

Formulation of Indigofera sp. Leaf Extract Nanoparticles Using Ionic Gelation and Freeze-Drying Method as a Feed Supplement for KUB Chickens

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Abstract: This study aimed to formulate nanoparticles of ethanol extract of Indigofera sp. leaves using the ionic gelation method combined with freeze-drying, and to evaluate their potential as a feed supplement for Kampung Unggul Balitbangtan (KUB) chickens. Indigofera leaves are known to be rich in bioactive compounds, particularly tannins and flavonoids, which act as natural antioxidants that can enhance poultry growth performance and health. However, their limited stability and bioavailability reduce their effectiveness when directly applied in the diet. The encapsulation process was carried out with two treatments: P1 = without sonication and centrifugation, and P2 = with sonication but without centrifugation. The best results were obtained with sonication and without centrifugation, which produced a fine powder without clumping.

Keywords: Indigofera, antioxidant, nanoparticles

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Introduction

The productivity of local chickens such as Kampung Unggul Balitbangtan (KUB) is often constrained by suboptimal feed quality, particularly due to the limited use of functional feed additives that can improve health and performance. Feed quality plays a crucial role in poultry productivity, influencing growth, immunity, and metabolic efficiency (Dibner and Richards, 2005; Gadde et al., 2017). The use of synthetic feed additives is increasingly restricted due to concerns regarding residues, antimicrobial resistance, and consumer demand for safer and environmentally friendly animal products (Gadde et al., 2017). Therefore, the development of natural feed additives with high biological activity has become a major focus in poultry nutrition.

Indigofera sp. leaves are recognized as a promising forage resource due to their high protein content and rich bioactive compounds. Previous studies reported that Indigofera leaf meal contains high crude protein and essential nutrients, making it a potential alternative feed ingredient (Palupi et al., 2014). In addition, Indigofera contains bioactive compounds such as flavonoids, tannins, and polyphenols, which act as natural antioxidants that can improve immune response, reduce oxidative stress, and enhance metabolic efficiency in poultry (Surai, 2014; Sugiharto, 2016). Despite its significant potential, the direct use of plant extracts is limited by the inherent instability of bioactive compounds when exposed to environmental factors such as heat, oxygen, and light, as well as their low bioavailability in the digestive tract (Shahidi and Ambigaipalan, 2015). Therefore, an advanced delivery system is crucially required to protect these valuable bioactive compounds and enhance their effectiveness.

Nanotechnology, particularly nanoparticle formulation using ionic gelation, has been widely studied as an approach to improve the stability and bioavailability of bioactive compounds. Chitosan is commonly used as an encapsulating agent due to its biocompatibility, biodegradability, and ability to protect active compounds while improving solubility and controlled release (Agnihotri et al., 2004; Grenha, 2012). Furthermore, the application of freeze-drying in nanoparticle preparation helps preserve structural integrity and produces a stable dry powder form suitable for feed applications

(Nowak and Jakubczyk, 2020). The combined approach of formulating *Indigofera* sp. extract into nanoparticles via ionic gelation and subsequently freeze-drying them, specifically for KUB chickens, represents an innovative strategy. To the best of our knowledge, a comprehensive study integrating all four elements – nanoencapsulated *Indigofera* extract, prepared via freeze-drying, and evaluated as a feed supplement specifically for KUB chickens – has not been previously reported. This novel approach aims to overcome the bioavailability and stability limitations of *Indigofera* while providing an effective natural feed additive to enhance the performance of KUB chickens.

Therefore, this study aimed to formulate nanoparticles of ethanol extract of *Indigofera* sp. leaves using ionic gelation combined with freeze-drying and to evaluate their potential as a natural feed additive for KUB chickens.

Materials and Methods

Materials: *Indigofera* sp. leaves were collected from farmer farm near of Universitas Mahaputra Muhammad Yamin, West Sumatra. Ethanol 96% (analytical grade, Sigma-Aldrich, USA), hydrochloric acid 37% (Sigma-Aldrich, USA), quercetin standard (Sigma-Aldrich, USA), chitosan (degree of deacetylation: 85–90%; molecular weight: medium; Sigma-Aldrich, USA), sodium tripolyphosphate (Na-TPP; Sigma-Aldrich, USA), glacial acetic acid (Sigma-Aldrich, USA), maltodextrin (food grade), Tween 80 (Sigma-Aldrich), deionized water (Bratachem, Indonesia), and DPPH (Sigma-Aldrich, USA) were used in this study. Equipment used included ultrasonic bath, rotary evaporator (Heidolph Heizbad Hal-VAP®), centrifuge, and freeze dryer.

