive enzyme activity and gastric secretion in the gastrointestinal tract, thereby affecting animal digestion [7]. Measurement is typically performed using a pH meter: after centrifugal separation of the liquid and solid phases of chyme, the pH of the liquid phase is determined.

Similar to viscosity measurement, sample pretreatment for pH determination varies among studies. Ai Xiaojie et al. [7] and Mao Zonglin et al. [8] performed no dilution of chyme samples, whereas Lü Qiufeng et al. [5] diluted samples tenfold. How to pretreat samples for chyme pH measurement and whether different pretreatments introduce bias remain unaddressed in the literature.

Particle Size

Particle size is an important physical characteristic of chyme, typically expressed as particle diameter or size distribution. The optimal particle size distribution of feed should match the physiological requirements of animals to maximize nutrient utilization and improve production performance [9]. Two primary measurement methods are commonly used: (1) Sieving method: This involves selecting a series of standard screens with different apertures, stacking them from largest to smallest, and determining the weight of particles retained on each screen after sieving. The mass fraction-based particle size distribution is then calculated

[10]. (2) Laser scattering method: In this approach, samples dispersed at adequate concentration in a suitable liquid or gas pass through a beam generated by a monochromatic light source (usually a laser). A multi-element detector measures light scattering at various angles, and scattering model-related values are recorded for subsequent analysis. These scattering data are transformed through appropriate optical models and mathematical processes (Mie scattering theory and Fraunhofer approximation theory) to generate the proportion of different discrete size fractions relative to total volume, constituting the particle size distribution [11].

Comparative studies of these two methods have shown that sieving is suitable for larger particles (>45 m), whereas laser scattering offers a wider measurement range (0.02–2,000 m), higher resolution, and faster detection, making it the more applicable method [12].

Flow Rate and Residence Time

Both chyme flow rate and residence time are important physical characteristics of chyme. Flow rate can indicate nutrient digestion and absorption efficiency; at a given digestive site, greater flow suggests higher absorption, while lower flow indicates reduced absorption [13]. Residence time typically characterizes how specific feed components (primarily fiber content) alter the physicochemical properties of chyme [14]. Currently, two internationally recognized methods exist for measuring chyme flow: total collection and indicator methods. Total collection involves gathering chyme through intestinal cannulation, providing accurate total chyme measurement, but the technique is cumbersome, labor-intensive, and results in poor animal care and short survival times [15]. The indicator method substitutes indicator flow and residence time in the gastrointestinal tract for those of chyme [16]. Since indicators cause minimal animal stress, this method is most popular [15]. The key to the indicator method is selecting appropriate markers. Numerous studies have compared endogenous and exogenous indicators. Gao Xinmei et al. [17] reported that using the exogenous indicator chromium (Cr) as a feed marker could not exclude interaction effects between feed factors and the test diet. Barnett et al. [18] used cobalt-EDTA (Co-EDTA) as an exogenous indicator to measure chyme flow, obtaining a coefficient of variation of 5.1$±$2.0, demonstrating its reliability as a marker. Zhang Naifeng et al. [19] compared chromium oxide (Cr2O3) and polyethylene glycol (PEG)-4000 as exogenous indicators, finding Cr2O3 yielded more reasonable results. Solà-Oriol et al. [20] observed that TiO2-marked chyme flowed faster than Cr-marked chyme. Zhang Naifeng [21] used Cr2O3 as a solid-phase marker and PEG as a liquid-phase marker, finding that dual-marker methods produced smaller coefficients of variation with better stability and reliability. Faichney [22] used Cr to study the effects of formaldehyde-treated diets on solute and particulate flow in sheep, finding 4.9% of Cr was absorbed into urine, indicating partial metabolism. These varying marker selections suggest that comparative studies on marker types would help identify more suitable options.

Water-Holding Capacity

Water-holding capacity refers to chyme' s ability to retain its own moisture and bind additional water, also known as water retention. This property affects chyme viscosity [23]. Two methods exist for measuring water-holding capacity: (1) Drying method: After centrifuging chyme samples, the precipitate is dried at 105°C, and water-holding capacity is expressed as the ratio of weight loss to final dry weight [24]. (2) Centrifugation method: Chyme samples are centrifuged at 2,500 r/min for 10 minutes, the supernatant is discarded, and water-holding capacity is calculated as the ratio of weight loss to precipitate weight after centrifugation [25].

Currently, no consensus exists on which method is superior. Comparative studies using both methods on the same material would help determine which is more scientific and applicable.

