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Nutritional Characterization of *Moringa oleifera* as a Potential Equine Feed and Human Application

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Abstract:
The demand for high-quality horse feed in Malaysia has led to a reliance on imported products. Locally grown *Moringa oleifera* is a promising protein source for horse feed and possible human application. Hence, this study aimed to characterize *Moringa oleifera* as a potential horse feed, projecting into possible human use. In this study, Moringa pellets were formulated to compose of rice bran, molasses, corn meal, palm kernel cake (PKC), calcium carbonate, mineral premix and salt. The formulation was designed based on the nutritional needs of maintenance horses (11% CP and 11 MJ/kg DM ME). The production process involved drying Moringa leaves, weighing of ingredients according to formulation followed by mixing, and pelletizing of the components. The nutritional composition of the Moringa pellets was analyzed following standard Association of Official Analytical Chemists (AOAC). It was found that the Moringa pellets contained 90.4% dry matter (DM), 11.7% crude protein (CP), 9.7% crude fiber (CF), and 4.9% ether extract (EE). The Moringa pellets have met the requirement for maintenance horse. Hence, the use of locally sourced ingredients ensures that the Moringa pellets provide balanced nutrition, supporting the overall health and wellness of horses while reducing dependence on imported feed ingredients. The individual protein and fibers compositions are also useful for human applications such as for topical use and oral consumption. However, further exploration and modification should be done for the purpose of human application.

Keywords: *Moringa oleifera*; Moringa pellet; Horse feed; Human application

1. INTRODUCTION

Moringa oleifera, a native plant from Africa and Asia, has gained attention from researchers in recent years due to its nutritional, anti-inflammatory, and medicinal properties in both people (1) and animals (2). It is renowned for its various useful parts, including edible seeds, flowers, and leaves, all of which have applications in herbal medicine (3). In many tropical and subtropical countries, *Moringa oleifera*, which includes leaves, fruits, immature pods, and flowers, is commonly added in people's foods. In animal diets, it is used as a supplement in the form of leaf meal (4). Across many nations, different parts of the *Moringa oleifera* tree are integrated into traditional diets, serving as a valuable source of nourishment for people (5).

Moringa oleifera offers significant nutritional benefits, boasting a high crude protein (CP) content of 20–35% (6). It is abundant in essential amino acids, antioxidants, vitamins A, C, and E, as well as minerals like calcium, potassium, and iron. Introducing Moringa into the diets of horses in Malaysia can enhance the overall protein quality and provide a well-balanced feed option utilizing locally available resources. Another researcher also stated that the CP content of Moringa leaf meal has been reported to range from 23% to 43% (7,8).

Calcium, a vital mineral found in *Moringa oleifera*, is essential for growth and development. It is one of the most important elements for a person growth. *Moringa oleifera* powder may serve as a replacement for iron supplementation in the treatment of anemia, as it contains 28 mg of iron (Fe) (9,10). A sufficient intake of zinc (Zn) is necessary for the synthesis of DNA and RNA, as well as for the regular division of sperm cells (9). The anti-inflammatory properties of Moringa leaves also can help with diarrhea and stomach ulcers. Due to its high protein, fiber, and iron content, it is a safe dietary option for people with anemia caused by malnutrition (11).

It has been found that adding *Moringa oleifera* to poultry diets can enhance the growth and antioxidant status of broilers (12). Some researchers have suggested the need for further studies to determine the optimal dosages of *Moringa oleifera* for promoting good health and performance in chickens, especially when included in experimental diets for poultry (5). Previous studies have also shown that supplementing the diets of nursing goats with *Moringa oleifera* can improve milk production and quality, as well as feed efficiency and ruminal fermentation (13). Research has also indicated that *Moringa*

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oleifera can improve lipid profiles, balance antioxidant levels (14), and reduce levels of triglycerides and total cholesterol when animals are exposed to heat stress (15).

Additionally, Moringa provides exceptional nutritional benefits for horses, with its leaves being a rich source of high-quality protein essential for muscle development and growth (11). It is packed with vitamins A, C, and E, as well as essential minerals like calcium (Ca), magnesium (Mg), and iron (Fe), which play a crucial role in maintaining a horse's bone strength, immune function, and skin health. Moringa also contains all the essential amino acids required by horses, supporting various physiological processes, including tissue repair and enzyme function.