The treatments applied in this study were: P1 = nanoparticle preparation without centrifugation after sonication; P2 = nanoparticle preparation with centrifugation after sonication.

Preparation of *Indigofera* Leaf Powder: Fresh *Indigofera* leaves were air-dried (without direct sunlight) for three days. The dried leaves were ground into fine powder and sieved.

Extraction: The dried powder was macerated with 96% ethanol (1:10 ratio) for two days with occasional stirring. The filtrate was collected and the process repeated until the final extract was colorless. All filtrates were pooled and concentrated with a rotary evaporator.

Nanoparticle Preparation: 100 mg *Indigofera* extract was mixed with 50 mL of 0.2% chitosan solution, homogenized at 1500 rpm for 30 min. Then 10 mL of 0.1% Na-TPP was added dropwise under stirring. Maltodextrin (2%) and 3 mL of 0.5% Tween solution were added, followed by sonication for 30 min. The supernatant was separated (with or without centrifugation), and the pellet was freeze-dried to obtain nanoparticle powder.

Results and Discussion

Effect of Centrifugation on Freeze-Dried Structure of *Indigofera* Leaf Extract

The results showed that the freeze-drying method significantly affected the physical characteristics of *Indigofera* leaf extract (Table 1). The treatment without centrifugation produced a finer, more homogeneous, and highly soluble powder compared to the centrifuged treatment, which resulted in a more compact and crystalline structure (Show on the figure 1 and 2). This difference is closely related to the initial matrix condition prior to freeze drying. Without centrifugation, the extract remains in a homogeneous colloidal system containing dissolved solids, proteins, and bioactive compounds. This uniform distribution allows the formation of evenly distributed ice crystals during freezing, which subsequently creates a highly porous structure after sublimation. Porous structures are known to enhance solubility and dispersibility due to increased surface area and water penetration capacity (Nowak and Jakubczyk, 2020).

Table 1. Comparison of Freeze-Drying Results of Indigofera Leaf Extract.

Treatment	Final Form	Texture	Homogeneity	Suitability for KUB Chicken Feed
With centrifugation	crystalline	Hard	low	Less suitable, difficult to mix)
Without centrifugation	Fine powder	Soft	high	Highly suitable and easy to homogeneously



Figure 1. The freeze drying by centrifugation



Figure 2. The freeze drying without centrifugation

In contrast, centrifugation induces phase separation and particle aggregation, leading to a denser matrix before freezing. This condition results in uneven ice crystal formation and reduced porosity after sublimation. Consequently, the final product becomes more compact and less soluble. Structural collapse and reduced porosity during drying have been reported to negatively affect the physical quality of dried plant materials (Ratti, 2001).

Freeze drying is widely recognized as a technique that preserves structural integrity and bioactive compounds due to its low-temperature process and sublimation mechanism. The formation of porous networks during freeze drying is a key factor influencing the physical and functional properties of the final product (Cieurzyńska and Lenart, 2011).

Implications for Solubility and Functional Properties

The higher solubility observed in the non-centrifuged treatment indicates better rehydration capacity and dispersion behavior. This is particularly important for feed applications, where homogeneous mixing ensures uniform nutrient intake. Powders with fine particle size and high porosity exhibit better mixing efficiency and improved bioavailability of nutrients and bioactive compounds. The improved physical characteristics of the non-centrifuged extract also suggest enhanced availability of antioxidant compounds. Porous structures facilitate the release and interaction of bioactive compounds such as flavonoids and phenolics, which are essential for antioxidant activity. Previous studies have shown that freeze drying can effectively preserve these compounds compared to conventional drying methods (Nowak and Jakubczyk, 2020).

The results demonstrated that freeze-drying without prior centrifugation yielded a significantly finer and more homogeneous powder compared to the centrifuged treatment (Figure 2). Such improved physicochemical properties promote better dispersion and uniform incorporation into feed, thereby enhancing the bioavailability of bioactive compounds. This, in turn, is expected to optimize antioxidant activity *in vivo*, leading to improvements in growth performance, feed efficiency, immune response, and overall product quality in chickens

Conclusion

The formulation of *Indigofera* sp. leaf extract nanoparticles using ionic gelation combined with freeze-drying successfully produced a stable nanoparticle powder. The absence of centrifugation prior to freeze-drying resulted in better physical characteristics, including finer particle size, higher homogeneity, and improved solubility.

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