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Evaluation of different minerals as mixing efficiency marker in commercial mixers

Raphaela Cenci Vidal
Doctor Scientiae

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Thesis submitted to the Animal Science Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

Adviser: Polyana Pizzi Rotta

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Assent:

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I dedicate this work to a dear friend whose presence I was blessed to share during a year of this journey and who forever changed the course of my life. Thank you, Tutu—your presence in my life gave it a deeper meaning.

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ABSTRACT

VIDAL, Raphaela Cenci, D.Sc., Universidade Federal de Viçosa, July, 2025. **Evaluation of different minerals as mixing efficiency marker in commercial mixers**. Adviser: Polyana Pizzi Rotta. Co-advisers: Alex Lopes da Silva and Edenio Detmann.

This thesis was divided into two products. Product 1 (Scientific article) - Performing a homogeneity test of the feed mixture is essential for quality assurance in feed mills. The aim of this study was to evaluate different minerals as test markers for the quality of feed mixtures in commercial mixers and to evaluate the sampling procedures for the standardization of homogeneity testing of mixtures in animal feed. Four horizontal, spiral-shaped, commercially available mixers were used for the test. Feed samples were taken from four different commercial mixers on five consecutive days to evaluate the quality of the mix. A total of 200 samples were taken during the entire test period (4 mixers × 5 days × 1 batch per day and mixer × 10 samples). Samples were taken sequentially during the mixer discharge time, with the total discharge time (4 minutes) divided into 10 equal 24-second intervals. All test portions were analyzed to determine the content of calcium (Ca), phosphorus (P), potassium (K), sodium (Na), magnesium (Mg), iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn). All statistical analyzes were performed using the GLIMMIX procedure of SAS 9.4 (a = 0.05) to check the variability of mineral markers for mixers, among batches and increments. Minerals K and Mg showed the lowest standardized total amplitudes and variation indices, which tended to zero, and are considered reliable for evaluating mix quality. In addition, a two-point sampling strategy was defined and proposed for animal feed. Product 2 (Book) – The aim of this literary work was to compile relevant data and make it available in the form of a book that will be published by Editora UFV under the title “The Fantastic World of Feed Mills”. The aim was to inform professionals involved in the operation of feed mills about the importance of feed quality and food safety. In addition, the book informs the reader about the importance of implementing Good Manufacturing Practices and other quality management tools, such as quality control of manufactured products, as well as the regulatory authorities and current legislation in Brazil.

Keywords: Feed mills; Food safety; Homogeneity; Legislation; Mineral markers

RESUMO

VIDAL, Raphaela Cenci, D.Sc., Universidade Federal de Viçosa, julho de 2025. **Avaliação de diferentes minerais como marcadores de eficiência de mistura em misturadores comerciais**. Orientadora: Polyana Pizzi Rotta. Coorientadores: Alex Lopes da Silva e Edenio Detmann.

Esta tese foi dividida em dois produtos. Produto 1 (Artigo científico) – A realização de um teste de homogeneidade da mistura de ração é essencial para a garantia da qualidade dos produtos nas fábricas de ração. O objetivo deste estudo foi avaliar diferentes minerais como marcadores de teste para a qualidade de misturas de ração em misturadores comerciais e avaliar os procedimentos de amostragem para a padronização do teste de homogeneidade de misturas em ração animal. Quatro misturadores comerciais, horizontais e helicoidais te foram utilizados. Amostras de ração foram coletadas dos quatro misturadores em cinco dias consecutivos para avaliar a qualidade da mistura. Um total de 200 amostras foram coletadas durante todo o período experimental (4 misturadores × 5 dias × 1 batida por dia e misturador × 10 amostras). As amostras foram coletadas consecutivamente durante o tempo de esvaziamento do misturador, levando em consideração o tempo total de esvaziamento (4 minutos) dividido em 10 intervalos iguais de 24 segundos. Todas as porções de teste foram analisadas para determinar a concentração de cálcio (Ca), fósforo (P), potássio (K), sódio (Na), magnésio (Mg), ferro (Fe), zinco (Zn), cobre (Cu) e manganês (Mn). Todas as análises estatísticas foram realizadas utilizando o procedimento GLIMMIX do SAS 9.4 (a = 0,05) para verificar a variabilidade dos marcadores minerais para misturadores, entre batidas e incrementos. Os minerais K e Mg apresentaram as menores amplitudes totais padronizadas e índices de variação, que tenderam a zero, sendo considerados confiáveis para a avaliação da qualidade da mistura. Além disso, uma estratégia de amostragem de dois pontos foi definida e proposta. Produto 2 (Livro) – O objetivo desta obra foi compilar e disponibilizar dados relevantes no formato de um livro a ser publicado na Editora UFV intitulado “O Fantástico Mundo das Fábricas de Ração”. Com o intuito de informar profissionais relacionados com o funcionamento de indústrias de alimentos para alimentação animal sobre a importância da qualidade de alimentos e segurança alimentar. Além disso, a obra instrui o leitor sobre a importância e necessidade da implementação das Boas Práticas de Fabricação (BPF) e outras ferramentas de gestão da qualidade como controle de qualidade dos produtos fabricados e as obrigatoriedades perante os órgãos fiscalizadores e à legislação vigente no Brasil.

Palavras-chave: Fábricas de ração; Homogeneidade; Marcador mineral; Legislação; Segurança alimentar

SUMMARY

GENERAL INTRODUCTION

The concept of formulated feeds, as understood today, emerged in the late 19th century, when the role of animal nutrition in agricultural production systems was first acknowledged. The industrial consolidation of feed manufacturing took place only after the identification and dissemination of the chemical composition of feed ingredients, along with the publication of the earliest works dedicated to animal nutrition (Coffey et al., 2016). Since then, diet formulation for livestock has emphasized the consistent provision of nutrients while minimizing variability in feed composition (Akintan et al., 2024).

In the livestock sector, production goals and feeding strategies adopted by producers may vary considerably, depending on management practices and the specific objectives of each farm. These variations directly affect the nutritional and physical quality of feeds, which depend not only on diet formulation but also on the feasibility of processing (Van der Poel et al., 2020). Consequently, the animal feed production chain is closely linked to the reutilization of plant by-products that are unsuitable for human consumption but, through appropriate technologies, can be converted into feed ingredients. These ingredients, in turn, contribute to the production of animal-derived foods for human consumption (meat, milk, and eggs) as well as for the pet food industry (Van der Poel et al., 2020; Akintan et al., 2024).

Given the direct connection between animal feed and the risks of contamination by pathogenic agents such as Salmonella, Listeria, Campylobacter, and Escherichia coli, the importance of ensuring the safety and quality of feed products becomes evident. Contamination with mycotoxins, heavy metals, dioxins, pesticides, among others, may be transferred to animal-derived products, thereby compromising both animal health and human consumer safety (Makkar, 2016; Đuragić et al., 2017).

The production of safe feed, particularly for food-producing animals, contributes to reducing operational costs while ensuring the quality of foods intended for human consumption (Baris, 2023). Moreover, contaminated feed often results in product recalls or disposal, generating considerable economic losses for companies in the sector (Makkar, 2016). As an integral part of the food chain, feed products for animal nutrition must comply with regulations and legislation comparable to those applied to human food production.

Currently, the feed industry has increasingly focused on enhancing the nutritional value of ingredients and final products, as well as on developing innovative technologies to improve production efficiency (Makkar, 2016). In this context, the accurate assessment of the nutritional

composition of feed ingredients is essential for achieving more effective formulations (Van der Poel et al., 2020).

Feed and supplement production requires the integrated expertise of specialists from multiple fields. The interaction between nutritionists and plant operators, for example, is critical to ensuring that the appropriate ingredients are selected and adequately processed according to the animals' nutritional requirements and the standards established by the producer, technician, or consultant (Van der Poel et al., 2020).

The production of these feeds involves methods and technologies related to formulation, transport, and storage, in addition to the implementation of quality control measures (Van der Poel et al., 2020). Therefore, investing in technological solutions for feed quality evaluation is essential to optimize nutrient utilization by animals (Akintan et al., 2024).

i) Formulation and Production of Animal Feed

Production lines in the animal feed industry are diverse, comprising different equipment, unit operations, production flowcharts, and processing systems (Axe, 1995; Van der Poel et al., 2020). A single feed mill may produce a wide variety of products, not only feeds for different animal species but also distinct products for the same species.

According to Normative Instruction (IN) No. 15 of 2009 from the Brazilian Ministry of Agriculture and Livestock (MAPA), the classification of products intended for animal nutrition is defined as the identification of the category to which the product belongs, which may include:

> *Art. 3º For the purposes of this Regulation, the following definitions apply:*
>
> ***I Classification of products intended for animal feed:*** *identification of the product category, which may be additive, feed, concentrate, ingredient, nucleus, premix, ration, supplement, and their variations, with possible indication of the species and animal category for which they are intended;*
>
> ***II. Vehicle or excipient:*** *an ingredient or substance added to another to facilitate dispersion, mixing, or dilution, without any nutritional or specific function within the product or in the animal;*
>
> ***III. Feed:*** *a mixture of ingredients intended exclusively for companion animals, constituting a ready-to-use product*

capable of meeting all or part of their nutritional requirements.

Art. 12. For the registration or manufacturing of feed products exempt from registration, the following classifications shall apply (as revised by Normative Instruction No. 42/2010/MAPA):

***I. Ingredient or raw material**: a component or constituent of any combination or mixture used in animal nutrition, with or without nutritional value, which may be of plant, animal, or mineral origin, in addition to other organic and inorganic substances;*

***II. Additive**: a substance, microorganism, or formulated product intentionally added, not normally used as an ingredient, with or without nutritional value, that improves the characteristics of feed products or animal-derived products, enhances the performance of healthy animals, meets nutritional requirements, or has anticoccidial effects;*

***III. Supplement**: a mixture of ingredients or additives, with or without vehicle or excipient, provided directly to animals to improve nutritional balance; in the case of mineral supplements for ruminants, these may also be intended for dilution;*

***IV. Premix**: a pre-mixture of additives and vehicles or excipients that facilitates dispersion in larger mixtures, and cannot be provided directly to animals;*

***V. Nucleus**: a pre-mixture composed of additives and macro-minerals, with or without vehicle or excipient, which facilitates dispersion in larger mixtures, and cannot be provided directly to animals;*

***VI. Concentrate**: a mixture of ingredients or additives which, when combined with other ingredients in appropriate proportions, constitutes a complete feed;*

***VII. Complete feed (ration)**: a mixture of ingredients and additives intended for production animals, constituting a*

ready-to-use product capable of meeting the nutritional requirements of the target species.

