

Review

Phosphorus for Cattle and Buffaloes in Brazil: Clinical Signs and Diagnosis of Its Deficiency and Relevance, and Recommended Strategies to Alleviate Issues Observed under Grazing Conditions

Pedro Malafaia ¹, José Diomedes Barbosa ², Marilene Farias Brito ³, Vinícius Carneiro de Souza ⁴ 
and Diogo Fleury Azevedo Costa ^{5,*} 

¹ Department of Animal Nutrition and Pastures, Federal Rural University of Rio de Janeiro (UFRRJ), Seropédica, RJ 23890-000, Brazil

² School of Veterinary Medicine, Federal University of Para (UFPA), Castanhal, PA 68746-360, Brazil

³ Department of Epidemiology and Public Health, Federal Rural University of Rio de Janeiro (UFRRJ), Seropédica, RJ 23897-000, Brazil

⁴ Department of Animal Science, University of California, Davis, CA 95616, USA

⁵ Institute for Future Farming Systems, CQUniversity, Rockhampton, QLD 4701, Australia

* Correspondence: d.costa@cqu.edu.au; Tel.: +61-4094-45454

Abstract: Phosphorus (P) deficiency impairs productivity of ruminants and is the most expensive mineral used in supplements for cattle and buffalo under Brazilian grazing conditions. There is a need for the rational use of P, particularly under extensive grazing conditions, as this mineral resource is expensive and the world's supplies are exhaustible. The diagnosis of P deficiency must start with careful and detailed history taking, followed by clinical examination of the herd and the use of ancillary tests. The latter are complementary to the diagnosis; they should be neither the first nor the most important steps when conducting a professional diagnosis. The indication of corrective or prophylactic measures necessarily involves the correct diagnosis of P deficiency (in herds), whether it be clinical or subclinical. This review discusses the main aspects related to P for cattle and buffaloes under grazing conditions in Brazil.

Keywords: ancillary tests; livestock; mineral deficiency; phosphate rock



Citation: Malafaia, P.; Barbosa, J.D.; Brito, M.F.; Souza, V.C.d.; Costa, D.F.A. Phosphorus for Cattle and Buffaloes in Brazil: Clinical Signs and Diagnosis of Its Deficiency and Relevance, and Recommended Strategies to Alleviate Issues Observed under Grazing Conditions. *Ruminants* **2023**, *3*, 55–75. <https://doi.org/10.3390/ruminants3010006>

Academic Editor: Valentina Busin

Received: 31 January 2023

Revised: 18 February 2023

Accepted: 27 February 2023

Published: 3 March 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The importance of phosphorus (P) and its deficiency in the nutrition of grazing beef cattle was first established in South Africa early in the last century [1,2] and was later recognized in other grasslands of North America [3], Australia [4–6], and Brazil [7,8]. Cattle and buffalo production occurs in a variety of ecosystems, representing an activity of great importance for the economy of many countries. Beef cattle farming is the main agricultural industry of Australia, where about 70% of the red meat is produced in extensive grazing systems in seasonally dry, tropical regions of the country [9]. In these systems, the majority of beef cows calve late in the dry season or early in the wet season, imposing a massive challenge to meet the higher demand for nutrients, including P. Higher amounts of P are required to support udder and foetal development during late pregnancy, and from the start to mid-lactation towards the onset of the wet season [10]. Brazil has a herd of approximately 215 and 1.5 million cattle and buffaloes, respectively. Over 90% of these animals are raised under a wide range of conditions in remote and vast areas, on pastures established in soils with low-to-moderate fertility. This may result in a low P concentration in the forage harvested by these animals and, consequently, may produce subclinical or clinical deficiencies in the animals [11].

The current review focuses on experiences and knowledge generated over the years in Brazil. A plethora of research initiated by Professor Tokarnia and, thereafter, by his collaborators, has consistently indicated three reasons to use P added to mineral supplements under strictly scientific and rational criteria: (a) Brazilian phosphate rock reserves are not very significant (1–2% of the world's reserves) and, as such, is a non-renewable resource deemed to reach depletion within the coming century [12,13]; (b) the current cost of P sources used in the diet of ruminants is high, and as times progresses and natural reserves are exhausted, the price of P is only going to increase; (c) the daily requirement of P by large adult ruminants, such as cattle and buffaloes, is high, i.e., above 15 g/day, as indicated by most feeding standards [14–16], which implies that irrational use of this nutrient would only increase dependence on P derived from external sources [11] and potentially increase the risks to environment, such as eutrophication of water bodies caused by overenrichment when excess P is excreted where animals gather to drink or rest under shaded areas.

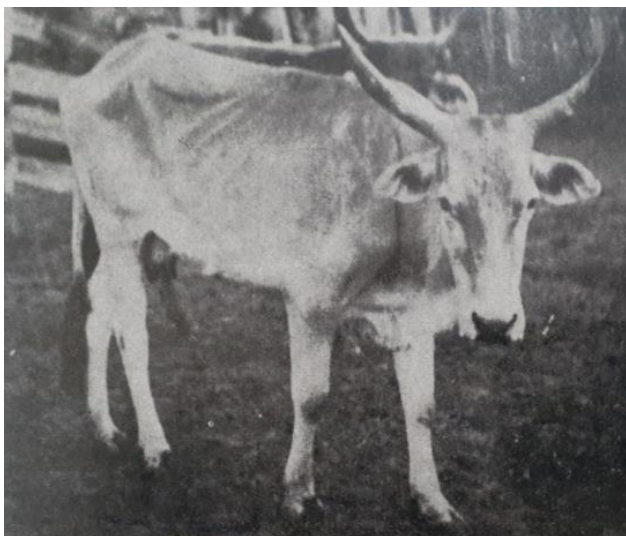
This review aims to present and critically assess the various aspects related to P nutrition of free-grazing cattle and buffalo with a particular focus on the Brazilian ecosystems. Conclusions presented in this publication are a reflection of the knowledge and experiences of the authors. As such, they are likely to be a reflection of the specific management and biophysical attributes of the situation they were observed under, and it is the view of the authors that careful consideration should be exercised when extrapolating to other regions and conditions, as appropriate adjustments are likely to be required.

