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Comparative Analysis of Total Phosphorus (P%) in Animal Feeds Available in the Libyan Market Using Spectrophotometry

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Abstract

Phosphorus is naturally present in a wide range of feed ingredients, including cereals, oilseed meals, animal-derived products, and mineral supplements. The research depends on conducting a general study of the phosphorus ratio in animals feed in the Libyan market including Broiler poultry feed, Egg-laying poultry feed, cow feed, sheep feed, Horses feed, growing pony, Bird Feed, Egg- laying birds feed. the present study aimed to confirm the amount of phosphorus and calcium in animal feed products available in the Libyan market using a spectrophotometric method. Significant difference in (P) concentration was observed between the feed categories. The comparatively large difference between feed kinds might reflect variance in feed formulation, ingredient composition, animal species, production stage, and the use of mineral P supplements.

Keywords: feed, local, animal, Phosphorus Concentration and Spectrophotometer technique

تحليل مقارن لمحتوى الفوسفور الكلي (P%) في أعلاف الحيوانات المتوفرة في السوق الليبي باستخدام تقنية القياس الطيفي

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الملخص

يوجد الفوسفور بصورة طبيعية في مجموعة واسعة من مكونات الأعلاف، بما في ذلك الحبوب، ووجبات البذور الزيتية، والمنتجات ذات الأصل الحيواني، والمكملات المعدنية. تعتمد هذه الدراسة على إجراء دراسة شاملة لنسبة الفوسفور في أعلاف الحيوانات المتوفرة في السوق الليبي، وتشمل أعلاف الدواجن اللاحمة، وأعلاف الدجاج البياض، وأعلاف الأبقار، وأعلاف الأغنام، وأعلاف الخيول، وأعلاف المهور النامية، وأعلاف الطيور، وأعلاف الطيور البياضة.

هدفت الدراسة الحالية إلى تحديد كمية الفوسفور والكالسيوم في منتجات الأعلاف الحيوانية المتوفرة في السوق الليبي باستخدام طريقة القياس الطيفي الضوئي. وقد لوحظ وجود اختلافات ملحوظة في تركيز الفوسفور بين فئات الأعلاف المختلفة. وقد يعكس التباين الكبير نسبيًا بين أنواع الأعلاف اختلافات في تركيب العليقة، ومكونات الأعلاف، ونوع الحيوان، ومرحلة الإنتاج، واستخدام مكملات الفوسفور المعدنية.

Introduction

Phosphorus (P) is an important mineral element and one of the major nutrients required for normal growth, development, and physiological function in animals. It plays significant roles in bone, energy metabolism, cellular structure, muscle function, and acid–base regulation. A Consequently, insufficient phosphorus intake can negatively affect skeletal development, rachitic (in young), low appetite, and overall animal health (NRC, 2005). Phosphorus is naturally present in a wide range of feed ingredients, including cereals, oilseed meals, animal-derived products, and mineral supplements. However, the concentration and biological availability of phosphorus can vary considerably among feed materials. Therefore, the distinction between total phosphorus and available phosphorus is important when evaluating the nutritional quality of animal feed.