The combined effect of these phytochemicals found in *Moringa oleifera* enhances feed intake, palatability, and disease prevention (16). Moringa pellets are typically well-received by horses because of their inherent sweetness and mild flavor, lowering the likelihood of feed rejection. This innate taste makes them appealing and appetizing to horses, ensuring that they are likely to be consumed willingly. The digestibility and absorption of nutrients are crucial for the overall health and performance of horses. In addition, the fiber content in Moringa promotes healthy digestion and reduces the risk of digestive disorders such as colic, supporting overall digestive health and well-being when added to horse feed. Therefore, the aim of this paper is to characterize the nutritional profile of *Moringa oleifera* for its potential use in formulating a balanced and nutritious horse feed.

2. MATERIALS AND METHODS

2.1 Selection of Feed Ingredients

The affordability, nutritional composition, and accessibility of these ingredients in the neighborhood all played a role in the selection. The palm kernel cake (PKC) and *Moringa oleifera* were utilized as sources of protein, whereas corn meal was used as an energy source. Rice bran was used as it contains high fiber and due to its hygroscopic qualities, which enable it to draw moisture from molasses and mold the pellets into desired shapes. Molasses serves as a binder, an energy source, and an enhancer of feed flavor due to its aroma. Calcium carbonate functioned as a binder for pellets. To keep the pellets fresh longer and give the horses the sodium (Na) and chloride (Cl) they need, salt was used a preservative. In the prepared pellet, a vitamin and mineral premix served as a multimineral and multivitamin source.

2.2 Formulation of Moringa Pellet

The Moringa pellet was designed for maintenance horses with 11 MJ/kg DM ME and 11% CP. It was formulated by trial and error using Microsoft Excel. The Moringa pellet's formulation is shown in Table 1.

Table 1. Moringa pellet's formulation

Ingredients	%
Moringa oleifera	5-15
PKC	5-10
Rice bran	30-40
Corn meal	10-20
Molasses	4-6
Salt	1-2
Calcium carbonate	4-8
Vitamin mineral premix	1-2

2.3 Processing and Production of Moringa Pellet

The Moringa leaves were gathered and stretched out on a large canvas to dry under the sun for approximately six hours. The leaves were processed into a fine, powdery texture using a heavy-duty blender after drying. There were two steps involved in the Moringa pellet preparation process, i.e., weighing and mixing of the ingredients. All the ingredients were hand-mixed after been weighed according to the formulation. A pelleting machine was then used to convert the ingredients to pellet form. The pellet measured 2 cm in length and 5 mm in diameter. To avoid becoming brittle and moldy, the pellets were allowed to cool after the pelleting process. To keep it dry and in good shape, it was then kept in a pack or an airtight barrel.

Figure 1. (a) Drying Moringa leaves; (b) Grinding process of *Moringa oleifera*

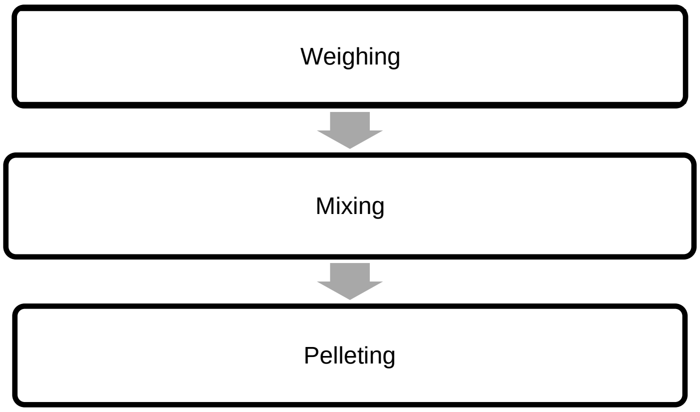

Figure 2. Processing and production of the TMR pellet

2.4 Proximate Analysis

The feed ingredients and the formulated pellet were analyzed by proximate analysis according to the standard method of the Association of Official Analytical Chemists AOAC (1997). The sample was analyzed for dry matter (DM), ash and CP (Method 984.13) (AOAC, 1997), ether extract (EE) (Foss Extraction System) (Foss, Gerhardt, Germany), and crude fiber (CF) (Method 973.18) (AOAC, 1997). The organic matter (OM), nitrogen free extract (NFE), and total digestible nutrients (TDN) were calculated as shown in the following Equations 1 - 4.