2. Phosphorus Deficiency in Cattle and Buffaloes: The Brazilian Experience

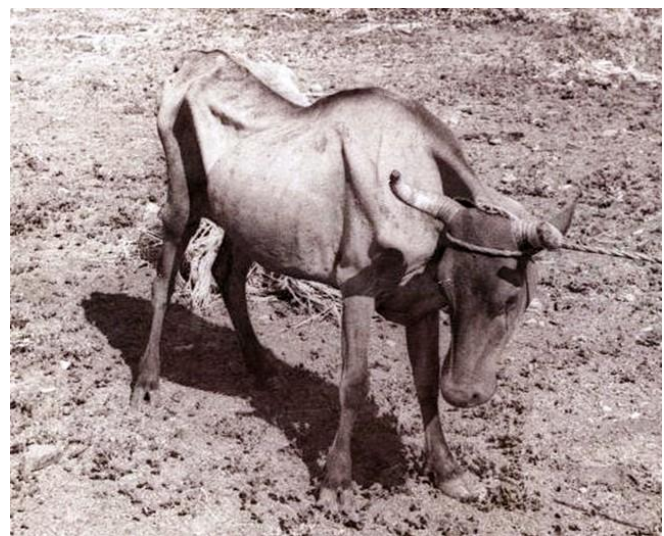
For the purposes of this review, an animal or herd is considered deficient in a mineral when there is a correct diagnostic of its deficiency and a positive response to its inclusion in the diet, e.g., increase in milk production, daily weight gain, fertility.

2.1. Brief History of the Study of P Deficiency

The first studies of P deficiency in Brazil were carried out in the central area of the state of Minas Gerais, during the 1940s [7,8,17] and, later, during the mid-1950s, it was also diagnosed in cattle raised in the semi-arid northeast region of the country [18]. Figure 1a,b show two examples of cows diagnosed with clinical P deficiency from the early studies in Brazil.



(a)



(b)

Figure 1. (a) Lean, dry cow that had failed calving for over two years and was reluctant to move when the photo was taken in Pará de Minas, MG, Brazil [17]. (b) Cow showing severe lordosis and hock deviations due to clinical P deficiency, photo taken in 1956, Altos, PI, Brazil [18].

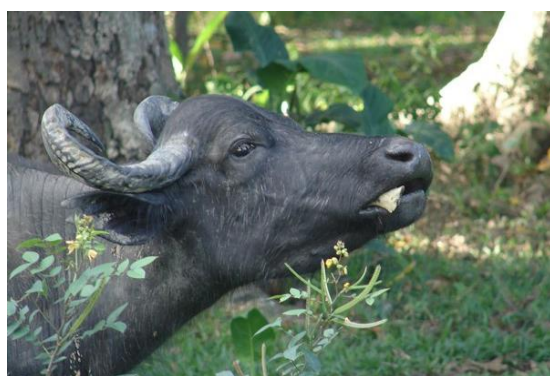
The importance and prevalence of P deficiency was consistently reaffirmed by studies conducted in the decades that followed [19–22]. Clinical and pathological pictures of severe phosphorus deficiency were observed in the western and southern regions of Minas Gerais, in the border and highland of Rio Grande do Sul, in the “lavrados” of Roraima, in Marajó island, in the tablelands of Maranhão and Tocantins, and in the grasslands of the cerrado biome (in the southern and northern areas of Mato Grosso, in the sandy soils of the eastern region of the Pantanal), in several regions of Bahia, in the northeast region of Espírito Santo, and in coastal areas in the north of the state of Rio de Janeiro [23].

2.2. General Aspects of P Deficiency

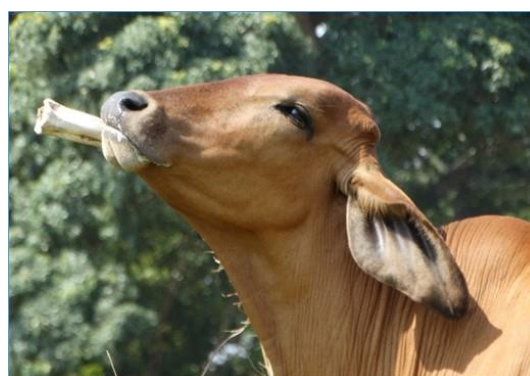
In many regions of Brazil, cattle and buffaloes consuming diets low in P may develop clinical or subclinical P deficiency. Phosphorus deficiency is always linked to certain geographical areas in Brazil [11,19,23], similar to what happens in other parts of the globe, such as South Africa [24] and Australia [4–6,25]. When severe, it can be responsible for low productivity and reduced income in certain regions, rural or urban, that are heavily reliant on livestock production. According to popular belief, unfortunately propagated even in the university environment, buffaloes are “hardy” animals and would be quite resistant to mineral deficiencies. However, this is far from reality, as in fact buffaloes are much more sensitive to developing clinical P deficiency than cattle, but the clinical signs in affected buffaloes are similar to those in clinically deficient cattle [22,23].

2.3. Clinical P Deficiency

Clinical P deficiency occurs when daily P ingestion is much lower than the amount required to meet the maintenance needs of an animal. For example, a 500 kg lactating beef cow producing 5 litres of milk per day requires 18–20 g/day of P [15]; if it is only consuming 8–9 g per day from the pasture under grazing conditions for an extended period, the cow is likely to develop clinical signs of P deficiency. Clinical P deficiency can be easily diagnosed due to its drastic consequences for the animal [11,23]. Among the more common manifestations associated with clinical P deficiency are osteophagia, i.e., the habit of chewing bones, a fact similarly observed in buffaloes (Figure 2a) and cattle (Figure 2b).



(a)



(b)

Figure 2. Clinically P-deficient buffalo (a) and cow (b) performing osteophagia [11,23].

Other characteristics commonly observed in clinically P-deficient animals are bone fragility and/or anomalies (joint enlargement, deviations, osteomalacia, etc.) (Figure 3a). Management fractures are considered side effects of the issue (Figure 3b).



(a)



(b)

Figure 3. (a) Buffalo steer over 3 years old, underdeveloped, with postural deviations and thickened tarsal articulation (hock joint) [11]. (b) Nellore steer with a metatarsal fracture that occurred while unloading the truck [11].