Regardless of the product or nomenclature adopted—since definitions used by regulatory authorities sometimes differ from those found in the scientific literature—the feed industry, whether producing complete feeds or intermediate products, must comply with the guidelines established by oversight agencies to ensure product quality and food safety.

Feed formulation has been defined as the combination of ingredients that meet the minimum requirements of animals to achieve baseline objectives or to improve productive performance (Saxena et al., 2012). The main goal of feed production is therefore to achieve an ideal composition so that, when provided to animals, it results in the expected performance outcomes. Formulations must be consistent, as the biological variability of ingredients must be accounted for to guarantee adequate and stable nutrient levels, regardless of fluctuations in ingredient chemical composition (Akintan et al., 2024).

While improving productivity and feed efficiency remains a major goal, consumer and societal demands must also be considered, as feed accounts for the largest cost factor in the production of animal-derived foods such as meat, milk, and eggs, and represents a critical link between crop production, animal rearing, and processing of animal protein (Hartog et al., 2013).

According to data from the Organisation for Economic Co-operation and Development and the Food and Agriculture Organization of the United Nations (OECD/FAO), global per capita meat consumption, which averaged 28.8 kg between 2020 and 2022, is projected to reach 29.5 kg by 2032. In the case of dairy products, an annual average growth rate of 0.8% is expected, reaching 15.7 kg per person. To meet this growing demand, world meat production is projected to reach 382 million tons by 2032, representing an increase of 41 million tons. Within this scenario, animal feed will continue to be one of the main destinations for global grain production, representing approximately 37% of total projected use by 2032 (OECD/FAO, 2023).

Thus, the formulation of balanced feeds is a key requirement to meet animal nutritional demands while adapting to the constant changes in global market demands (Uyeh et al., 2018). To provide rations that meet animal requirements, it is necessary to use ingredients containing nutrient levels aligned with productive potential (Van der Poel et al., 2020). Consequently, supplementation through products specifically formulated for species and categories plays a crucial role in livestock production, ensuring that animals receive adequate nutrition for their requirements at different production stages (Akintan et al., 2024), as well as for companion animals.

ii) Nutrition and Animal Welfare

Nutrition plays a decisive role in animal health, performance, and welfare, directly influencing growth, reproduction, and immune responses (Baris, 2023). Animals fed diets formulated according to their nutritional requirements develop stronger immune systems, making them less vulnerable to diseases and, consequently, less dependent on pharmacological treatments (Akintan et al., 2024). This reduced reliance on medication, in turn, contributes to lowering antimicrobial resistance and minimizing drug residues in animal-derived foods.

Beyond directly affecting animal health, the nutritional composition of feeds also impacts the quality of products consumed by humans, such as milk, meat, and eggs. Thus, proper feed formulation is a key element not only in ensuring animal welfare but also in safeguarding the quality and safety of food of animal origin for human consumption (FAO, 2010).

Another important aspect is that the balanced supply of nutrients is directly linked to compliance with internationally recognized animal welfare principles, defined as the Five Freedoms by the United Kingdom Farm Animal Welfare Council (FAWC): freedom from hunger and thirst; freedom from discomfort; freedom from pain, injury, or disease; freedom to express normal behavior; and freedom from fear and distress. In ruminants, when diets are designed solely to increase productivity and reduce costs, nutrient deficiencies or excesses may lead to metabolic disorders and welfare-related health issues (Makkar, 2016).

Therefore, any nutritional imbalance—whether due to deficiency or excess—can compromise at least one of the pillars of animal welfare, such as the right to be free from hunger (FAO, 2010).

In general, practices that promote animal welfare yield benefits such as lower disease incidence, reduced mortality rates, and increased productivity, generating positive impacts on production efficiency and operational cost reduction (FAO, 2010).

Within this context, feed and feed supplements occupy a strategic position in the global food supply chain, contributing to the sustainable and safe provision of animal-derived foods. In many agricultural systems, whether industrial or small-scale, commercially manufactured or locally produced feeds and supplements represent the primary input in raising animals for diverse purposes—including the pet food sector (FAO, 2020).

The growing complexity of production systems and increasing consumer demands have driven governments and regulatory bodies to implement stricter monitoring and control

policies. This has led to a restructuring of production standards and heightened requirements for food traceability and safety (Hartog et al., 2013). These changes also reflect shifts in consumer preferences, as people are increasingly attentive to the quality, origin, and impacts of the foods they consume.

In this competitive environment, where consumers associate quality with cost-effectiveness and product safety (Paladini et al., 2006), the animal feed industry faces the challenge of maintaining quality standards while under pressure to reduce costs. In a constantly evolving setting, adaptability and innovation become essential differentiating factors for the sector's long-term sustainability (Castro Marino, 2006).

iii) Quality Assurance

Ensuring product quality throughout the production process is an essential strategy to reduce unwanted variability, minimize failures, and strengthen food safety (Montgomery, 2004). Standardization of processes, combined with effective communication across different links in the production chain, facilitates risk identification and supports more informed decision-making. The more structured this communication process is, the better the decisions regarding risk management and loss reduction will be.

The concept of quality is associated with desirable attributes that consumers or users expect to find in a product or service. For this reason, quality plays a decisive role in choosing between competing products, regardless of the type of consumer—whether an individual, a company, a farmer, or a commercial establishment (Montgomery, 2004).

As a concept, "quality" may vary depending on the perspective of each social group, and is therefore a subjective construct. From this perspective, quality is more closely linked to people's perceptions than to the intrinsic properties of products (Aluddin et al., 2019). This implies that customer satisfaction is the true driver of quality, requiring companies to be prepared to adapt to changing market expectations.

Understanding, monitoring, and improving quality thus becomes a determining factor for business growth and competitiveness. This requires a thorough understanding of the entire production chain, its components, and its variables, in order to adopt effective methods that ensure product consistency and performance. As Montgomery (2004) emphasized, quality and variability are inversely proportional—the lower the variability in the process, the higher the quality and the lower the costs involved.

The quality cost approach, in turn, reveals that investments made in preventive and assessment stages generate significant financial benefits. For every dollar allocated to prevention, it is estimated that savings of between 10 and 100 dollars—or more—can be achieved in internal and external failure costs (Montgomery, 2004). Moreover, resources directed toward prevention tend to be more effective than those aimed at problem detection, as they prevent hidden failures from manifesting after the production process is completed. Thus, practices such as detailed planning, problem anticipation, and actions focused on "getting it right the first time" have a direct impact on reducing waste and rework.

To ensure quality systematically, companies must define clear strategies, adopt effective controls throughout production, and conduct periodic internal audits. The objective is to make processes stable and predictable, which requires reducing variability through tools adapted to the industry's reality.

Currently, a wide range of instruments are available to create and maintain a Quality Management System (QMS), in accordance with the guidelines established by ISO 9000, 9001, and 31000 standards, as well as food-specific regulations. In the context of animal feed production, the most widely used tool—also required by regulatory agencies in Brazil—is the set of Good Manufacturing Practices (GMP), which serves as the foundation for self-monitoring in industrial plants.

iv) Good Manufacturing Practices (GMP)

Good Manufacturing Practices (GMP) comprise a set of standardized rules, procedures, and routines designed to ensure the safety of feed intended for animal nutrition. These practices apply to the entire production chain, from the receipt of raw materials to the delivery of the final product, including storage, processing, and distribution stages.

The primary objective of GMP is to prevent and control potential sources of contamination—such as physical, chemical, and biological hazards—that may compromise feed quality and animal health. Cross-contamination, for example, is one of the most recurrent risks in feed production and must therefore be strictly controlled (e.g., the use of ingredients such as urea and ionophores in ruminant formulations may cause fatalities in non-ruminant animals if cross-contamination occurs). Moreover, GMP serve as prerequisites for the implementation of more robust safety systems, such as Hazard Analysis and Critical Control Points (HACCP), which is required in several countries as an international sanitary control standard (FAO, 2020).

According to Decree No. 12.031/2024 from the Brazilian Ministry of Agriculture and Livestock (MAPA), GMP are defined as “systematized hygienic-sanitary and operational conditions and procedures applied throughout the production flow, with the aim of ensuring the safety, identity, quality, and reliability of products.” This regulation establishes that establishments manufacturing animal feed must be registered with the regulatory authority and implement GMP as part of their self-monitoring programs.

The decree further defines self-monitoring programs as “procedures described, implemented, maintained, monitored, and verified by the establishment, aimed at ensuring the safety, identity, quality, and reliability of its products.” Thus, in addition to complying with legal requirements, companies must maintain updated technical documentation and conduct their activities in accordance with pre-established parameters.

The effective implementation of GMP and self-monitoring programs is essential not only for regulatory compliance but also to guarantee the quality and safety of feed products, regardless of the target market—domestic, international, or production-oriented.

v) Feed Mixing Quality

In the context of implementing Good Manufacturing Practices (GMP) and self-monitoring programs to ensure product quality, one of the requirements established by regulatory authorities is the mixing quality of the manufactured product, whether intended for domestic or international markets, the pet food sector, or livestock production.

Animal feed industries are therefore required to conduct mixing quality tests, also known as homogeneity tests, to ensure that animals consume the specified levels of nutrients regardless of the portion ingested (Rocha et al., 2015). The mixing stage is one of the most critical and essential steps in feed manufacturing (Abo-Habaga et al., 2017), as nutrient concentrations and other feed components are expected to be similar in any portion or sample collected from a batch (Axe, 1995). Mixing is one of the most commonly employed operations in feed production, where homogeneity is expected to be achieved through the dispersion of two or more ingredients (Premi et al., 2022).

Thus, there is a complex relationship between product quality characteristics and mixing patterns, which are directly influenced by the choice of mixer. Mixers play a significant role in determining mixing quality in terms of mixing time, product yield, and operational costs (Premi et al., 2022).

However, perfect mixing cannot be achieved, since mixing is essentially the rearrangement of two or more ingredients under kinetic energy, which varies depending on the

equipment used. Furthermore, mixing is subject to ingredient segregation caused by differences in particle size, density, shape, moisture, and electrostatic charges (Axe, 1995; Fahrenholz, 2019; Sindirações, 2023).