$$NFE(\%) = 100 - (\%\ CP + \%\ CF + \%\ EE + \%\ Ash) \tag{1}$$
$$OM\ (\%) = 100 - \%\ Ash \tag{2}$$
$$TDN\ (\%) = 5.31 + 0.412\ CP + 0.249\ CF\% + 1.444\ EE\ \% + 0.937\ NFE\ \% \tag{3}$$
$$ME\ (MJ/kg\ DM) = 0.185\ TDN - 1.89 \tag{4}$$

2.4.1 Dry matter (DM)

The DM was analyzed by drying the sample in a drying oven. Firstly, the empty container was weighed and denoted as *W1*. Then, approximately 2 g of sample (*W2*) was weighed. The sample was then dried in the force air oven at 110°C for 24 hours. The final weight of the dried sample was recorded as *W3*. The DM (%) was calculated following Equation 5:

$$DM\ \% = \frac{W3 - W1}{W2} \times 100\% \tag{5}$$

2.4.2 Ash

The ash was analyzed by incinerating the sample in the incinerator. Firstly, the empty crucible was weighted, and the weight is denoted as *W1*. Then, approximately 2 g of sample (*W2*) was weighed. The sample was then incinerated in the incinerator at 600°C for 6 hours. The final weight of the incinerated sample was recorded as *W3*. The ash (%) was calculated following Equation 6:

$$Ash\ \% = \frac{W3 - W1}{W2} \times 100\% \tag{6}$$

2.4.3 Crude Protein (CP)

Kjeldahl analysis was used to determine CP which was "derived through calculation using nitrogen (N) value" (17). Approximately 1g of sample was weighed and transferred into Kjeldahl flasks. The samples have then been digested in 12 ml concentrated sulphuric acid (H_2SO_4) with two Kjeldahl tablets as catalysts used as a digester. The digestion process continued for an hour and a half until the solution color became clear. Then, the tubes were allowed to cool followed by distillation process by Gerhardt distillation system. After that, N in digested samples was collected in 4% of boric acid. Then 1 ml of bromocressol green and 0.7 ml of methyl red were added as an indicator to observe color changes. The final step was a titration where the sample was added to 0.1N hydrochloric acid (HCl) drop by drop until the color changed from green to pink. The CP was determined using Equation 7 and 8.

$$N\ (\%) = \frac{[V - V\ (blank)] \times n \times 14.007}{W} \quad (7)$$

where *V* is the volume of acid neutralized sample (ml); n is the concentration of HCl; and *W* is the weight of sample (g).

$$CP\ (\%) = N\ (\%) \times 6.25 \quad (8)$$

2.4.4 Ether Extract (EE)

To determine the EE, the FOSS Extraction System was used. An aluminum cups were heated at 103℃ for 30 minutes and cooled in a desiccator for 20 minutes. The initial weight of the cups was recorded and marked as *W1*. Approximately 1g of sample was weighed into the thimbles and the weight was marked as *W2*. Then, the aluminum cups were filled with 80 ml petroleum ether and place them in the extraction unit. During the extraction process, the samples had to undergo immersion, rinsing, and recovery process. After the extraction process was complete, the cups were heated in the oven at 103℃ for 30 minutes and cooled down in a desiccator for 20 minutes. Then the final weight was recorded and marked as *W3*. The following Equation 9 shows how EE was determined.

$$EE\ (\%) = \frac{W3 - W1}{W2} \times 100 \quad (9)$$

where *W1* is the weight of empty aluminum cups (g); *W2* is the weight of sample (g); and *W3* is the weight of residue after extraction (g).

2.4.5 Crude Fiber (CF)

The CF was determined using the Fiber Bag System. Firstly, the fiber bags were dried for an hour at 105℃ and let cool for 30 minutes in a desiccator. The weight of the bag was recorded and represented as *M1*. Then, the samples were weighed approximately at 1g and inserted to glass spacers (*M2*). The samples were washed with petroleum ether 40/60 cold to de-fat the sample dried for 2 minutes. The samples were boiled with 0.13 mole sulphuric acid (H_2SO_4) for 30 minutes followed by boiling the sample in 0.313-mole sodium hydroxide (HCl) for 30 minutes. After boiling process, the samples were washed with hot water for three times. The fiber bags were removed from the carousel and dried at 105℃ for 4 hours and transferred in a desiccator for 20 minutes. Then, the fiber bags were weighed with an incinerating crucible and marked as *M3*. The empty incinerating crucible and incinerating crucible with empty fiber bags as blank were weighed and marked as *M6* and *M5* respectively. The fiber bags were heated at 600℃ for 4 hours in the furnace and allow to cool in the desiccator for 30 minutes. The crucible that contained ash was weighed and the weight was marked as *M4*. The CF% was determined as using Equation 10 and 11.