Bone fragility may cause postural deviations that compromise locomotion, a situation known by the colloquial names of “peg-leg” or “stiff gait” (Figure 4a,b). In these instances, the animals tend to avoid flexing the joints of the forelimbs, which exaggerates the flexion of the hock joints and causes a tendency for the limbs to be held underneath the body; as a result of these postural anomalies, a shortened stride occurs at the front and the rear. These animals are always reluctant to move when stimulated to walk, possibly, due to pain. The arching of the back (kyphosis) is interpreted as an anti-pain position (Figure 4b).



(a)



(b)

Figure 4. (a) Clinically P-deficient Nellore cow with stiff gait [11]. (b) Buffalo cow with “peg-leg” and arched back (kyphosis) [11].

In cases of acute P deficiency, lordosis has also been observed (Figure 5a,b).

Another manifestation that draws attention is that the cows show a sharp drop in body condition after calving, even becoming cachectic, as seen in Figure 6a,b.

The fragility of bones, cited above, is easily observed during postmortem examination. In severe cases, rib bones can be cut relatively easily during the necropsy (Figure 7a), and due to their increased porosity are able to float in water (Figure 7b).



Figure 5. Clinically P-deficient buffalo cow (a) and crossbred cow (b) with severe lordosis [11,23].



Figure 6. (a) Very lean Nellore cows (approximately 30 days of calving) with distinct difficulty in walking when the photo was taken [11]. (b) Cachectic buffalo cow that had recently calved, with severe difficulty in moving [23].



Figure 7. (a) Rib from buffalo with clinical P deficiency being easily cut by the necropsy knife [23]. (b) Bones of cattle with clinical P deficiency floating in water [23].

Epizootic botulism is one of the most important causes of death in adult cattle in Brazil [26,27]. It is a fatal disease caused by the ingestion of the botulinum toxin, types C and D, produced under certain environmental conditions by the bacterium *Clostridium botulinum*. In some regions, osteophagia, triggered by P deficiency, can result in many cases of botulism (Figure 8a,b).



(a)



(b)

Figure 8. (a) Cow in the initial phase of botulism, in sternal decubitus and with sensorium in a state of alert. (b) A few days later, unable to retract the tongue when this organ is pulled out of the oral cavity ("the tongue test" is a common procedure among veterinarians to confirm a suspected case of botulism) (Photos courtesy of Prof. José D. Barbosa).

In buffaloes, the signs and pathological picture of clinical phosphorus deficiency are not dissimilar to those observed in cattle [22,23]; this is a very important consideration that is contrary to widespread belief.

All of these clinical manifestations result in considerable economic losses for producers. An important detail is that the clinical deficiency of P is not commonly found in Brazilian farms [11], although there are obviously many regions in the country where the P deficiency is clinical, e.g., Marajó Island, and in large areas of the Pará, Maranhão, and Roraima states. However, of the total area dedicated to national livestock production (especially cattle), only a small percentage is on soils that would lead to the severe clinical deficiency of P.

2.4. Subclinical P Deficiency

Subclinical, i.e., below clinical detection, occurs when the amount of P consumed is slightly below the requirement for maintenance and/or production, and for prolonged periods. Despite the lack of clinical symptoms, this form of the disease has severe economic impacts due to reduced growth and delayed maturity, subfertility and low carcass yield, or low milk production leading to poor liveweight of calves at weaning. Unfortunately, the deleterious consequences caused by subclinical P deficiency are often found in other problems that herds may be affected by, such as (a) uncontrolled parasitism, (b) low forage mass on offer, (c) a high number of cows with teat problems, (c) brucellosis or other diseases affecting reproduction, (d) a deficiency of another mineral, e.g., Co, (e) a high proportion of old cows (i.e., more than six deliveries) or primiparous cows in the herd, and (f) the use of sires and cows of low genetic merit.

This form of the disease has severe economic impacts and, in the Brazilian conditions, is aggravated by advertisements for purely commercial purposes which say that the low performance of ruminants raised on tropical pasture systems is due to P deficiency, and less experienced professionals may be persuaded to believe that the main causal agent of low animal performance or any decrease in cow fertility is always linked to a mineral deficiency, e.g., P.

Contrary to what is said about the clinical deficiency of P, the subclinical form is the most common form found in Brazilian farms, and it requires skilled professionals to understand the causes and correctly diagnose this form of deficiency [11].

3. Diagnosing P Deficiency

Diagnosis is a complex process of human thought that serves to identify, through data and hypothesis, what is affecting a particular animal or herd. In human medicine and veterinary sciences, any diagnosis is based on a strict order: (a) detailed history taking, (b) physical examination, and (c) the utilization of ancillary or complementary tests. The rupture or inversion of this order may result in no diagnosis, or in the worst scenario, an incorrect diagnosis.

For the diagnosis of P deficiency, as well as that of any other disease, it is necessary to study its various manifestations and to explore the issue from different “angles”; only then can conclusions be drawn by analyzing the set of data obtained [11,19,23].

The first and crucial step is to obtain a careful and detailed history of the herd and property management. It is important to emphasize that only with this information and its correct interpretation can the diagnosis of clinical P deficiency be reached. An example of the latter affirmation would be the presence of osteophagia, associated with cases of fractures during herd management, low fertility rate (e.g., <40%), and many cases of animals showing “peg leg”: these are all strong indications of clinical P deficiency.

After history taking, the professional must perform a detailed inspection/examination of the property/herd environment, which involves: (a) the search for bones left on pastures and close observations to verify if they have been chewed; (b) the presence of animals performing osteophagia; (c) identification of postural alterations or animals with fractures; (d) the presence of plants that indicate soils of low fertility, e.g., “mexerico” (*Leandra hirtella*), bracken fern (*Pteridium aquilinum*), West Indian foxtail grass (*Andropogon bicornis*), and “ciganinha” (*Memora peregrina*); (e) the presence of plants indicative of moderate soil fertility, i.e., some legumes, which require moderated soil pH, low toxic aluminum and moderate levels of P in the soil; in the Brazilian cerrado, the “capitão do cerrado” (*Terminalia argentea*), “balsemim” (*Diptychandra aurantiaca*) and “baruzeiro” (*Dipterix alata*) are considered to be “good” land indicators; and (f) examination of the mineral supplementation scheme, i.e., whether supplements are kept in covered troughs, whether the troughs are kept in suitable areas with sufficient bunk space; and (g) the recommendation that if animals with fractures or *in extremis* are found, euthanasia and subsequent necropsy should take place, with an emphasis, above all, on the investigation of the skeleton and signs of osteomalacia, as well as the collection of material for histological examination.