Digestive Enzyme Activity

Digestive enzyme activity in chyme is one of its most important characteristic indicators and a key focus in animal nutrition research. It is a critical factor affecting nutrient digestion and absorption, with increased enzyme activity improving digestibility [26]. Current measurements focus on activities of amylase, lipase, trypsin, chymotrypsin, maltase, sucrase, lactase, and carboxymethyl cellulase [27-29].

The principle of enzyme activity measurement involves utilizing the specific and efficient catalytic properties of enzymes to determine their content and activity by measuring reaction rates. Various methods exist for different enzymes: amylase activity is measured by iodine-starch colorimetry, lipase by turbidimetry, trypsin by N-benzoyl-L-arginine ethyl ester (BAEE) method, and total protease by Folin-phenol method. These can be categorized as spectrophotometry, polarimetry, fluorometry, and chemical reaction methods, with spectrophotometry being most common. Overall, enzyme activity assays are trending toward simplicity, high precision, and strong repeatability, making kit-based methods using microplate readers increasingly popular. Examples include Shi Dongjie et al. [30] measuring trypsin, lipase, and amylase in koi; Lin Xiajing et al. [31] measuring lipase, amylase, trypsin, and chymotrypsin in broilers; Wang Guoxia et al. [32] measuring protease, lipase, and amylase in perch; and Fu Xu et al. [33] measuring pepsin, lipase, and amylase in black tilapia. This approach involves sample pretreatment followed by using commercial kits according to manufacturer protocols. It offers simplicity, high specialization, and accuracy. The main steps include: diluting and homogenizing chyme samples with 0.86% sodium chloride solution (homogenization buffer), centrifuging at 3,000 r/min for 20 minutes at 4°C in a low-temperature ultracentrifuge, collecting the supernatant, following kit instructions, and measuring absorbance with a microplate reader to determine enzyme activity.

Microbial Flora

The microbial flora in chyme, representing the intestinal microecosystem, is one of the most important indicators of chyme characteristics. A balanced microbial flora plays vital roles in nutrient metabolism, immune function, animal health promotion, and nutrient utilization, with research on this topic helping to elucidate mechanisms of gastrointestinal digestion, absorption, and metabolism [34-35].

Traditionally, microbial flora has been measured by plate counting. In this method, aseptically collected intestinal contents are serially diluted 10-fold with phosphate buffer to 10^-6, and appropriate dilutions are plated on selective media. Bacterial counts are expressed as log (CFU/g) of intestinal content. Bacteria are identified based on colony characteristics on selective media combined with Gram staining and microscopic examination. With advances in molecular biology, molecular methods have become widely applied, including denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP), real-time quantitative PCR, gene chip technology, and metagenomics. These techniques are increasingly used in animal nutrition research: Simpson et al. [36] applied DGGE to analyze porcine fecal microbiota composition; Kocherginskaya et al. [37] studied DGGE profiles of rumen fluid from steers; Zhang Yongjing et al. [38] used T-RFLP to investigate effects of different fiber sources and cell wall-degrading enzymes on diversity and composition of porcine intestinal microbiota; Pan Yanyan et al. [39] analyzed bacterial community characteristics and diversity in the anterior, middle, and posterior intestinal walls and feces of perch using T-RFLP; Shtriker et al. [40] analyzed intestinal microbiota in mice using real-time quantitative PCR; gene chip technology, building on traditional blotting hybridization, can monitor expression of thousands of genes in a single experiment, with Luo et al. [41] using it to explore differences in porcine fecal microbiota diversity; Wu Peng et al. [42] applied metagenomics to study rumen microbial diversity and function; and Nielsen et al. [43] used metagenomics to investigate microbial diversity and function in pig feces. Although molecular methods offer irreplaceable advantages over traditional approaches, they face challenges including sample preservation, DNA quality, and purification issues [44].

Conclusion

Chyme and the intestinal tract are the two most important aspects of animal digestion and absorption. While intestinal research is extensive, studies on how chyme status and characteristics affect nutrient utilization remain relatively limited. Currently, few indexes evaluate chyme characteristics, measurement methods vary, and indexes that can objectively reflect chyme properties require further exploration. Chyme formation and changes are influenced not only by feed composition but also significantly by the intestine itself. Scientific evaluation of chyme' s physicochemical and rheological properties represents a direction for animal nutrition and feed processing research, which will help identify

chyme characteristics optimal for nutrient absorption and provide theoretical foundations and scientific guidance for feed formulation, processing, ingredient selection, and additive application.

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Note: Figure translations are in progress. See original paper for figures.