$$CF\ (\%) = \frac{M3\text{-}M1\text{-}M4\text{-}M5}{M2} \times 100\% \quad (10)$$

where *M1* is the fiber bag (g); *M2* is the Initial sample weight (g); *M3* is the Incinerating crucible and dried fiber bag after digestion (g); *M4* is the Incinerating crucible and ash; and *M5* is the weight of empty fiber bag (g).

$$Blank\ value = (m7 - m6) \quad (11)$$

where *M6* is the Incinerating crucible with empty fiber bag (g); and *M7* is the incinerating crucible and ash of the empty fiber bag (g).

3. RESULTS AND DISCUSSION

The DM of feed ingredients ranged from 87-99% for rice bran, PKC, corn meal, mineral premix, and salt. However, the DM of *Moringa oleifera* and molasses was low at 20% and 72% respectively. The ash of feed ingredients ranges from 1% - to 11% for *Moringa oleifera*, PKC, rice bran, molasses, and corn meal. However, salt and mineral premix contained high ash at 98% and 73% respectively. Besides, the OM of feed ingredients ranged from 88-99% for rice bran, PKC, corn meal, molasses, and *Moringa oleifera*. However, the OM of salt and mineral premix was low at 2.02 % and 27.37 % respectively.

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The rice bran, PKC, corn meal, and molasses also contained a CP range from 4-15 %. The *Moringa oleifera* contained the highest CP at 26.3 %. The EE of feed ingredients ranges from 4-7 % for rice bran, corn meal, and *Moringa oleifera*. Nevertheless, the EE of PKC is the lowest at 1.37%. The CF of feed ingredients ranges from 10-12 % for rice bran and *Moringa oleifera*. Besides, the amount of CF is the highest at 39.65 % for PKC and lowest at 2.52 % for corn meal.

Additionally, the NFE of feed ingredients ranges from 27-58 % for rice bran, PKC, mineral premix, and *Moringa oleifera*. However, the salt contained the lowest NFE at 2.02%. The highest NFE was found in corn meal and molasses, at 84.81% and 91.81% respectively. The TDN of feed ingredients ranges from 60-95 % for rice bran, PKC, corn meal, molasses, and *Moringa oleifera*. Yet, the TDN of mineral premix and salt were low at 30.95 % and 7.20 % respectively.

Table 2. Chemical composition of feed ingredients.

Ingredients	Nutrient Content (%)						
	Moringa oleifera	Rice Bran	PKC	Corn Meal	Molasses	Mineral Premix	Salt
DM	19.7	90.09	90.33	87.37	72.03	94.29	98.78
Ash	9.8	11.14	4.36	1.38	3.70	72.63	97.98
OM	95.3	88.86	95.64	98.62	96.30	27.37	2.02
EE	26.3	12.13	15.57	6.98	4.49	0	0
CP	5.9	6.56	1.37	4.31	0	0	0
CF	10.8	12.17	39.05	2.52	0	0	0
NFE	47.2	58.01	39.65	84.81	91.81	27.37	2.02
TDN	71.22	77.16	60.58	94.50	93.19	30.95	7.20

Generally, it is crucial to consider the nutrient composition of feed ingredients when formulating pelleted feed. It is imperative to establish a connection between the nutritional makeup of feed ingredients and the dietary needs of horses. To maintain their health and performance, the feeding requirements and digestive systems of the horse need to be considered precisely. The Moringa pellets contain 90.4% DM suggests that the moisture content is low (<15%) to prolong the feed's shelf life and prevent the mold growth. It was found that a good moisture content should be less than 15% and the DM should be higher than 85%. Maintaining the DM and moisture level at these ranges were crucial to prevent mold growth (18). Similar research shows that feedstuff have an average DM of 87 to 98%, which is indicative of high-quality feed that does not easily decay or lose its nutrients. According to Vereecken and Roels (19), one of the key elements affecting the growth of mold is moisture.

In addition, 72% DM of molasses is regarded as a good molasses quality. According to a Heuzé *et al.* (20), molasses with a DM concentration of less than 70% are of low quality and have a high-water content. It was recommended to add 2-5% of molasses in the formulation which is consistent with present findings as the formulated pellet contain 4-6% of molasses. A formulation with more than 6% molasses may have an impact on the quality of the pellet, making it more prone to breaking and molding.