The suspected cases of subclinical P deficiency can only be diagnosed after the correct execution of complementary (ancillary) tests on the animals, with its confirmation obtained by experimentation, in which a group of animals receives supplementation containing P and their biological responses are evaluated over a few months, for up to a year. These procedures, both diagnostic and confirmatory, should only be performed by trained professionals who have the skills and knowledge to understand the subject [11].

Lastly, the professional can carry out the so-called ancillary diagnostic tests that serve to support the decision-making process in relation to the fact/problem. However, it is important to note that such tests should neither be the first nor the most important step when conducting a professional diagnosis. Ancillary tests are important, especially when a subclinical or marginal P deficiency condition is suspected, the manifestations of which are more subtle and difficult to perceive [11]. Below we discuss the main ancillary tests used in the support of diagnosis of P deficiency in cattle and buffaloes.

3.1. Collection of Soil Samples

As the deficiency of P in the animal is linked to the concentration of P in the forage ingested, this, in turn, is dependent on the P content of the soil. Results from soil testing provide an indication of the potential for P deficiency and are commonly recommended.

However, sampling errors must be considered. Soil sampling is a laborious process that requires human effort and specific equipment. Another important aspect is that often within the same farm, there is a huge variation in the concentration of P in the soils over short distances, often within the same paddock. It is important that soil samples represent the complete grazing area, but due to the selective grazing nature of livestock, this may still not be representative of the diet consumed.

The analytical results are quite complex to interpret, regarding the part referring to the status of P in animals. It must be considered that not all the amounts of P in the soil are absorbable by the roots of the forage plants. In well-managed pastures, with stocking rates that leave a good amount of plant litter, i.e., mainly dead leaves, much of the P is recycled almost directly from the litter to the roots of the grasses in close proximity to the soil surface; thus, much of the recycled P does not enter the deepest layers of the soil. Therefore, although soil samples taken between 0 and 10 cm and between 10 and 20 cm can correctly be used for establishing a new pasture area or fertilization program in existing pastures, they may not reflect the real concentration of P in the soil when the primary objective is its use as an ancillary test for the diagnosis of P deficiency in ruminants (Table 1).

Table 1. Analysis of soils sampled at different depths. The subsamples ($n = 12$) were taken with a probe and bulked to a composite sample for each depth. The 0 to 3 cm samples were taken about 4 cm from the place where the 0 to 10 cm samples were taken ¹.

Soil	Depth (cm)	P ² mg/dm ³	Organic Carb. (%)	K mg/dm ³	Ca	Mg	H+Al Cmol _c /dm ³	pH	BS ³ (%)
A	0–3	22	1.53	393	1.5	1.2	6.6	5.6	36
A	0–10	21	1.04	118	0.70	0.90	8.2	4.9	19
B	0–3	26	2.08	202	3.4	2.9	6.6	5.8	51
B	0–10	23	1.44	145	3.0	1.9	7.9	5.1	40
C	0–3	4.0	1.39	78	0.40	0.20	6.1	4.1	12
C	0–10	2.0	1.10	31	0.20	0.10	6.1	3.9	6
D	0–3	15	1.91	191	2.5	2.0	3.8	5.1	57
D	0–10	9.0	1.22	129	2.2	1.4	3.3	5.0	54

¹ Data previously cited in Malafaia [11]; ² exchange resin used as extractor; ³ base saturation.

A suggestion drawn from the issue described above is to sample the soil to approximately 3 cm in depth when desiring to better understand P content in soils, but to use the sample exclusively as an ancillary tool [11]. These latter aspects greatly increase the difficulty of drawing valid conclusions about the use of soil analyses for the diagnosis of P deficiency in ruminants.

3.2. Collection of Forage Samples

In Brazil, for decades, forage sampling and analysis have been used to establish whether or not its composition is adequate for different minerals, and thus propose a “diagnosis of the situation” and even formulas for mineral mixtures for a given farm [28–30]. However, from a medical point of view, i.e., considering the conceptual postulates of semiology, a “diagnosis” should not be made based only on an ancillary diagnostic test. The practice of using of forage samples to diagnose P or other mineral deficiency in certain instances is currently taught in universities and adopted in research centres in Brazil [11]. Nevertheless, we strongly recommend for any diagnosis that it should require a strict order, starting with a detailed history taking, followed by physical examination and completing with ancillary exams. It is worth mentioning that forage sampling is not without value, but it is relative, and this methodology cannot be seen as the only or the first option to establish a diagnosis of any mineral deficiency (e.g., P, Co, Cu, Zn, Se).

As limitations of the “diagnosis” of P deficiency, established by forage analysis, it is worth mentioning: (a) in the tropical grassland ecosystem it is extremely difficult to make a representative sample of the forage that cattle and buffaloes actually eat, as animals

in tropical pastures also eat plants others than grasses [11], which may contain several nutrients, including P; (b) it is rarely possible for such sampling to be reliable, given the great heterogeneity of the pastures and the immense areas to be sampled; (c) there may be considerable variation in the analytical results, i.e., from the same forage sample analyzed in two different laboratories; and (d) there are variations in the levels of P in samples from the same pasture at different seasons of the year (dry vs. wet season). These discrepancies make the interpretation of the forage analyses rather subject to inexact results and invalid conclusions [11].

The differences in results sometimes found among laboratories, as described above, is probably due to the lack of standardization. An example of the latter statement can be seen in Table 2.

Table 2. Variation in P concentration between two laboratories. The forages were collected, dried (60 °C over 72 h), ground and then 50% was sent to each of the two laboratories ¹.