The goal of the mixing stage is therefore to produce a product in which the probability of finding a particle of a given substance is equal at any point within the mixture (Axe, 1995). Ensuring uniform nutrient concentrations across portions is essential, since insufficient homogeneity in a batch not only leads to economic losses for the industry but also results in undesired animal performance, financial losses for producers, and/or nutritional imbalances that may trigger health disorders in different animal categories (Sindirações, 2023).

Another important factor influencing mixing quality is the sequence of ingredient inclusion and their pre-processing. Ingredients incorporated at very low inclusion rates, such as vitamins or additives—typically the most expensive components—are especially vulnerable to losses. Due to their small amounts, they may be lost before mixing through suspension in the air, or during mixing due to electrostatic charges, causing them to adhere to the walls of the mixer (Axe, 1995). Additionally, if these are added before bulk ingredients, they may become trapped in dead spots at the bottom of the mixer (Fahrenholz, 2019), thereby compromising the final formulation.

According to Van der Poel et al. (2020), processing conditions such as grinding, extrusion, and steam treatment can significantly affect the nutritional value of feeds. This highlights the importance of understanding interactions between prior ingredient processing and mixing processes, for example, the toasting of soybeans for feed production.

Knowledge of the precise effects of processing on the inclusion levels of specific ingredients will allow more accurate feed formulations (Van der Poel et al., 2020), ensuring that animals consume diets that are both safe and appropriate for their species and production stage.

Conventional feed production techniques, although historically significant and still widely used in the global market, present limitations when it comes to ensuring feeds that are safe and nutritionally adequate for each animal species and category. According to Akintan et al. (2024), feeds available on the market rely on fixed ingredient proportions, which may lead to nutritional imbalances if variability arises at the time of ingredient procurement. Many conventional formulation methods overlook the dynamic nature of ingredient prices and availability throughout the year, potentially resulting in cost inefficiencies.

Furthermore, conventional feed formulation and production techniques may fail to meet the specific dietary requirements of different species, breeds, or life stages. The lack of precision in these methods may also contribute to environmental concerns, such as nutrient excretion

through feces and urine resulting from excessive supplementation (Akintan et al., 2024), while occasionally failing to meet the nutritional demands of the animals.

Overall, feed production processes individual ingredients or ingredient mixtures to improve nutrient availability in a physical form that meets animal requirements. However, the extent to which macro- and micronutrients are available depends on the processing parameters, production methods, and equipment employed (Van der Poel et al., 2020). Several production stages (e.g., grinding, mixing, thermal processing) can influence the final nutrient availability in feeds (Dimaiwat et al., 2018).

Thus, animal feeds may be considered mixtures of ingredients, some of which readily expose their nutrients for absorption at specific sites, while others require balancing between processing demands, animal nutritional requirements, and production costs to be incorporated into formulations (Makkar, 2016; Van der Poel et al., 2020). Certain components, such as vitamins, are included at very low levels (e.g., µg to mg/kg) but contribute substantially to total diet costs and are essential nutritional cofactors in all biological functions (Van der Poel et al., 2020). Their depletion may therefore lead to severe effects on animal health and performance.

Ensuring proper feed mixing ultimately means guaranteeing a quality product for consumers, one that meets animal requirements as long as the formulation is properly designed for the target species and production stage. To achieve this, industries must comply with current regulations and periodically perform mixing homogeneity tests as part of their self-monitoring programs, thereby ensuring a safe product for consumers.

vi) Mixing Homogeneity Tests

Mixing homogeneity tests assist in evaluating and demonstrating the uniform distribution of ingredients throughout a feed batch. From a nutritional standpoint, they ensure that animals receive the nutrients specified in the formulation, thereby preventing both nutrient overexposure and deficiencies. The mechanism for verifying feed homogeneity essentially involves statistically assessing the distribution of a given component in relation to the formulated mean, quantifying its variation, and comparing it with standards established by regulatory agencies and/or predetermined scientific references.

With the continuous rise in ingredient costs, strict monitoring of nutritional balance—commonly referred to as precision feeding—has become increasingly essential (James et al., 2008).

To conduct homogeneity tests, feed manufacturers must establish a method, preferably supported by scientific data, that employs a reliable marker and appropriate sampling procedures. This ensures that samples are representative and that the marker is not influenced during mixing and/or by the analytical method (Sindirações, 2023). Because different markers may be used to evaluate mixing homogeneity, critical analysis is required when selecting them, taking into account ease of use, cost, and analytical accuracy. It is also important to determine whether the marker originates from a single ingredient in the mixture or from a common component.

According to Clark (2007), several criteria should guide the choice of a marker: (i) select ingredients with similar physical properties, such as density and particle size; (ii) avoid nutrients that are common across several ingredients; (iii) do not select markers whose variation in the mixture will not influence animal performance; and (iv) although minerals can serve as useful markers due to their physical properties, their analytical costs must be considered, as some may be economically disadvantageous.

Given the various factors that may affect mixing quality, the definition of a reliable marker—one that remains stable regardless of processing variations (e.g., equipment differences, ingredient composition variability, or labor-related factors)—is a crucial step in conducting homogeneity tests.

Mixing uniformity analysis allows inference about mixer performance and helps determine minimum and maximum mixing times. Typically, this analysis is based on the concentration of an indicator (Fahrenholz, 2019). Indicators are often low-inclusion ingredients, such as amino acids, microminerals, microtracers (colored iron particles), or antimicrobials (Clark et al., 2007). For an indicator to be effective, its analysis must be practical, precise, and economical, and it should be used at its normal inclusion level within the formulation (Fahrenholz, 2019). According to Eisenberg (2004), inclusion levels of up to 100 g/ton are recommended, and the concentrations measured can then be extrapolated to other ingredients in the feed to estimate mixing efficiency (Rocha et al., 2015).

Minerals have been proposed as reliable nutritional markers for verifying feed homogeneity due to their physical and chemical properties (Behnke, 1996; Carneiro et al., 2021). In Brazil, the regulatory authority commonly recommends testing homogeneity based on the coefficient of variation (CV) of zinc and/or manganese concentrations in collected samples. However, the literature also points to the use of other minerals.

Salt (NaCl) is a commonly included component in feed formulations and is the most widely used indicator for mixing efficiency assessment, as its analysis is simple and relatively

inexpensive (Çiftci et al., 2003; Dimaiwat et al., 2018). Accordingly, both Na and Cl can be used as indicators (Çiftci et al., 2003). Conversely, Rocha et al. (2015) do not recommend the use of mineral-containing molecules such as Mn, Cu, Zn, and Cl for assessing mixing efficiency, as these minerals are also present in several feed ingredients, potentially leading to misinterpretation. In such cases, the authors suggest the use of specific amino acids to evaluate efficiency in dry and wet feeds. However, the use of amino acids as markers is limited, since their analyses are often costly and less accessible.

Therefore, quality control methods are essential for evaluating particle dispersion throughout the production process. Mixing efficiency is assessed by comparing the variation in composition across different samples with that of an ideal mixture (Weiss et al., 2024).

Homogeneity tests conducted as part of quality control programs in feed manufacturing assess the effectiveness of mixing by verifying particle dispersion uniformity within a mixing cycle and consistency across successive cycles (Cleary et al., 2008). For statistical analysis of these data, the Pearson CV is the most commonly used indicator, as it is a standard tool for analyzing relative dispersion across different distributions.

Mixing process evaluation can also be performed by measuring nutrient levels or other chemical components in multiple feed samples (Kuo et al., 1986). Thus, the use of markers aims to ensure homogeneity in ingredient dispersion, taking into account their physical properties (Weiss et al., 2024). In non-ruminant feed manufacturing, for instance, manganese (Mn) is often used as a marker for mixing quality evaluation, where a CV below 10% indicates effective mixing (Behnke, 1996; Johnston et al., 2000). Groesbeck et al. (2004) reported that CV values below 10% indicate excellent homogeneity, values between 10–15% are considered acceptable, and values above 20% indicate non-homogeneous mixing, requiring reevaluation of the process or equipment. According to Ordinance No. 798/2023 of MAPA, the concentration of the evaluated substance is considered acceptable if the CV is ≤10% when medication is included in the formulation.

Given the importance of the feed industry and the need to ensure the quality of animal diets, standardization of indicators, CV thresholds, and evaluation processes is imperative. However, the available literature created above presents diverse and sometimes conflicting results regarding the most appropriate markers for assessing homogeneity, as some (e.g., minerals) are easier and less costly to analyze, while others (e.g., amino acids) may provide more precise results but are less accessible due to high analytical costs. Furthermore, confounding effects may occur when markers are part of the chemical composition of ingredients used in the formulation.

Therefore, studies aimed at standardizing accessible, reliable, and accurate markers for mixing evaluation are necessary to ensure food safety and animal welfare, while preventing contamination of animal-derived products destined for human consumption.

Accordingly, the objectives of this thesis were: (i) to evaluate different minerals as indicators of mixing quality in commercial mixers, verifying their stability across different equipment, batch cycles, and sample collections; (ii) to standardize sampling and evaluation procedures for mixing homogeneity, and; (iii) to compile and provide relevant data in a reference book to assist the animal feed industry in complying with current Brazilian legislation and ensuring the delivery of safe and high-quality products to consumers.

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Product 1 (Paper): ***Evaluation of Different Minerals as Mixing Efficiency Marker in Commercial Mixers***
Submitted to Animal Feed Science and Technology

Highlights

- Standard protocol is needed to assess feed mixing homogeneity in feed mills
- New sampling protocol: two increments at second and eight tenths of mixer emptying time.
- Homogeneity achieved when max/min ratio of marker content is < 1.26.

Evaluation of different minerals as mixing efficiency markers for concentrate rations

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Abstract

Carrying out a homogeneity test of the feed mixture is essential for quality assurance in feed mills. We aimed to evaluate different minerals as markers for the quality of concentrate ration mixtures in commercial mixers and to assess sampling procedures to support the standardization of the homogeneity test of mixtures in animal feed. Four horizontal, spiral-shaped, commercially available mixers were used in the experiment. Increments were taken sequentially during the mixers emptying time on five consecutive days, totalling 200 increments (4 mixers × 5 days × 1 batch per day and mixer × 10 samples). We evaluated nine potential markers: calcium (Ca), phosphorus (P), potassium (K), sodium (Na), magnesium (Mg), iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn). The minerals K and Mg showed simultaneously low variabilities among batches, among mixers, and among increments, being considered reliable markers for evaluating mix quality. In addition, a two-point sampling protocol was proposed along with its statistical support. In summary, the proposed protocol uses K or Mg as markers for concentrate ration-mixing efficiency. Two increments per ration batch are used to evaluate marker contents in the ration mixture, which are taken at second and eight tenths of the mixer emptying time. For the ration mixing operation to be considered efficient, the ratio between the highest and lowest marker contents in the increments must be lower than 1.26. Otherwise, the concentrate ration cannot be considered properly mixed and either the mixing process or the equipment should be re-evaluated.