The accurate determination of phosphorus in animal feed is important for both nutritional assessment and feed formulation. Insufficient phosphorus may result in impaired bone mineralization, reduced growth, poor feed efficiency, and skeletal abnormalities, whereas excessive dietary phosphorus can increase mineral excretion and contribute to environmental pollution. Determination of the phosphorus content of feed ingredients therefore provides useful information for evaluating their nutritional value and ensuring. Several analytical techniques have been used to determine P in feed and food materials. Spectrophotometric is predominantly useful method because they are relatively simple, rapid and suitable for the concentration of phosphorus in unknown feed samples to be quantitatively determined. Previous studies have investigated phosphorus concentrations and phosphorus use in major feed ingredients such as corn and soybean meal. For instance, Jeong et al. (2015) found phosphorus concentrations of approximately 2.0–2.9 g/kg among different corn products, demonstrating variation in phosphorus content amongst feed materials. Additional studies have reported the importance of phosphorus digestibility and the effects of phosphorus in animal diets (Akinmusire and Adeola, 2009; Bohlke et al., 2005; NRC, 2012). However, information regarding the phosphorus content of commercially animal feeds in the Libyan market remains limited. Therefore, the present study aimed to To confirm the addition of the amount of phosphorus and calcium in animal feed products available in the Libyan market using a spectrophotometric method for the physical health of the animal, as well as to emphasize the economic performance of animals. The study provides comparative data on phosphorus concentrations among locally available feed products and contributes to the assessment p of their mineral nutritional quality. The findings may also provide useful information for feed quality control, nutritional evaluation, and future studies of mineral composition and phosphorus availability in animal feeds in Libya.

2. Materials and Methods

2.1 Reagents and chemicals.

All reagents solutions were used in analytical grade. The reagents and chemicals included: Sensitive scal (Mettler Toledo, Switzerland), distilled water, calcium carbonate (Sigma-Al,drich UK), 6 N of HCl Hydrochloric acid (HCl) (Sigma-Al,drich UK), 1N of HNO3 (Sigma-Al,drich UK), Potassium Pyrosulfate (KGaA, Germany), Ammonium molybdate reagent (KGaA, Germany), Ammonium metavanadate (Sigma-Al,drich UK), All prepared reagents were stored in an appropriate conditions recommended for each reagent.

2.2 Apparatus and laboratory equipment.

The analytical procedure was performed using standard laboratory equipment, including: UV–Visible spectrophotometer (Shimadzu Corporation, Japan), Electric furnace/muffle furnace capable of reaching approximately 550 °C, Electric heater/hot plate, Water bath, 250-mL and 100-mL beakers, Conical flasks, Volumetric glassware, Filter paper, Dark reagent-storage bottles.

2.3. Study design and animal feed samples.

Animal feeding samples was taken from different areas (Tripoli, Zawia, Libya from January–June, 2025). A total of n= 3 of each animal feed samples were included in the study. Each product was assigned a unique identification code according to its manufacturer and/or feed type. Samples were collected transported to the laboratory under ambient conditions for analysis. Prior to analysis, commercial feed samples were homogenized and stored in clean, dry, airtight containers until further analysis.

2.4 Sample Preparation and Digestion

Each animal-feed sample was homogenised to obtain a homogeneous material and then passed through a sieve. The all sieved samples were transferred into clean plastic bags, which were labelled with the sample information. Using a sensitive analytical balance, 1.0 g of each prepared feed sample was accurately weighed into a labelled crucible. The samples were subjected to controlled dry ashing in a muffle furnace. About 1.0 g of calcium carbonate ($CaCO_3$) was added to each sample and mixed thoroughly. The crucibles containing the feed samples were then placed in a combustion furnace and heated at 500–600 °C as described in this protocol (Manika, & Ayele, (2022). After that, crucibles were allowed to cool to room temperature. The cooled residues were transferred into clean 250-mL beakers and added 30 mL of dH_2O. Then, 10 mL of 6 N HCl was added gradually to each sample to control the reaction and prevent excessive foaming, and the addition was continued until the reaction ceased completely. The beakers were then placed on a heated bath until the solutions were evaporated to dryness. After cooling, 10 mL of 1 N nitric acid (HNO_3) was added to each samples residue, and the beakers were covered with watch glasses for 10 min. The resulting solutions were filtered, and each beaker was rinsed twice with hot dH_2O to ensure quantitative transfer of the soluble components. The filtrates were collected in 100-mL volumetric flasks, and the volume was made up to the calibration mark with dH_2O. Each flask was shaken to obtain a homogeneous solution prior to the determination of phosphorus by UV–Visible spectrophotometer (Manika & Ayele, 2022).