Forage	P Concentration (g/kg Dry Matter)		Variation (%)
	Laboratory 1	Laboratory 2	
<i>Cynodon nlenfuensis</i>	1.05	1.10	−5
<i>Melinis minutiflora</i>	0.87	1.32	52
<i>Paspalum notatum</i> —sample a	0.48	1.50	212
<i>P. notatum</i> —sample b	0.35	1.00	186
<i>Panicum maximum</i> , cv. Tanzania	2.37	1.70	−28
<i>Urochloa brizantha</i>	1.44	1.50	4
<i>U. decumbens</i> —sample a	0.96	1.30	35
<i>U. decumbens</i> —sample b	0.13	0.80	515
<i>U. decumbens</i> —sample c	0.23	1.00	335

¹ Data previously cited in Malafaia [11].

The fact that cattle and buffaloes graze other plants and not just the grasses is well known by researchers and people working with livestock. Figure 9a shows a heifer grazing *Hyptis pectinata*, from the *Lamiaceae* family (Figure 9b). These plants are widespread in South America and have a different root system, which goes much deeper in the soil horizons than grasses of the *Poacea* family. The latter is the most likely reason for a much higher P concentration in a plant that is capable of seeking the mineral from deeper layers of the soil.

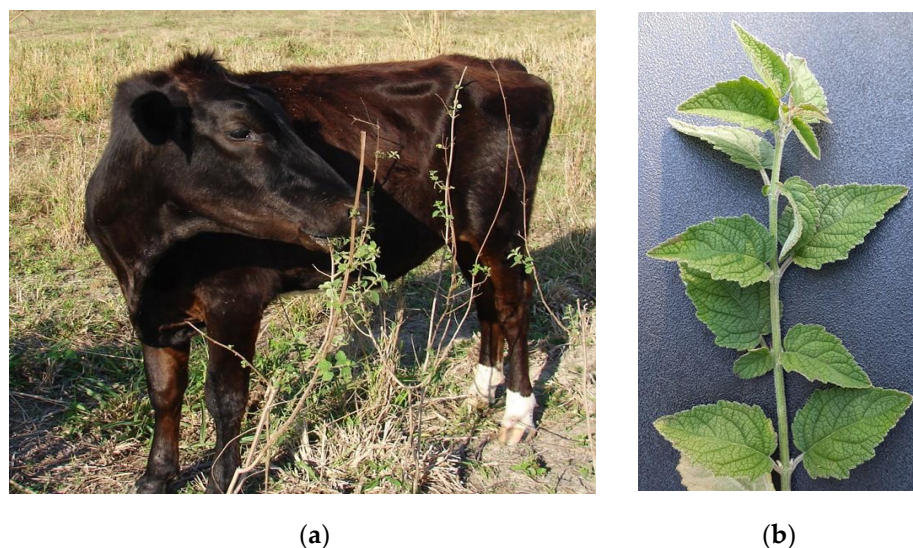


Figure 9. (a) Heifer grazing a non-grass plant (*Hyptis pectinata*) [11]. (b) Closer view of an example of *Hyptis pectinata* with concentrations of crude protein, P and Ca of 108, 2.13, and 12.9 g per kg of dry matter, respectively [11].

In conclusion, both soil and pasture samplings have their relative values; however, they should be considered only as a complement to the study of P deficiency and should not be used in isolation from the herd's history and physical examination.

3.3. Blood Sampling

Blood samples can be useful in the diagnosis of P deficiency, when collected at the right moment and in the right way, and when the evaluation is always used in association with other information about the animals/herd. Ruminants have an efficient system to regulate phosphatemia, which keeps blood P within the normal reference values (3.5 to 7.0 mg/dL or \cong 1.1 to 2.3 mM). When the daily intake of P, for a short period, is less than the animals' need, they will mobilize P from the bones and, thus, maintain the normophosphatemia status. If daily P consumption is below the requirements for long periods, then animals can accelerate the depletion of their bone P reserve; in this situation the blood P concentration is reduced (hypophosphatemia).

During the dry season, owing to the fact that forage has low P levels, plasma inorganic phosphorus (Pi) values are more correlated with bone mobilization and recycling of salivary P than with daily phosphorus intake. For this reason, Pi values obtained during the dry season (forage naturally limited in P, crude protein (CP), and energy) have no diagnostic value.

Hypophosphatemia is accentuated when the animals are eating diets that are adequate in protein and energy and low in P—as is the case in forage deficient in P—during the rainy season. For this reason, as an ancillary test, the Pi dosage should be measured in cattle and buffaloes (avoiding cows that have just calved and those in the final stage of pregnancy where, in both phases, there is characteristically high mobilization of P from the bones), not supplemented with P (for at least one month) and kept in “green” pastures, i.e., with higher levels of crude protein and energy, from the middle to the end of the wet season. During that time, values below 3.5 mg/dL are indicative of clinical deficiency, values between 3.5 and 4.0 mg/dL are suggestive of subclinical deficiency, and values above 4.0 mg/dL are compatible with non-deficient animals [11,23]. Another important fact is that blood Pi values, obtained in the middle to the end of the wet season, should always be compared with faecal P and nitrogen (N) (Table 3).

Table 3. Interrelationship between the values of blood P and faecal N of cattle raised on pastures during the middle to the end of the rainy season and not supplemented with P.

Blood P (mg/dL)	Faecal N (%DM)	Probable Herd Status ¹
<3.5	1.25–2.5	Clinical deficiency
3.5–4.0	1.25–2.5	Subclinical deficiency
3.5–4.0	>2.5	Clinical deficiency
>4.0	>1.25	Not deficient

¹ Criteria used and recommended by Malafaia [11], always in association with history taking and clinical examination of the herd. Faeces with less than 1.25% N are found in animals during the dry season.

The main limitations to using the Pi measurements in support of a diagnosis of P deficiency are that the Pi concentration is influenced by: (a) stress and/or physical exercise prior to collection, (b) hemolysis (during collection, storage, and transport); (c) the elapsed time between sample collection and separation of the serum; and (d) the blood collection site, i.e., jugular vs. coccygeal vein. The blood collected from the jugular vein has a lower concentration of Pi, i.e., up to 20% lower than blood from the coccygeal vein; this may be due to the fact that most of recycled P for the rumen is extracted by the salivary glands from the blood that passes through the jugular vein.

Another limitation is that blood samples taken days after the first rains, subsequent to the end of the dry season, give higher Pi values, even in animals known to be deficient in P [23].