Keywords: feed mill, homogeneity test, magnesium, potassium, ration sampling protocol

1. Introduction

Feed manufacturers should use standardized techniques and tests to assess the quality of their products. Mixture homogeneity tests verify that each feed or ration fraction ingested by the animal contains the amount of nutrients indicated on the product label (Rocha et al., 2015). The mixing phase is crucial in the production of rations (Abo-Habaga et al., 2017), since it is expected that the concentration of nutrients and other substances in each aliquot from the same batch should be similar (Axe, 1995). However, perfect mixing is unattainable due to factors such as the order of ingredient addition in the mixer, ingredient moisture, particle size, and density, as well as electrostatic charges, which are potential causes of segregation (Axe, 1995; Fahrenholz, 2019; SINDIRAÇÕES, 2023).

The homogeneity of a concentrate ration mixture is generally assessed by the dispersion of a marker, normally evaluated using Pearson's coefficient of variation (CV; Premi and Sharma, 2022). As broadly recommended, an effective mixing process should exhibit low CV values, indicating good homogeneity and uniformity within or between batches (Cholette and Cloutier, 1959; Fahrenholz, 2019). In such cases, the CV of the marker distribution among increments taken within a ration batch should be lower than 10%, which would indicate an effective mixture of the ration ingredients (Behnke, 1996; Johnston and Southern, 2000). When the CV value falls between 10 and 15%, the mixing process would be considered acceptable. A CV above 20% would indicate an inhomogeneous mixing process, suggesting that either the process or the equipment should be re-evaluated (Groesbeck et al., 2004). However, those overall CV ranges seem rather subjective, as they do not take into account the influence of marker characteristics, sampling procedures, or the ration intrinsic properties.

The markers used are generally ingredients with a low content in the mixture, such as amino acids, minerals, micro tracers (e.g., coloured iron particles), or antimicrobials (Clark et al., 2007). Even though specific amino acids are recommended as markers, their analysis is expensive and less accessible to commercial feed mills (Fahrenholz, 2019). Minerals are commonly used in feed formulations and are the most frequent markers to evaluate mixing efficiency, as their analyses are relatively simple and inexpensive (Çiftci and Ercan, 2003; Dimaiwat et al., 2018). However, some minerals may occur in high concentrations in feeds, which may compromise their effectiveness as markers of ration mixing (Rocha et al., 2015) and the effectiveness of mixture diagnostics.

A crucial aspect of evaluating the effectiveness of a ration mixing process is the sampling protocol (ICCF, 2020). Taking adequate increments and primary samples assures the

representativeness of the analytical sample (AAFCO, 2015), which provides information regarding marker concentration. Currently, a sampling protocol based on ten increments per ration batch is commonly used, although this approach lacks strong scientific support.

To perform the homogeneity test, feed mills should use a valid method with a scientific background, a stable and effective marker, and a rigorous sampling protocol (SINDIRAÇÕES, 2023). Frequently, homogeneity tests in commercial feed mills in Brazil are performed based on the CV of zinc (Zn) and, or manganese (Mn) concentrations. However, to our knowledge, there is still no data in the literature identifying which markers are most efficient for assessing ration mixing homogeneity. Therefore, further research is needed to develop methods and standardize processes that may assure a robust evaluation of repeatability (i.e., within the same mixer) and reproducibility (i.e., between mixers and, or feed mills).

Studies focusing on the standardization of methods to evaluate the effectiveness of the ration mixing process are needed to indicate markers with more accessible analyses and sensitivity to detect mixing effectiveness, as well as sampling protocols capable of detecting failures in the mixing process. Therefore, our objective was to evaluate different minerals as markers for concentrate ration mixing effectiveness and propose a ration sampling protocol for feed mills.

2. Material and methods

The experiment was conducted in a commercial feed mill in Luziânia, Goiás, Brazil. No ethics committee approval was required, as the study involved only the analysis of animal feed under routine factory conditions, with no interference in operations. Furthermore, employees were not instructed to change or modify their daily management practices.

2.1. Equipment, mixing procedures, and sampling

Four horizontal helical mixers were used: two with a capacity of 1,000 kg (Wadin, MetaGril, Goiatuba, Goiás, Brazil) operating at 32 revolutions per minute (rpm), and two with a capacity of 800 kg (Discometal, Ind. Com. Maq. e Metais Perfurador, Goiânia, Goiás, Brazil) operating at 30 rpm. To evaluate the mixing quality, a standard concentrate ration for lactating dairy cows was produced in each mixer for five consecutive days. The ration was composed of ground maize, soybean meal, limestone, urea, sodium chloride, dicalcium phosphate, and a mineral-vitamin mix, formulated to contain 240 g of crude protein/kg as-fed.

The ingredients were weighed and placed in the mixer according to their inclusion rate. Major ingredients were added first, followed by minor ones. Mixing time was set to 4 minutes for all mixers (Figure 1). All mixers were operated strictly according to the manufacturers' instructions to ensure an optimised mixing process.

For all mixers, sampling began at the time of packaging. All study materials were collected directly from the storage silo after the 4-minute mixing time. The emptying time of the silo was estimated for each mixer and then divided into ten equal intervals (i.e., a total emptying time of 4 minutes resulted in ten 24-second intervals). Hereafter, these intervals are referred to as tenths of the mixer emptying time.

An increment was defined as the individual portion of material collected by a single sampling operation from a decision unit (i.e., ration batch; AAFCO, 2015). Accordingly, beginning with the start of emptying, an increment of the concentrate mixture was taken at each tenth of the emptying time directly from the outlet of the filling silo, totalling ten increments per batch. Each increment weighed approximately 200 g and was individually packed in a clean plastic bag and sealed (Figure 1). At the ending of the sampling procedures, a total of 200 increments were taken (4 mixers × 5 days × 1 batch per day and mixer × 10 increments).

2.2. Laboratory analysis

After the trial period, all increments were sent to the laboratory for mineral analysis to evaluate potential markers of mixing efficiency and homogeneity. Nine potential minerals were quantified: calcium (Ca), phosphorus (P), potassium (K), sodium (Na), magnesium (Mg), iron (Fe), Zn, copper (Cu), and Mn. Analyses followed the methods described by Sarruge and Haag (1974) and Detmann et al. (2025). Mineral contents were expressed on an as-fed basis to avoid bias introduced by additional dry matter quantification.

2.3. Statistical analysis

Initially, results of each mineral marker were subjected to an analysis of variance according to the model:

$$Y_{ijk} = \mu + M_i + B_{(i)j} + S_k + \varepsilon_{ijk} \quad (1),$$

where Y_{ijk} is the mineral content in the increment taken from batch j in mixer i at time k (i.e., k-tenth of the mixer emptying time); μ is the general constant (fixed effect); M_i is the random effect of mixer i, assumed to be NIID (0, σ^2_M); $B_{(i)j}$ is the random effect of batch j nested within the mixer i, assumed to be NIID (0, $\sigma^2_{B/M}$); S_k is the fixed effect of the increment taken at k-tenth of the mixer emptying time; and ε_{ijk} is the random error, assumed to be NIID (0, σ^2_ε).

The increments were considered as repeated measurements during the mixer emptying process. Therefore, the residual (co)variance matrix was modelled assuming different variances for each time and covariances between times [i.e., an unstructured (co)variance matrix], allowing the assessment of variability of the markers along the mixer emptying process. All variance components were estimated using the restricted maximum likelihood method (Littell et al., 2006).

The variances obtained from the statistical model (Equation 1) were standardized relative to the average marker content across all sampling times as follows:

$$RSD_M = \frac{\sqrt{\hat{\sigma}_M^2}}{\hat{\mu}} \times 100 \quad (2),$$

$$RSD_B = \frac{\sqrt{\hat{\sigma}_{B/M}^2}}{\hat{\mu}} \times 100 \quad (3),$$

$$RSD_{Ik} = \frac{\sqrt{\hat{\sigma}_{\varepsilon_k}^2}}{\hat{\mu}} \times 100 \quad (4),$$

where RSD_M and RSD_B are the relative and standardized standard deviations between mixers and batches, respectively (%); RSD_{Ik} is the relative and standardized standard deviation between increments taken at the k-tenth of the mixer emptying time (%); $\hat{\mu}$ is the average estimate of mineral marker content; and $\hat{\sigma}_M^2$, $\hat{\sigma}_{B/M}^2$, and $\hat{\sigma}_{\varepsilon_k}^2$ are the estimates of their respective variance components.

The maximum range of mineral content was estimated as:

$$R_{max} = \max(\hat{\mu}_k) - \min(\hat{\mu}_k) \quad (5),$$

where R_{max} is the maximum range of the mineral or marker content, and $\hat{\mu}_k$ is the average content of the marker at the k-tenth of the mixer emptying time.

The statistical significance of the R_{max} was assessed using the total studentized range considering a null hypothesis based on a parametric value of 0 and a two-tailed alternative hypothesis ($\alpha = 0.05$).

The estimated R_{max} was then standardized as:

$$RR_{max} = \frac{R_{max}}{\hat{\mu}} \times 100 \quad (6),$$

where RR_{max} is the relative or standardized R_{max} (%).

To enable graphical comparison among mineral markers, their contents at different tenths of the mixer emptying time were standardized so that all minerals had the same overall mean (i.e., 100) Standard errors of the means at each time point were also expressed as a percentage, as follows:

$$RMV_k = \frac{\hat{\mu}_k}{\hat{\mu}} \times 100 \quad (7),$$

$$VI_k = \pm \frac{s_{\hat{\mu}_k}}{\hat{\mu}_k} \times 100 \quad (8),$$

where RMV_k is the relative or standardized mean content at the k-tenth of the mixer emptying time (%), VI_k is the variation index or relative standard error of the mean content at the k-tenth of the mixer emptying time (%), $\hat{\mu}$ is the overall mean mineral marker content, $\hat{\mu}_k$ is the mean mineral marker content at the k-tenth of the mixer emptying time, and $s_{\hat{\mu}_k}$ is the standard error of $\hat{\mu}_k$.

All statistical analyses were performed using the GLIMMIX procedure of SAS 9.4 (α = 0.05).