2.5 Preparation of Standard Phosphorus Solutions.

1. Preparation of the 1000 mg/L (1000 ppm) phosphorus stock standard solution: About 4.4 g of KH_2PO_4 was accurately weighed and dissolved in a beaker containing a small volume of dH_2O. The resulting solution was quantitatively transferred into a 1-L volumetric flask**,** and the beaker was rinse three times with distilled water to ensure complete transfer. The rinsing was added to the volumetric flask, and the solution was diluted to the calibration mark with distilled water and mixed thoroughly. The resulting solution contains 1000 mg/L (1000 ppm) of phosphorus**,** equivalent to 1.00 mg of phosphorus per mL of solution**.**

2. Preparation of the 100 mg/L (100 ppm) phosphorus working standard solution:

A 100 mg/L (100 ppm) phosphorus working solution was prepared by adding 100 mL of the 1000 mg/L stock solution into a 1L volumetric flask. The volume was then made up to the calibration mark with dH_2O and mixed carefully to ensure homogeneity. The resulting standard contains 100 mg/L (100 ppm) phosphorus, equivalent to 0.10 mg of phosphorus per 1mL of solution**.**

2.6 Standard Solutions and Determination of Phosphorus:

A series of phosphorus standard solutions was prepared for standard calibration curve. Aliquots of 0.0, 5.0, 10.0, 15.0, and 20.0 mL, respectively, of the standard P solution prepared according to Debnath & Tsegaye, (2022) were transferred into separate 100-mL volumetric flasks. To each flask, 20 mL of the ammonium molybdo-vanadate reagent was added. Then, the solutions were diluted to the calibration mark with dH_2O and mixed. These solutions represented the phosphorus standards used to create the calibration curve.

For sample analysis, a suitable aliquot of the prepared sample solution was transferred into volumetric flask, ensuring that the expected phosphorus concentration fell within the concentration range of the calibration standards. Subsequently, 20 mL of ammonium molybdo-vanadate reagent was added, and the solution was diluted with distilled water and thoroughly mixed.

The absorbance of the standard solutions was measured spectrophotometric at a wavelength of 400 nm**,** using an appropriate reagent blank for baseline correction. A calibration curve was constructed by plotting the measured absorbance values against the phosphorus concentrations of the solutions. The phosphorus concentration in the analysed samples was determined from the calibration curve and subsequently used to calculate the percentage of total phosphorus (P%) in the original samples.

Table 1 a and b: show the intensity of optical absorption of phosphorus standard solution.

(a)

intensity of optical absorption	**concentration (PPm)**
Zero	0 (PPm)
0.16	5 (PPm)
0.17	10 (PPm)
0.18	15 (PPm)
0.19	20 (PPm)

(b)

Absorbance intensity	**concentration (PPm)**
Zero	0 (PPm)
0.16	5 (PPm)
0.19	8 (PPm)
0.18	10 (PPm)
0.18	15 (PPm)
0.17	20 (PPm)

3. Results and discussions

3.1 Absorbance Measurements of Feed Samples

The phosphorus content of the selected animal-feed samples was investigated using the spectrophotometric molybdovanadate method. Absorbance was measured at 400 nm as control sample**,** and three replicate measurements were performed for each sample. The absorbance values obtained for the poultry, cattle, and sheep feed samples collected from three different sources are presented as shown in Table 2.

Table 2: shows the absorption intensity at a wave length of 400nm. Feed samples were taken from three sources as follows: poultry feed, cattle feed, sheep feed, Horse feed, and bird feed.