3.4. Sampling of Fresh Faeces for the Measurement of P Concentration

Faecal P concentration is directly related to forage P concentration, as pioneered demonstrated by K.W. Moir, in 1960 [31]. Faeces are the main route of excretion of P, especially in ruminants that are not producing milk or in those producing little milk, i.e., less than 8 kg/day. In this context, cattle and buffaloes raised extensively on tropical pastures are the obvious candidates to have their faeces sampled for the determination of faecal P. In diets not limited in P, there is a direct relationship between daily intake of CP (N) and faecal excretion of P; therefore, an increase in daily intake of CP increases the faecal excretion of P. In other words, during the rainy season, pastures that are established in soils not deficient in P—as they have a higher concentration of CP—will result in a higher content of faecal N and P. However, if the forage is grown in P-deficient soil, in the wet season they will guarantee a higher intake of CP and a lower intake of P; this will produce faeces with a lower P concentration, and in this situation the measurement of faecal P can be useful as a complement to the diagnosis of P deficiency in herds. On the other hand, during the dry season, forage naturally reduces the concentration of CP and P, which leads to a natural reduction in the values of fecal N and P.

Thus, it seems logical that the measurement of faecal P is a reasonable ancillary test which may help greatly in the diagnosis of P deficiency (in herds), but only if the faecal samples are collected in the middle to the end of the wet season and come from animals not supplemented with P for at least one month.

The collection of faeces must be performed from the top of a fresh faecal pie, without contamination with the soil or with another source of organic material. Each faecal pie is sampled with the aid of a tablespoon or with the fingertips. For good sampling, about 10 faecal pies are required per paddock. The samples must be collected directly from the pasture to be investigated and placed in the same plastic bag to form a composite sample. After the sampling procedure, the composite sample is homogenized and preserved, under refrigeration, until it is sent to the laboratory. The professional should request the measurement of P and N in faecal dry matter (DM). If the values of N and P are not expressed on the faecal DM basis, their interpretation will be compromised.

The authors assume, based on their own data, that (in the wet season) when animals produce faeces with more than 1.25% N (in faecal DM) and less than 2.4 g of P per kg of DM, they have some degree of P deficiency (Table 4).

Table 4. Interrelation between faecal N and P values obtained from cattle not supplemented with P and kept in pastures of *Urochloa decumbens* or *U. brizantha*, during the middle to the end of the wet season.

Faecal N [% Dry Matter (DM)]	Faecal P (g/kg DM)	Probable Herd Status ¹
1.25–2.0	<2.2	Clinical deficiency
	2.2–2.4	Subclinical deficiency
	>2.4	Not deficient

¹ The criteria are always used in association with history taking and clinical examination of the herd.

It is important to understand that the higher the faecal N and the lower the faecal P, the more severe the deficiency and the more effective will be the supplementation with P. In addition, faeces with less than 1.25% N are commonly found in animals during the dry season, as the intake of N is drastically reduced.

The concentration of P in the diet of grazing ruminants can be estimated with the use of near infrared spectroscopy (Faecal NIRS) [32]. The latter technique also allows the estimation of metabolizable energy (ME) and nitrogen (N) concentrations in the diet. Combining the estimates of dietary ME, N and P would theoretically indicate whether the current requirements of the animals are supplied by the diet [32]. Despite being very promising, the latter technique has not been applied in Brazil.

It is important to keep in mind that a given faecal P value should be interpreted with caution and always corroborated with as much data as possible about the herd.

3.5. Bone Biopsy

Among the different ancillary tests, rib biopsy in adult ruminants stands out as a very useful procedure [33,34]. Once the rib sample has been collected, the variables that would be measured are the cortical thickness (CT) and specific bone density (SBD) [11].

The cortical thickness (CT) is expressed in mm. In this evaluation, it is required to make the measurement in the thickest region of the cortical layer, i.e., for the rib, the CT of the caudal border is naturally thinner, whilst the CT of the cranial border is naturally thicker. To avoid diagnostic confusion, it is recommended to always measure the CT on the cranial border of the lateral side of the rib (Figure 10) [11].



Figure 10. A twelfth rib from an adult bovine clinically deficient (left) and one from an animal not deficient in P [11]. Note that the cortical thickness of the cranial border of the lateral side of the rib (upper red mark) is thicker than the caudal border (lower red mark). In summary, the professional should always measure the CT on the lateral side of the rib and on its cranial border.

As the CT varies with age, body weight and the physiological status of the animals, i.e., onset of lactation vs. mid-lactation, it should not be used as the only parameter to establish a diagnosis of P deficiency, especially the subclinical form [11]. In addition to history taking, clinical examination of the herd, and necropsies in adult animals, the authors use values below 2.5 mm for clinical P deficiency, between 2.5 and 3.0 mm for subclinical P deficiency and greater than 3.0 mm for non-deficient animals.

The specific bone density (SBD) is obtained following the Archimedes principle. The SBD is estimated by the ratio between the mass and the volume of water displaced by the bone sample. Densities less than 1.4, between 1.40 and 1.50 and greater than 1.50 g/cm³, are, respectively, suggestive of clinical, subclinical and P-adequate animals.

As stated for the other ancillary tests, CT and SBD values (given the considerable source of variation in the data) should not be used alone in the diagnosis of P subclinical deficiency.

3.6. Needle Test

The needle test (NT) is a point-of-care test developed in Brazil to evaluate the degree of P deficiency in adult cattle or buffaloes raised in pastures. Based on bone resistance, the NT is a very inexpensive method which allows for the diagnosis of any degree of P deficiency in a fast and simple way, in vivo and directly on the farm. Cattle or buffaloes must be physically restrained in the crush before performing the NT. For the NT, a needle (18G × 11/2") is inserted through the skin, subcutaneous and muscle layers until it reaches the transverse process (TP) of the lumbar vertebrae L₃ or L₄. Once positioned about halfway

through the TP, the operator pushes on the needle gun using the index finger to try to make the needle penetrate through cortical layer of the transverse process [35]. The NT measures three levels of resistance in the transverse process (TP) of the lumbar vertebrae: (a) TPs that are impenetrable and result in warping or bending of the needle (P-adequate animals); (b) TPs offering some resistance to the penetration, resulting in use of great effort by the operator to pierce the TP, leaving a pronounced mark on the operator's finger (animals with some degree of P deficiency, often subclinical); and (c) TPs which have minimal resistance to penetration (clinical P deficiency).