3. Results and discussion

The results obtained in our study are based on a set of assumptions necessary for their correct interpretation. First, we assumed that the entire mixing process was carried out optimally. Moreover, we adopted the assumption that the final concentrate ration mixture can be considered an infinite element material. Thus, the ration mixture would consist of a practically infinite number of indiscernible elements that cannot be individually identified (AAFCO, 2015). From this, the ration mixture is interpreted as a continuous and homogeneous matrix or medium (i.e., with minimal distributional and compositional heterogeneities). In contrast, the different mineral markers would theoretically be considered as a finite element material (AAFCO, 2015), consisting of individually identifiable elements whose distribution within the ration mixture would be discernible by an adequate analytical method. Therefore, the markers' concentration in the increments could conceptually be interpreted as the frequency of occurrence of these finite elements per unit of mass of a continuous medium formed by infinite elements. Based on these assumptions, the ration mixture is homogeneous, and any deviation from homogeneity indicated by a marker would reflect its intrinsic limitation to be adequately diluted in the matrix (i.e., to show a homogenous frequency of occurrence). Therefore, all results presented in this study and their respective interpretations are based on the aforementioned assumptions.

Overall, the mineral markers exhibited different patterns of variability between mixers and batches (Table 1). Considering the previously presented assumptions, a high variability among mixers and batches would indicate limitations of the marker for evaluating the reproducibility and repeatability of the mixing process, respectively. In this sense, it is reasonable to expect

that an ideal marker should indicate estimates of variability tending to zero for a suitable mixing process. In the case of inter-mixer variability (i.e., an approach to the mixing process reproducibility), near-zero variability was observed for P, K, Mg, Cu, and Mn. In contrast, high variability was observed for Na, Zn, Fe, and Ca. Furthermore, a value tending towards zero for an ideal marker is expected to indicate inter-batch variability within the same mixer, which could allow a direct association with the repeatability of the mixing process. In this case, low or close to zero variability was observed for Na, P, K, Mg, and Mn.

In addition to the variability between mixers and batches, the importance of a low variation between increments taken under similar sampling conditions is also emphasized. Assuming that the medium (i.e., the ration matrix) is continuous and homogenous, as previously highlighted, a high variability in mineral marker content between increments taken under the same conditions could indicate limitations in the dispersibility of the marker in the ration mixture, which could lead to a false diagnosis of a non-homogenous mixture. In our study, the sampler and sampling procedures were the same for all mixers and batches. This means they were performed by the same sampler and sampling device and were compared within the same sampling moment (Table 1). In this sense, the lowest variability between increments was observed for K and Mn.

Therefore, an intersection of the results described above can be made based on the three evaluated dimensions (i.e., between mixers, between batches, and between increments). Under this perspective, only K and Mg fulfilled all desired characteristics.

In our study, the measures of location of the mineral marker contents were used as a complementary information to the measures of variability. In general, none of the total ranges of the mineral marker contents differed from zero ($P>0.08$, Table 2, Figures 2 to 4). At first glance, this result would indicate that maximum oscillation among the contents evaluated at the different tenths of mixer emptying time is null, which would lead us to conclude that all markers indicate a homogeneous mixing process. However, this pattern does not suggest that the marker choice should be discretionary. On the contrary, it gives greater weight to variability measures for the sampler's decision-making. If the mineral markers are similarly accurate in indicating homogeneity, the choice should rely on the one that behaves more precisely (see the previous presented assumptions). In this sense, and in line with the statements above, the lowest total range and variabilities were observed for K (Figure 2c) and Mg (Figure 3b). Despite the narrow total range, P is not recommended due to the high variability between increments compared to the two mineral markers mentioned above (Table 1).

To provide an adequate diagnosis of ration-mixing efficiency, a marker must indicate both the accuracy and precision of the process and have a feasible and inexpensive analytical method (Fahrenholz, 2019). Moreover, it should present physical properties comparable to those of the other feed components so it can disperse easily in the medium (Paiano et al., 2014; ICCF, 2020). In theory, markers for mixing efficiency may be internal, when intrinsic to the ration feed components, or external, when not naturally present. However, when evaluating ration mixing efficiency, a marker can also be simultaneously internal and external. This hybrid classification applies to minerals that are naturally present in feeds, usually in low concentrations, but also added through mineral premixes. That is the case of Mg, suggested as one of the two potential markers in this study. Magnesium is predominantly external (i.e., most Mg comes from mineral premix), but also internal, as feeds contain Mg naturally. On the other hand, K tends to be only an internal marker, as it is abundant in plant-based feeds, such as maize grain and soybean meal (Clover and Mallarino, 2013; NASEM, 2021; Valadares Filho et al., 2024) and is not commonly added via premixes.

When a marker is predominantly internal to the feed components, another relevant characteristic must be highlighted: its contents across feed ingredients should be as different as possible. An internal marker whose contents across the ration feed components are similar is not capable to diagnostic the mixing efficiency. In this case, regardless of mixing degree, the final content tends to be similar, providing insufficient sensitivity to detect any deviation from an ideal mixing. Considering this, markers with predominantly external characteristics seem to be slightly more reliable, as their contribution on the final ration derives from mineral premixes. Therefore, Mg seems more advantageous than K for most commercial concentrate rations. However, in our case, using K posed no constraint, as its average content differ substantially among the ingredients used (maize grain, 3.5±0.5 g/kg dry matter; soybean meal, 20.2±1.0 g/kg dry matter; Valadares Filho et al., 2024).

A common recommendation for a ration mixing-efficiency test is to take at least 10 representative and independent samples (i.e., increments) from each ration batch, where the marker content is individually evaluated using an appropriate analytical method (ICCF, 2020). However, it seems that literature is not totally clear regarding a standard sampling procedure to instruct the sampler on how increments should be collected. Moreover, when considering a set of increments within a ration batch, the CV of the marker content among increments has been widely used to diagnose the adequacy of the ration-mixing process. However, the CV's interpretation is partially subjective, as it depends on the marker, analytical uncertainty, and the chemical and physical properties of the feed ingredients (ICCF, 2020). This means that any

adopted CV range (Behnke, 1996; Johnston and Southern, 2000; Groesbeck et al., 2004) can be seem as discretionary and partially subjective. Nevertheless, a robust protocol to evaluate ration mixing efficiency should be as objective as possible to ensure reproducibility across samplers, feasibility under different industrial conditions, and its utilisation as a reliable tool for comparisons among batches, mixers, and feed mills.

Under the assumptions presented at the beginning of this section, and considering that the recommended markers (i.e., K and Mg) presented a stable pattern along the mixer's emptying time (Figures 2 and 3), it seems that 10 increments are not necessary to diagnose mixing efficiency. Form this, we propose here an objective protocol to evaluate the ration mixing efficiency based on two increments taken independently during the mixer emptying operation. Our main objective is to produce an evaluation protocol that has scientific background, and maximum objectivity and reproducibility.

Our method is based on two simple assumptions: 1. the marker used is suitable for representing mixing efficiency, and 2. the two increments to be evaluated are independent and taken at different and appropriate times during the mixer emptying.

Consider X_1 and X_2 as the marker contents in increments 1 and 2, respectively, taken during the mixer emptying operation. Thereafter, it is assumed that X_1 represents the higher marker content between the two increments. The values of X_1 and X_2 can be standardized based on their mean content as follows:

$$\bar{X} = \frac{X_1 + X_2}{2} \quad (9),$$

$$P_1 = \frac{X_1}{\bar{X}} \times 100 \quad (10),$$

$$P_2 = \frac{X_2}{\bar{X}} \times 100 \quad (11).$$

Equations (9) to (11) show that the mean of the standardized values equals 100 arbitrary units. Therefore, the value 100 corresponds to the mean value and the midpoint between P_1 and P_2. The following properties of the difference between P_1 and P_2 can be derived from these statements:

$$d = |P_1 - P_2| = \left|\frac{X_1 - X_2}{\bar{X}} \times 100\right| \quad (12),$$

$$|P_1 - 100| = |P_2 - 100| = \frac{d}{2} \quad (13),$$

where d is the difference between the standardized values (i.e., P_1 and P_2).

From the standardization, the following assumption is adopted: the marker, when expressed as arbitrary units (Equations 10 and 11), can be considered as a finite element material (i.e., discrete material) distributed along a medium that corresponds to an infinite

element material (i.e., the concentrate mixture is assumed to be a continuous medium). Under this assumption, the marker presents an expected frequency of occurrence equal to 100 arbitrary units per unit of mass of the continuous medium (Equations 9 to 11). Therefore, once the marker is assumed to be a finite element material or a discrete material, its standardized content per unit of medium mass can be interpreted as a frequency of occurrence, whose numerical pattern follows the χ^2 distribution. Thus, the expected frequency of markers occurrence (i.e., the finite or discrete element) is 100 arbitrary units per unit of medium mass, whereas its observed frequencies are represented by the standardized values P_1 and P_2. In an ideal ration mixing process, the observed frequencies P_1 and P_2 should be equal and converge to 100. From this, the evaluation of the mixing efficiency becomes analogous to a hypothesis test in which the null hypothesis is that the observed frequencies P_1 and P_2 are equal to the expected frequency 100.