Molasses increases feed palatability and functions as a binding agent which allows to produce pellets under low pressure (21). It also provides sufficient energy and protein for animals (22). The benefit of adding molasses to the pellet can increase its hardness and lifespan. In opposite findings, addition of 10% molasses in the formulation resulted in a tougher and longer-lasting pellet than a 5% molasses. Adding molasses to feed can also improve palatability, promote development, reduce dust, and improve palatability (23). The overall mineral content of the pellet is indicated by its 10.4% ash percentage.

Besides, higher mineral levels indicated higher ash content. Ashing include oxidizing organic materials, vaporizing water, and volatile substances, and converting minerals into silicates, phosphates, oxides, sulfates, and chlorides (24). It is critical to comprehend the requirements for chemical composition in order to provide diets that suit the needs of the animals (25). Since animals unable to digest ash, this could make the nutritional assessment of animal diets less accurate (26). The Moringa pellets typically include 9.0% ash which is made up of natural, external, and supplemental minerals. The ash concentrations have occasionally reached 17.0% in extreme circumstances (27). According to Duberstein and Johnson (28), excessive quantities of minerals can be toxic leading to serious health problems or interfere with the absorption of other minerals

Table 3 shows the nutrient composition of formulated Moringa pellets. It was found that the Moringa pellet contains 90.4% DM, 10.4% ash, 89.6% OM, 11.7% CP, 9.7% CF, 4.9% EE, 11.4 MJ/kg DM ME energy, 63.3 % NFE, and 78.9% TDN. The Moringa pellets also contain 4.9% EE which is close to the maximum standard feed sources (2-5% EE). Horses can digest and use up to 20% (by weight) of their feed as oil. But the majority of "high fat" horse diets usually include less than 10% (29). Pellet durability and palatability frequently place restrictions on the amount of liquid fat that can be added to animal diets. Therefore, the maximum amount of fat in feed pellets should be 15% as it reduces horses' palatability (30).

The Moringa pellet contains 9.7% crude fiber (CF), which is near the recommended level of over 10%, helping to minimize digestive issues. While fiber is not as easily absorbed as fats, carbohydrates, or proteins, it still contributes some energy to the diet. Modifying the type of fiber consumed, without reducing its quantity or weight, can regulate energy intake. For instance, feeding horses with high-energy high-quality forage could reduce the amount needed, thereby shortening feeding time while still maintaining optimal gut fill and saliva production (31). Alternatively, feeding low-quality, low-energy forage allows for the maintenance of the necessary fiber levels without increasing energy intake (32).

In this study, the Moringa pellet designed for horses provides a comprehensive blend of nutrients, including energy, protein, minerals, and vitamins, which are essential for their maintenance. The Moringa pellet contains 11.7% crude protein (CP), which is comparable to certain commercial horse feed products such as stabilized rice bran and endurance feeds that offer a CP content between 10% and 11%. However, other commercial products for high performance horses provide a higher CP content, reaching 13% and 14%, respectively. Despite these variations, the Moringa pellet is specifically formulated for maintenance purposes and has been proven to meet all the nutritional requirements necessary for producing a high-quality, balanced feed for horses.

Table 3. Nutrient composition of TMR pellet.

Components	%
DM	90.4
Ash	10.4
OM	89.6
CP	11.7
CF	9.7
EE	4.9
Energy (MJ/kg DM)	11.4

4. CONCLUSION

In conclusion, the findings of this study led to the development of a unique pellet formulation using locally available feed ingredients such as *Moringa oleifera*, rice bran, molasses, and PKC. The formulation, based on Moringa pellets, effectively met the nutritional requirements of maintenance horses in Malaysia. Additionally, the study demonstrates the potential of locally produced Moringa pellets as a concentrate-based horse feed in Malaysia. Furthermore, the results highlight *Moringa oleifera* as a viable protein alternative to imported soybean meal. Its application for human application also needs further exploration due to the high abundance of protein composition. For future research, it is recommended to explore other legumes, such as mulberry leaves, to replace *Moringa oleifera* incorporation.

AUTHORSHIP CONTRIBUTION STATEMENT

Maisarah Madihah Matriffin: writing - original draft, formal analysis; Mira Panadi: writing - review & editing, conceptualization, funding acquisition; Ahmad Zahran Md Khudzari: supervision, validation

DATA AVAILABILITY

Data are openly available in a public repository.

DECLARATION OF COMPETING INTEREST

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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