Although this test is useful in helping to diagnose P deficiency, similarly to other ancillary tests it should not be evaluated in isolation. As taught in semiology, any complementary tests have to be performed in conjunction with history taking and examination of the herd [11,35]. It is also important to emphasize the need to perform the NT in animals over 300 kg and halfway across the transverse process of the L₃ or L₄ vertebrae, as the distal end of the transverse process (marked with an "x" in Figure 11) is more cartilaginous and may allow the needle to penetrate quickly and without resistance, leading to false-positive results.

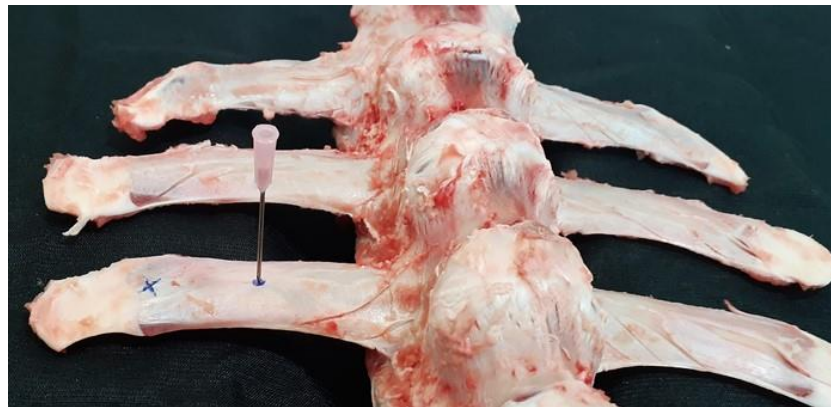


Figure 11. Needle inserted at the correct location for testing in the lumbar vertebrae, i.e., approximately half the length of the transverse process, and the more fragile (cartilaginous) end of the transverse process (X = incorrect test site) where if tested on a P-adequate animal would generate a false-positive result [11].

Figure 12 illustrates the NT performed on animals with various degrees of P deficiency, from non-deficient (top), subclinical P deficiency (middle) and acute P deficient (bottom), respectively.

A study carried out on five farms showed a good association between NT results, cortical thickness, and specific density in water of bone fragments obtained from the twelfth rib of healthy cattle and animals with clinical and subclinical P deficiency [35].

3.7. Experimentation

Experimentation in field studies measuring production gains from supplemental P under controlled situations is an extremely valuable resource that allows for the diagnosis of P deficiency, especially the subclinical type. It essentially consists of administering P (which is suspected to be deficient in the herd) to a group of animals, kept on pasture suspected of being deficient in P. Another group of animals must remain as a control; that is, without receiving the P supplemented to the first group. The studies carried out by several Brazilian researchers are excellent examples of the use of experimentation in the comparison of different mineral supplementation schemes for cattle [35–40].

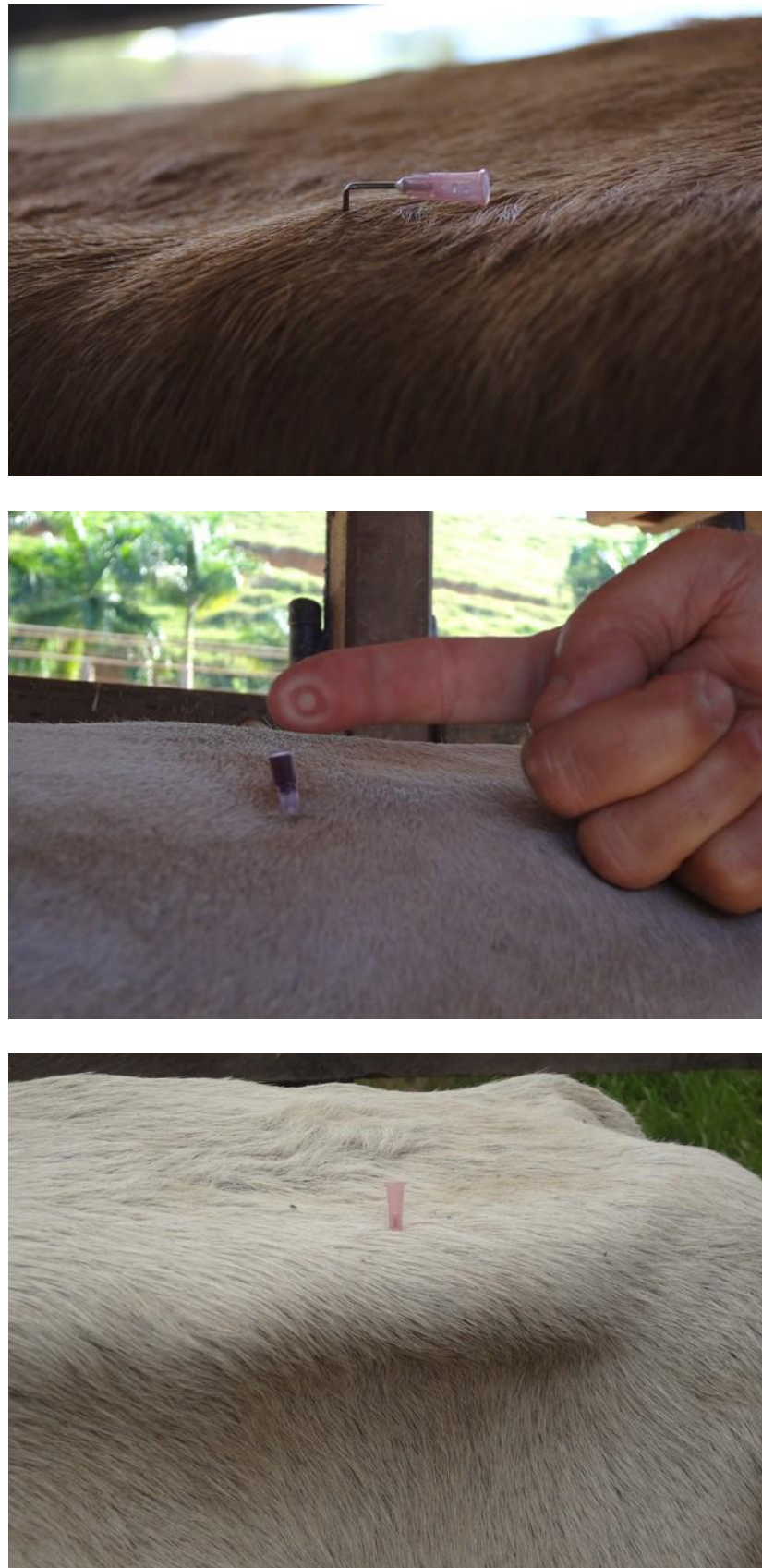


Figure 12. (top) Cow with no P deficiency (warped the needle); (middle) cow with subclinical P deficiency (note the pronounced mark on the operator's finger); and (bottom) cow with clinical P deficiency [34].