Feed type	Source 1 Samples:	Absorption intensity (A) Mean ± SD	Source 2 Samples	Absorption intensity (A) Mean ± SD	Source 3 Samples	Absorption intensity (A) Mean ± SD
Poultry feed	0.11, 0.10, 0.09	0.100 ± 0.010	0.09, 0.10, 0.11	0.100 ± 0.010	0.09, 0.10, 0.11	0.100 ± 0.010
Cattle feed	0.06, 0.07, 0.08	0.070 ± 0.010	0.06, 0.07, 0.08	0.070 ± 0.010	0.06, 0.07, 0.08	0.070 ± 0.010
Sheep feed	0.08, 0.08, 0.07	0.077 ± 0.006	0.07, 0.08, 0.08	0.077 ± 0.006	0.07,0.08, 0.08	0.077 ± 0.006

Formulas used in Calculations

$$\text{Average Absorption intensity(A)} = \frac{X1+X2+X3}{3}$$

Cppm= ppm C ppm= Concentration part per million

$$\text{Cxppm} = \frac{\text{Cppm} \times \text{V} \times \text{D.f}}{\text{Wt}}$$

$$\text{D.f} = \frac{100}{5} = 20 \qquad \text{(D . f) dilution factor}$$

$$\text{Cxppm} = \frac{\text{Cppm} \times 100 \times 20}{1\,g} = \text{concentration (ppm)} \qquad \text{Cxppm= unknown Sample}$$

$$\text{P \%} = \text{Cxppm} \times 10^{-4}$$

The results showed a relatively narrow range of absorbance values among replicate measurements. Poultry feed exhibited the highest absorbance (0.100 ± 0.010) across all three sources, followed by sheep feed (0.077 ± 0.006), whereas cattle feed exhibited the lowest absorbance (0.070 ± 0.010). The same pattern was observed in all three sources, indicating good measurement absorbance values. However, the low observed among the three replicate measurements indicate good repeatability of the spectrophotometric measurements in all the experimental conditions. In particular, the sheep-feed samples showed the smallest variation between replicates, whereas poultry and cattle feed showed slightly greater variation.

Table 3: Shows the percentage of phosphorus concentration in the animals feed samples.

<table>
<tr><th>Feed Types</th><th>Average Sources Feed</th><th>CPPM From the graph</th><th>weight of sample</th><th>Volume of sample 100 ml</th><th>Dilution Factor D.f = 100/5</th><th>Semple Concentration part per million Cx PPM 100 ml</th><th>P %</th></tr>
<tr><td>Broiler poultry feed</td><td>0.08</td><td>3.8</td><td rowspan="9">1gm</td><td rowspan="9">100ml</td><td rowspan="9">20</td><td>7600 ppm</td><td>0.7% 6</td></tr>
<tr><td>Egg-laying poultry feed</td><td>0.6</td><td>20.0</td><td>4000 ppm</td><td>0.4%</td></tr>
<tr><td>Beef Cattle Feed</td><td>0.07</td><td>3.4</td><td>6800 ppm</td><td>0.68 %</td></tr>
<tr><td>Dairy Cattle Feed</td><td>0.1</td><td>13</td><td>26000 ppm</td><td>2.6%</td></tr>
<tr><td>Sheep Feed</td><td>0.07</td><td>3.4</td><td>6800 ppm</td><td>0.68 %</td></tr>
<tr><td>Horse Feed</td><td>0.083</td><td>4</td><td>8000</td><td>0.80 %</td></tr>
<tr><td>Bird Feed</td><td>0.01</td><td>1.2</td><td>2400</td><td>0.24 %</td></tr>
<tr><td>Egg- laying birds</td><td>0.011</td><td>1.3</td><td>2600</td><td>0.26 %</td></tr>
<tr><td>Growing Ponies</td><td>0.02</td><td>2</td><td>4000</td><td>0.4%</td></tr>
</table>