Using the Neyman-Pearson lemma, the null hypothesis described above would be considered as the conclusion of the hypothesis test if the calculated χ^2 statistic value does not exceed a critical χ^2 statistic value. Consequently, for a homogenous ration mixture, the following condition must be observed:

$$\chi^2_{calculated} < \chi^2_{critical} \quad (14a),$$

$$\sum_{i=1}^{2} \frac{(f_{observed} - f_{expected})^2}{f_{expected}} < \chi^2_{critical} \Rightarrow \sum_{i=1}^{2} \frac{(P_i - 100)^2}{100} < \chi^2_{critical} \quad (14b),$$

$$\frac{(P_1 - 100)^2}{100} + \frac{(P_2 - 100)^2}{100} < \chi^2_{critical} \quad (14c).$$

By applying the property defined in equation (13), we obtain:

$$\frac{(d/2)^2}{100} + \frac{(d/2)^2}{100} < \chi^2_{critical} \Rightarrow \frac{1}{4}d^2 + \frac{1}{4}d^2 < 100 \times \chi^2_{critical} \quad (15a),$$

$$\frac{1}{2}d^2 < 100 \times \chi^2_{critical} \Rightarrow d < \sqrt{200 \times \chi^2_{critical}} \quad (15b).$$

The critical χ^2 statistic is defined by the number of degrees of freedom - which is intuitively equal to 1 in the present situation - and by the α value, which represents the maximum tolerated probability of undue rejection of the null hypothesis. Here, the rejection of the null hypothesis implies concluding that the mixture cannot be considered homogeneous. An analogous pair of statistical errors can be derived from our reasoning: type I error will consist in identifying a homogenous ration mixture as non-homogenous, and type II error will consist in identifying a non-homogenous ration mixture as homogenous. In feed mills operations, a type II error is clearly more hazardous for commercial purposes and feed safety. As so, an α value of 0.10 is suggested to provide stricter control over type II error. Considering that the

region corresponding to null hypothesis rejection is unilaterally located in the right tail of χ^2 distribution, we have:

$$\chi^2_{critical} = \chi^2_{\alpha(d.f.)} = \chi^2_{0.10(1)} \cong 2{,}71 \quad (16).$$

By applying (16) in (15), we obtain:

$$d < \sqrt{200 \times 2{,}71} \cong 23.28 \quad (17).$$

If we consider the d value obtained in (17) as the maximum tolerable range between P_1 and P_2 and assume that 100 represents the midpoint between these values, we obtain under ideal ration-mixing conditions:

$$\max P_1 = 100 + \frac{d}{2} = 100 + \frac{23.28}{2} = 111.64 \quad (18),$$

$$\min P_2 = 100 - \frac{d}{2} = 100 - \frac{23.28}{2} = 88.36 \quad (19).$$

Based on these values, the initial standardization process is reverted using equations (9) and (10):

$$\max P_1 = \frac{\max X_1}{\bar{X}} \times 100 \Rightarrow \max X_1 = \frac{\max P_1 \times \bar{X}}{100} = \frac{111.64 \times \bar{X}}{100} = 1.1164 \times \bar{X} \quad (20),$$

$$\min P_2 = \frac{\min X_2}{\bar{X}} \times 100 \Rightarrow \min X_2 = \frac{\min P_2 \times \bar{X}}{100} = \frac{88.36 \times \bar{X}}{100} = 0.8836 \times \bar{X} \quad (21).$$

Calculating the ratio between the maximum permissible value of X_1 and the minimum permissible value of X_2 gives the maximum critical ratio (MCR), which allows conclusions to be drawn about the sufficient homogeneity of a mixture:

$$MCR = \frac{\max X_1}{\min X_2} = \frac{1.1164 \times \bar{X}}{0.8836 \times \bar{X}} = \frac{1.1164}{0.8836} \cong 1.26 \quad (22).$$

Thus, if X_1 and X_2 are the marker concentrations in increments 1 and 2 taken independently at appropriate times during mixer emptying time, with $X_1 > X_2$, a ration mixture is considered homogeneous ($\alpha = 0.10$) if:

$$\frac{X_1}{X_2} < 1.26 \quad (23).$$

All markers evaluated in this study satisfied this criterion (Table 2), but the lowest ratios were observed for K and Mg, reinforcing their suitability as ideal markers. However, one point in the evaluation protocol still needs to be defined, which is the moment to take the two increments to be used in the evaluation described above. The moment of occurrence of maximum and minimum contents was rather variable across all markers (Table 2). However, considering only the information concerning K and Mg, we can state that their contents were stable throughout the mixer emptying operation (Figures 2 and 3). Therefore, at least in theory, the moments of taking the increments would not influence the statistical diagnosis. Nevertheless, a free-choice recommendation here could lead to a lack of robustness and

reproducibility of the protocol, and in some cases, increments could be taken at very close moments of the emptying operation, which could lead to a false diagnosis of homogeneity. To avoid this type of inconvenience, we suggest a protocol based on taking increments in second- and eighth-tenths of the mixer emptying time. These specific tenths represent symmetrical times from the beginning and end, and from halfway through the emptying operation, and are adequately spaced apart to assure sensitivity to detect differences in marker content. For example, suppose the total emptying time of a ration mixer is 600 seconds. Then, the increments would be taken at 120 (i.e., 2 × 60 seconds) and 480 (i.e., 8 × 60 seconds) seconds from the beginning of the emptying operation. Both are symmetrical with respect to the beginning and the end of the emptying operation (i.e., equally far from 0 and 600 seconds, respectively) and are also symmetrical with respect to half the emptying time (i.e., equally distant from 300 seconds).

In summary, the proposed protocol uses K or Mg as markers for ration-mixing efficiency. Two increments per ration batch are used to evaluate marker contents in the ration mixture, which are taken at second and eight tenths of the mixer emptying time. For the ration mixing operation to be considered efficient, the ratio between the highest and lowest marker contents in the increments must be lower than 1.26 (Equation 23). Otherwise, the concentrate ration cannot be considered properly mixed and either the mixing process or the equipment should be re-evaluated.

4. Conclusions

The minerals K and Mg must be used as markers to evaluate the efficiency for concentrate rations mixtures. These markers should be used following a standard protocol that is proposed herein. Two increments (i.e., samples) must be used per ration batch, which are taken at second and eight tenths of the mixer emptying time. For a concentrate ration mixture to be considered homogeneous, the ratio between the highest and lowest marker contents in the increments must be lower than 1.26.

CRediT authorship contribution statement

Raphaela C. Vidal: Investigation, Conceptualization, Writing – original draft. **Edenio Detmann:** Writing – original draft, Writing - review & editing, Conceptualization, Formal analysis, Supervision. **Marcia O. Franco:** Writing – review & editing. **Daiana F. Quirino:** Writing – review & editing. **Marcos I. Marcondes:** Writing – review & editing. **Alex L. Silva:**

Writing – review & editing. **Laiane C. Silva:** Investigation, Writing – review & editing. **Polyana P. Rotta:** Writing – review & editing, Supervision, Funding acquisition, Project administration.

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Declaration of Competing Interest

The authors report no declarations of competing interest.

Data Availability

Readers may request the corresponding author to provide data supporting the findings of this study under reasonable circumstances.

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Table 1

Relative standard deviations (%) of mineral markers across mixers, batches, and sampling increments collected at different tenths of the mixer emptying time in a ration mixing process

	Mineral marker								
Source of variation[a]	Ca	P	K	Na	Mg	Fe	Zn	Cu	Mn
Between mixers	5.4	0.0	0.0	17.5	0.0	12.9	6.4	0.0	0.0
Between batches	0.4	2.3	0.3	0.9	2.0	3.0	6.2	16.2	2.1
Between increments									
1	20.4	10.0	8.1	23.8	9.5	29.7	28.8	86.2	35.3
2	12.2	8.1	8.5	39.0	9.1	21.9	13.8	73.1	54.6
3	12.4	8.6	6.0	28.0	6.6	28.3	17.4	46.2	25.7
4	17.4	13.5	9.0	24.4	7.6	28.9	18.8	123.4	18.3
5	17.4	9.2	6.0	24.9	12.2	23.0	16.2	28.8	31.6
6	15.8	14.3	9.7	33.1	9.5	21.6	21.2	74.2	37.6
7	14.1	10.6	7.1	31.4	5.2	28.3	17.1	76.9	29.7
8	23.3	9.2	6.8	29.3	7.6	47.4	17.7	99.9	25.2
9	15.4	9.5	6.6	28.9	8.5	30.6	17.2	89.8	20.1
10	13.8	10.4	8.3	31.1	7.5	37.6	15.6	35.8	22.0

[a] For details, report to Equations (1) to (4).

Table 2

Descriptive statistics of mineral marker contents in the ration mixing process

	Mineral marker								
Item	Ca[a]	P[a]	K[a]	Na[a]	Mg[b]	Fe[b]	Zn[b]	Cu[b]	Mn[b]
Overall mean	17.3	3.44	9.57	4.64	0.246	175	109	30.7	54.5
Maximum mean	18.7	3.53	9.86	5.08	0.251	199	115	40.4	59.1
Position of the maximum mean[c]	8	10	10	7	1	8	1	8	2
Minimum mean	16.5	3.35	9.37	4.24	0.238	160	104	19.9	50.6
Position of the minimum mean[c]	9	3	8	1	9	7	10	5	4
Total range	2.2	0.18	0.49	0.84	0.013	39	11	20.5	8.5
P-value	0.523	0.190	0.651	0.168	0.357	0.690	0.327	0.085	0.866
Standardized total range (%)	12.8	5.3	5.2	18.3	5.3	22.6	10.5	66.9	15.7
Maximum/Minimum	1.13	1.05	1.05	1.20	1.05	1.24	1.11	2.03	1.17

[a] g/kg as fed.

[b] mg/kg as fed

[c] It indicates the tenth of mixer emptying time. For details, refer to Figures 2 to 4.