The spectrophotometric determination showed substantial variation in total phosphorus (P) concentration among the different animal-feed samples. The measured phosphorus concentrations ranged from corresponding to approximately 0.24–2.60% P. The highest P concentration was observed in cattle feed, with about 2.60%. This was followed by broiler poultry feed 0.76%. In contrast, beef cattle feed and sheep feed was reported with 0.68% each, and horse feed was measured at 0.80%. The egg-laying poultry feed showed concentration 4.0% as showed in the table (3), while bird feed and egg-laying bird feed contained 0.24% and 0.26% respectively. Growing pony feed was absorbed at 0.40%. Overall, the data prove considerable differences in P concentration among feed categories for animals. The high value recorded for dairy cattle feed indicates that this formulation contained substantially more phosphorus than the other investigated feeds, while bird feed and egg-laying bird feed showed the lowest measured concentrations. The phosphorus concentration of each sample was obtained from the calibration curve prepared using phosphorus standards. So, the differences observed between animal samples represent differences in measured phosphorus content under the experimental conditions.

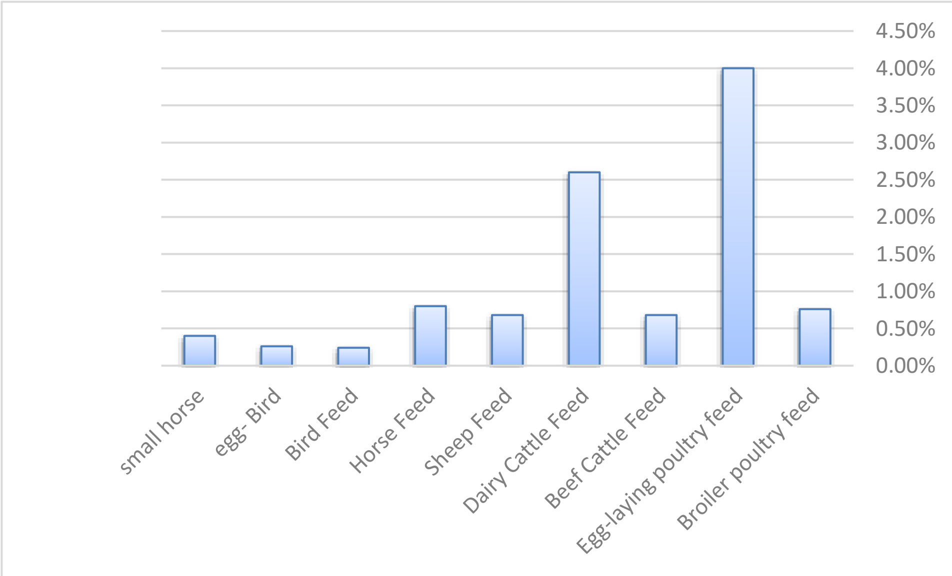

Figure 1: The phosphorus content (%) determined in the investigated animal-feed samples using spectrophotometric method. The results demonstrate considerable difference in phosphorus concentration between the different feed animal types.

The phosphorus content of the investigated feed animal samples was detected at 400 nm following reaction with ammonium molybdo-vanadate reagent. As shown in Figure 1, phosphorus concentration was observed between the examined feed types. The measured values ranged from about 0.20% to about 4.00%. The highest P concentration was recorded in egg-laying poultry feed with approximately 4.00%, followed by dairy cattle feed around 2.60%. P concentrations were observed at the high rate in horse feed and broiler poultry feed about 0.80%, followed sheep feed with approximately and beef cattle feed (approximately 0.70%. However, the lowest phosphorus concentrations observed in bird feed (approximately 0.20%) and egg-laying bird feed (approximately 0.25%). In this study, the small-horse feed was an approximately 0.40% phosphorus. These findings demonstrate marked differences in phosphorus content between feeds in different animal, reflecting differences in formulation and mineral requirements.

4. Discussion

In this study, phosphorus concentrations of the various feed types are consistent with the observed variations in absorbance to be greater than those reported by others (Manika, & Ayele, 2022). The photometric measurement of phosphorus in animal feed utilizing molybdovanadate chemistry and spectrophotometric detection at about 400 nm was reported in animal feed. Based on the results obtained it can be confirmed that the UV-VIS technique is more reliable for the phosphorus determination from different types of feed animals. The accuracy of this method was used to measure P in animal food (Latimer, 2016). The relatively higher absorbance observed in poultry feed suggests a higher phosphorus concentration than that observed in the cattle and sheep feed samples, assuming that all animal feed samples were subjected to the same preparation, dilution, reagent conditions, and calibration procedure. On the other hand, the lower absorbance recorded for cattle feed suggests a lower phosphorus concentration within the analytical range used in this experiment. These data were supported and validated for phosphorus concentration using the ICP-MS technique which is more reliable for the phosphorus determination from different type of food (Catrina, et al 2020). The current results are reliable for feed phosphorus determination by molybdovanadate method. Previous collaborative investigations of mixed feeds confirmed photometric molybdovanadate method provide results in reasonable agreement with established data for phosphorus determination (Mattos, et al., 2026). The absorbance values were essentially identical for each feed type across the three sources: 0.100 for poultry feed, 0.070 for cattle feed, and approximately 0.077 for sheep feed.

This similarity may indicate comparable phosphorus-containing formulations detected in feed (Gliszczyńska & Rybicka, 2021). Nevertheless, because only three samples per feed category were examined, this observation should be considered descriptive rather than evidence of statistical equivalence among commercial sources. It is important to highlight that the nutritional significance of phosphorus depends not only on P concentration but also other minerals, predominantly calcium is demonstrated in food animals. So, the measured phosphorus concentration should be taken in relation to the formulation and intended animal species rather than as an isolated indicator of feed quality. The European regulatory analytical framework likewise recognizes spectrophotometer determination as a method for measuring total phosphorus in animal feeding stuffs (Park, et al., 2019).). Total, the current findings demonstrate that the method and procedures were produced reproducible absorbance measurements for the investigated feed samples. The differences observed among poultry, sheep, and cattle feeds provide evidence of variation in P level, even though the actual P concentrations and percentages should be obtained from the calibration equation before making quantitative nutritional comparisons.

Significant difference in (P) concentration was observed between the feed categories as illustrated in figure 1. The comparatively large difference between feed kinds might reflect variance in feed formulation, ingredient composition, animal species, production stage, and the use of mineral P supplements. The present results can be compared with the study which reported total phosphorus concentrations in broiler feed (Dou et al., 2014). A further confirmation comparison for our data which is provided that evaluated phosphorus in horses, poultry, feed samples representing cattle and quantified accurately using the same techniques (Sørensen, etal., 2022). This supports the use of analytical methods for monitoring phosphorus in commercial feeds.

Conclusion

The present study demonstrated that in the phosphorus (P) concentration of the animal-feed samples obtained from the Libyan market. The concentration of P was different according to the type of feed. In comparison, the concentrations were measureable in poultry, cattle, sheep, and horse feeds were generally with the low range. These results determine that the levels vary substantially between commercial feed formulations and may depend on the ingredients, mineral supplementation, animal species, age, and physiological or production stage. Overall, the data pointed out the importance of regular monitoring of P concentrations in animal feed, because together insufficient and excessive phosphorus intake might impact animal health, productivity, mineral balance.

Recommendations and Suggestions

- Training animal feed handlers to accurately mix and prepare feed to ensure adequate phosphorus levels and maintain animal health.
- Regularly monitoring bone health and nutritional status to ensure adequate phosphorus intake and support optimal animal growth and production.
- Avoiding excessive phosphorus supplementation, as unnecessarily increasing phosphorus content raises feed costs and increases phosphorus excretion in manure, which may contribute to environmental contamination and represent an economic loss.
- Poultry and laying hens may require higher phosphorus levels than some other animals because of the increased demand associated with eggshell formation. Similarly, dairy cows and sheep may require higher phosphorus levels during lactation to support milk production and maintain adequate mineral balance.

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