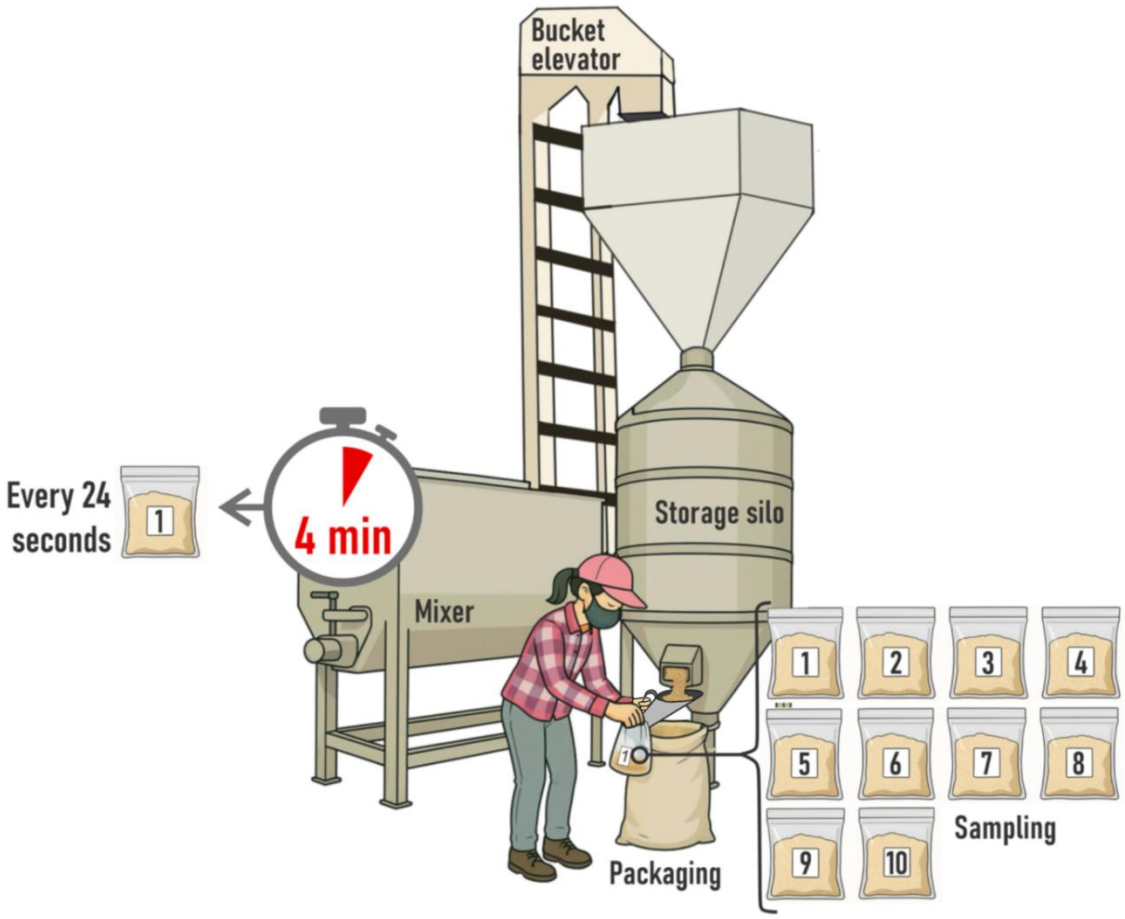
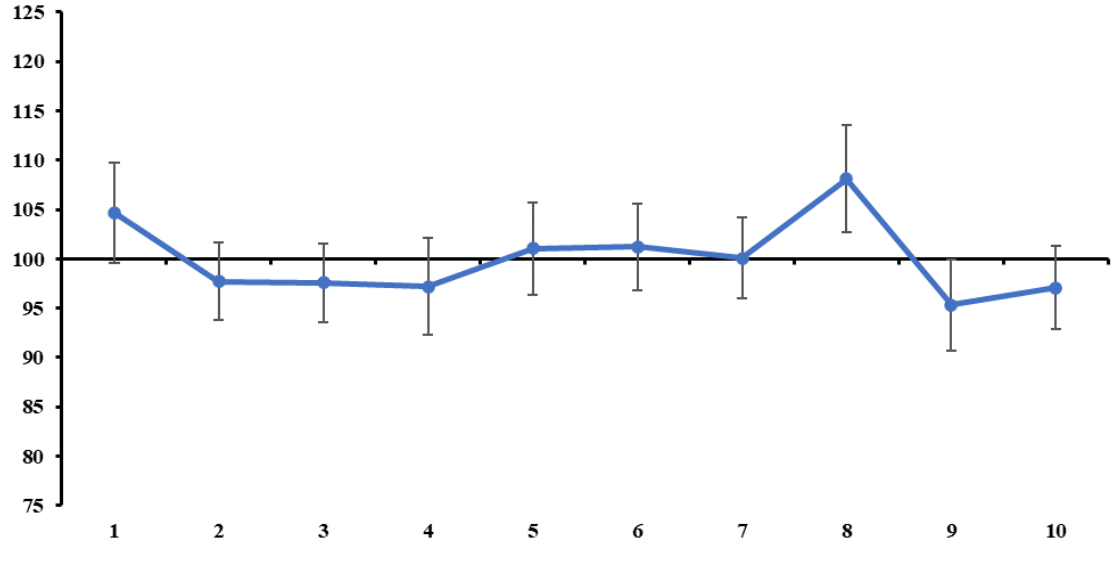


Figure 1

Experimental design and sampling protocol for evaluating four commercial horizontal spiral mixers.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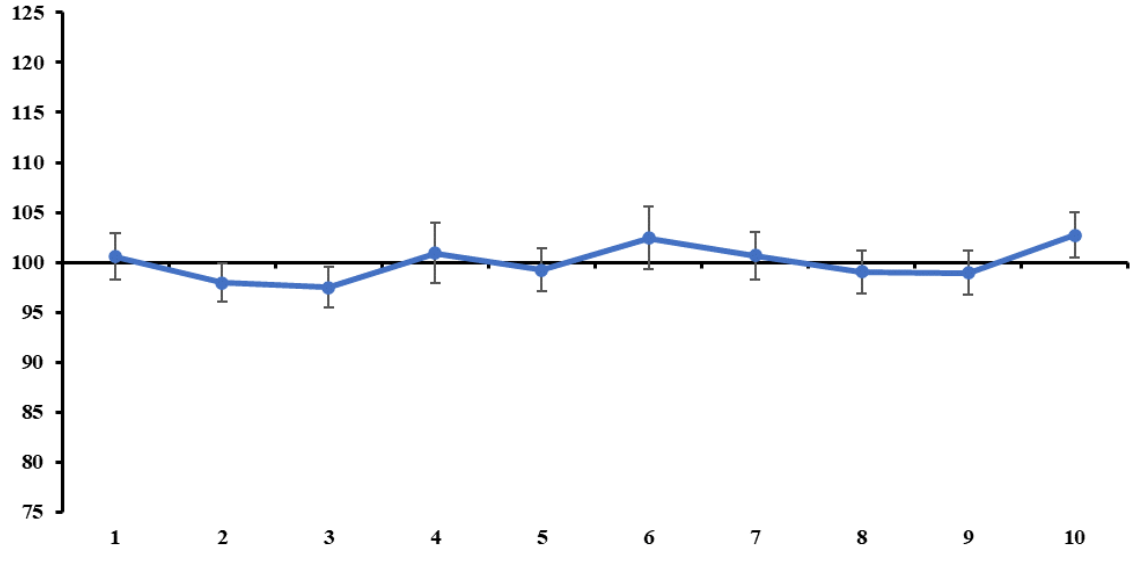
(a)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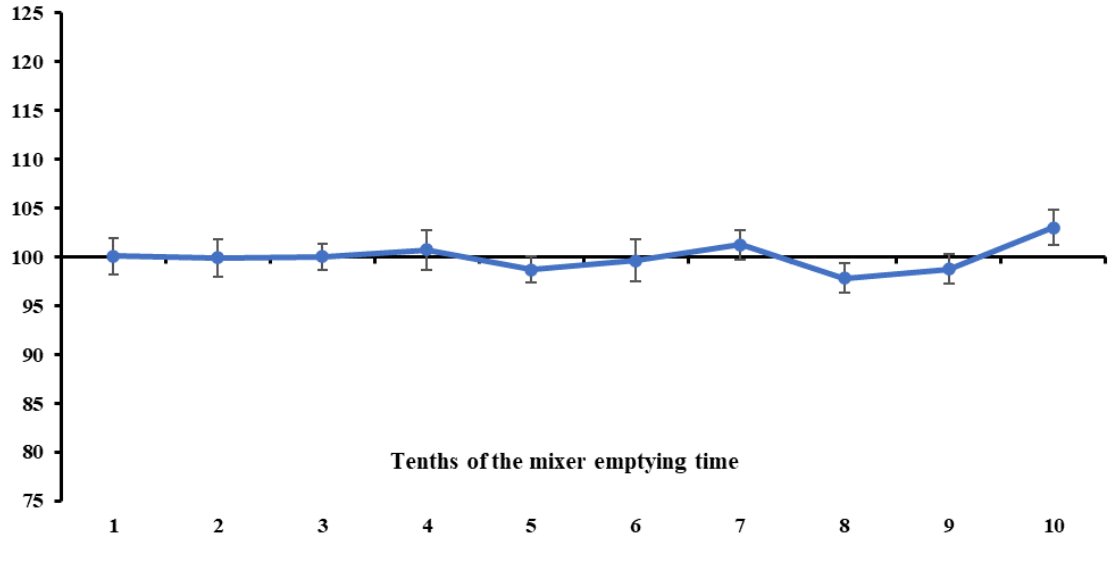
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Tenths of the mixer emptying time
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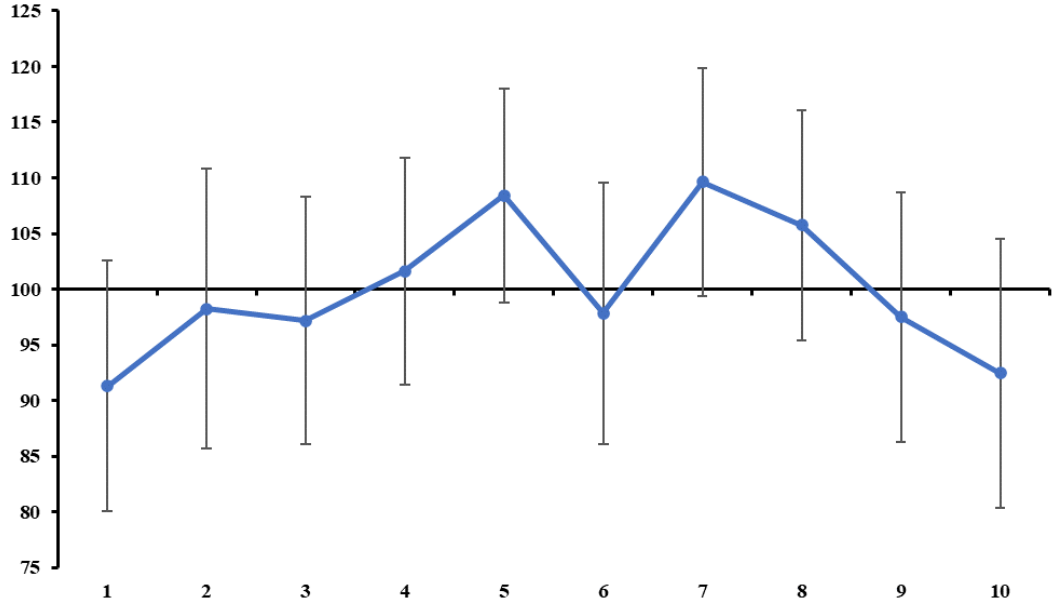
(c)

Figure 2

Standardized mean values of Ca (a), P (b), and K (c) contents across increments taken at different tenths of mixer emptying time. Vertical bars represent the variation index (refer to Equations 7 and 8).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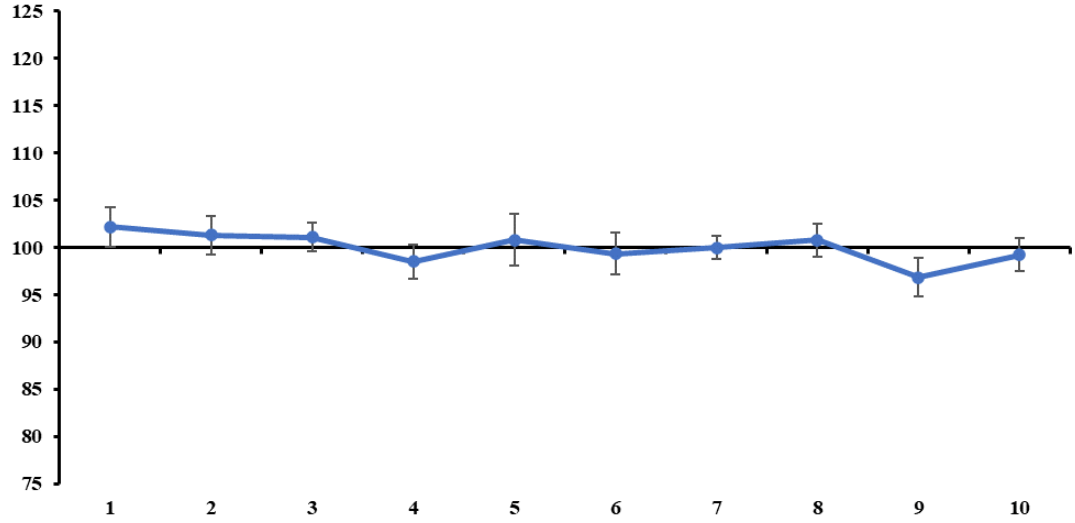
(a)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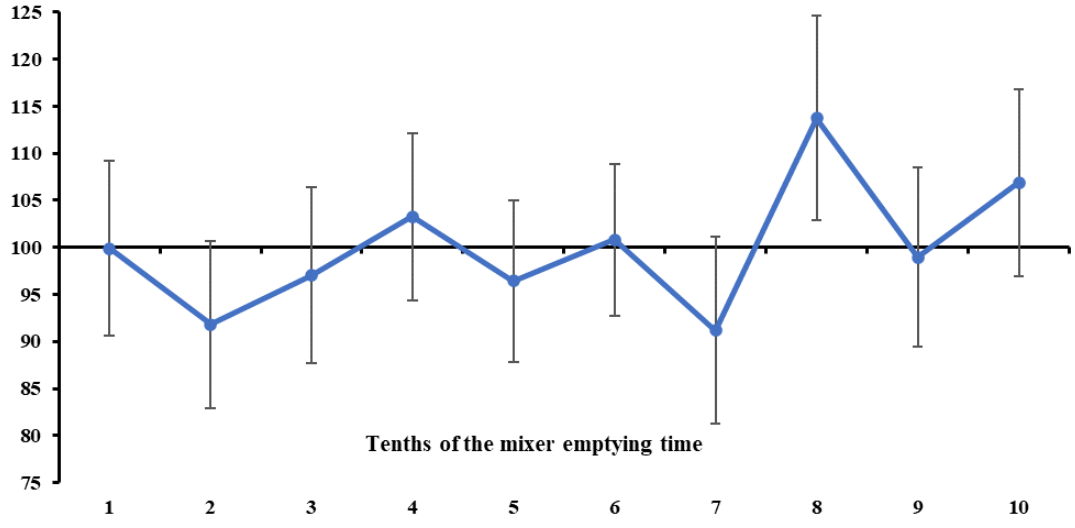
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Tenths of the mixer emptying time
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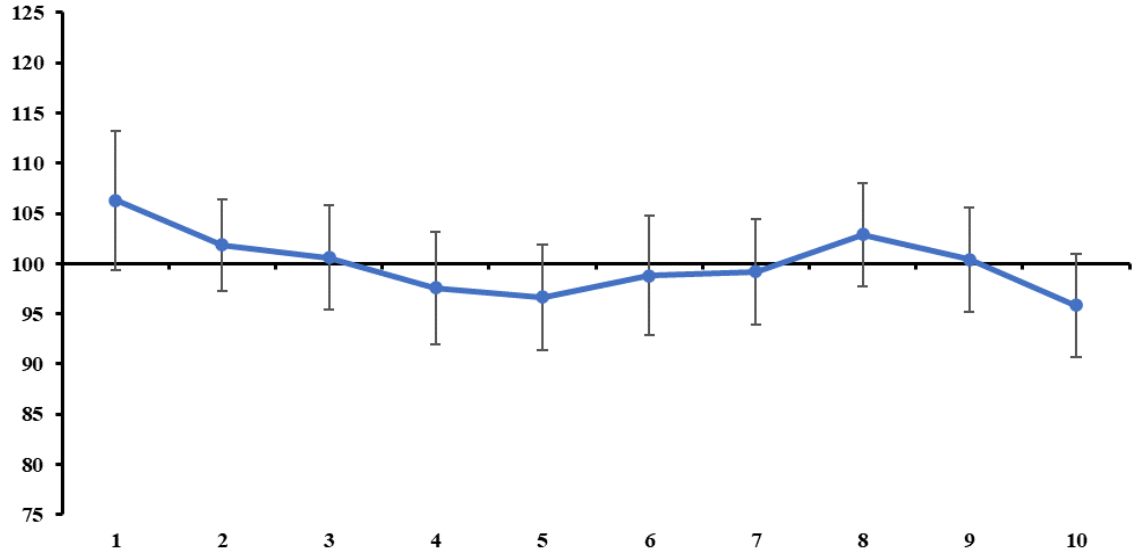
(c)

Figure 3

Standardized mean values of Na (a), Mg (b), and Fe (c) contents across increments taken at different tenths of mixer emptying time. Vertical bars represent the variation index (refer to Equations 7 and 8).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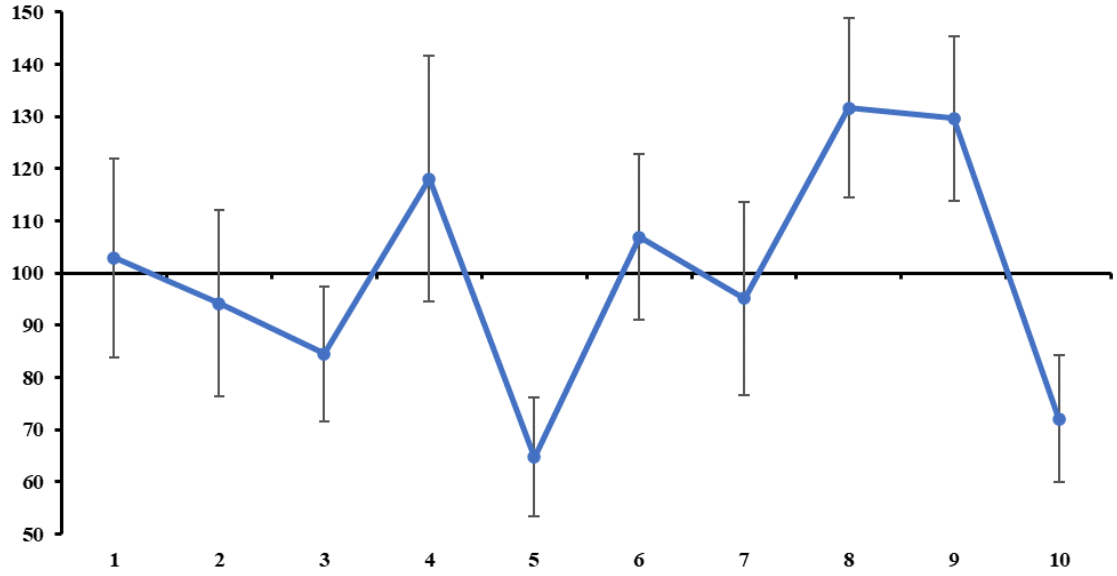
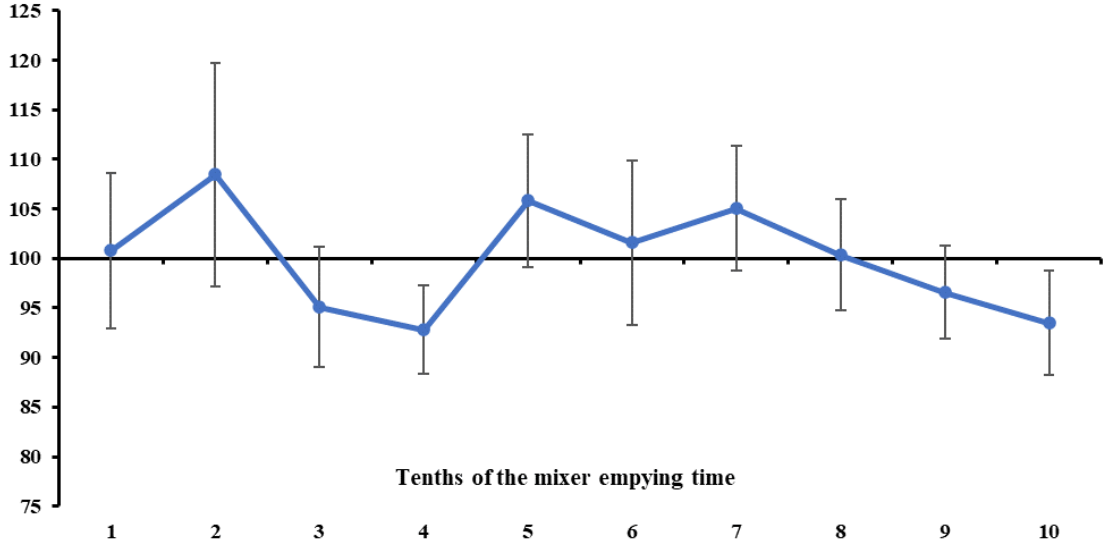
10

(a)


150
140
130
120
110
100
90
80
70
60
50
1
2
3
4
5
6
7
8
9
10

(b)


125
120
115
110
105
100
95
90
85
80
75
Tenths of the mixer empying time
1
2
3
4
5
6
7
8
9
10

(c)

Figure 4

Standardized mean values of Zn (a), Cu (b), and Mn (c) contents across increments taken at different tenths of mixer emptying time. Vertical bars represent the variation index (refer to Equations 7 and 8).

Product 2 (Book*)*: *The Fantastic World of Feed Mills*
Book submitted to be published by the Federal University of Viçosa Press and to avoid duplication of content is not included in this material.

O FANTÁSTICO MUNDO DAS FÁBRICAS DE RAÇÃO

Uma abordagem sobre a produção de alimentos para animais

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