This type of experiment may only be carried out if some basic requirements are met: (a) it is performed in the wet season (where the metabolic need for P is greater); (b) it is undertaken using animals of the same sex, breed, genetic group and similar in weight; (c) the groups must be kept in separate pastures; (d) the forage must be of the same species; (e) the forage mass on offer must be adequate, i.e., the availability of dry matter of the forage must be high, as there can be no limitation in the forage intake; (f) the pastures must have a similar topography; and (g) the duration of the experiment should not be less than 150 days, since the responses that indicate when there is a subclinical deficiency are verified later. The main variables for evaluating the results are the average daily weight gain, the fertility rate, and the average weight at weaning.

Experimentation, although an excellent option in helping to diagnose subclinical P deficiency, has the inconvenience of being laborious and requiring more time for diagnosis. However, it is the best way to confirm a diagnosis and to arrive at the ideal mineral formulation for a specific farm. Unfortunately, it is a method that is rarely used in Brazilian farms; perhaps because it is not properly taught in animal sciences and veterinary courses or because it might be unfeasible for some/most farm operations.

3.8. Concluding Remarks about the Ancillary Tests

In short, regarding the ancillary tests described above, the greater the number of data collected and correctly interpreted, the greater the chances of reaching a correct diagnosis [11,23]. The baseline values for faecal P, blood, rib parameters and the NT were obtained from hundreds of animals with clinical and subclinical P deficiency from cattle operations in Brazil with distinct nutritional managements, soils, and climate. These values were used to reach the correct diagnosis of P deficiency and should be used with care when applied elsewhere.

The safest and most correct procedure in the diagnosis of P deficiency is to obtain an excellent history taking, to carry out a detailed examination of the herd (based on clinical and pathological principles) and to complement it with ancillary tests of material(s) coming from the animals (blood, faeces, and bone). The final certainty will be given by experimentation [11]. As previously mentioned, forage and soil analyses have secondary importance.

4. Prophylactic Measures after P Deficiency Is Diagnosed

Once the correct diagnosis of P deficiency (clinical or subclinical) in a herd is established, the professional should indicate the appropriate corrective and prophylactic measures as described below.

4.1. Fertilizing the Soil with Phosphate Fertilizers

Fertilizing the soil with phosphate fertilizers would be the ideal measure because, in addition to solving the problem of P deficiency in the herd, in some cases, it also increases the forage production and the farm's stocking rate capacity. Unfortunately, it is a very expensive method of correcting P deficiency in farms where only livestock is raised; especially in those located far from grain-producing areas or in places with difficult access, i.e., on Marajó Island, most of which can be flooded during the rainy season.

In Brazil, large areas destined for grain production adopt the so-called crop–livestock integration system, which consists of using cattle to graze areas where grass has been planted alongside annual crops. These grass species serve as soil-cover for protection from erosion, improve soil-plant health by breaking the lifecycles of plant pathogens, and add organic matter to the system, improving the yield of the next agricultural crop (Figure 13a,b).



Figure 13. (a) Combined use of corn and *Urochloa brizantha* in a crop–livestock mixed system. (b) Corn being harvested and the grass left for subsequent grazing (Photos courtesy of Dr. Rodrigo Almeida).

Areas subjected to cropping (e.g., corn, soybeans, sunflowers, beans, potatoes, tomatoes, etc.) require prior soil preparation (e.g., lime, fertilization, etc.) (Figure 14a), and a range of different agricultural practices, often requiring the use of roads between plots (Figure 14b). Among the fertilizers, P is often a mandatory component in these crops.



Figure 14. (a) Soil preparation for cropping in which use of lime and P fertilizers are the norm [11]. (b) Tomatoes in full vegetative stage, with intermediate rows for traffic of people and machinery between plots [11].

After use of the area for cropping, it is then managed with pastures. Despite not requiring as much fertilization, the grass benefits from residual fertilization, becoming more concentrated in P in comparison with pastures in conventional systems. This represents a way to provide this expensive mineral to the ruminants that subsequently graze these areas. An example of the latter can be clearly seen in Figure 15 where the grass was sowed just before the end of the tomato harvest in a crop–livestock system.



Figure 15. *Urochloa brizantha* in a vegetative phase, after being sown just before the end of tomato harvest; the pasture on the left had been not submitted to crop–livestock integration [11].

The latter aspect emphasizes the importance of understanding the previous use of the area when gathering information for a P-deficiency diagnosis. In summary, professionals need to know that in some situations, depending on previous use of the area (which will depend on a correct diagnosis) the P supplementation of herds could be achieved with mineral supplements with no added P or with lower concentrations of this mineral.

4.2. Supply of P to Animals, Orally, Mixed with Common Salt (NaCl)

In Brazil the supply of P + NaCl to animals orally is the most used and indicated method, either with commercial mineral mixtures (CMMs) containing several minerals (including P) or in the simple P + NaCl mixture. Whatever the product, it is left in troughs, so that the animals ingest it. In general, supplementation of cattle and buffaloes with commercial mineral mixtures (CMMs) has been advocated for decades in Brazil. Popularly and mistakenly, the CCMs are called “complete”. It is often believed that this practice would dispense with all laborious diagnostic work, as described above, and it would be great if the widespread use of CMMs, for all cattle and buffaloes, solved the problems related to P deficiency. However, unfortunately, this procedure is not the best way to address the problem, as it is an uneconomic method [41]. According to the knowledge of our research team, the only minerals in which deficiency has been correctly diagnosed in cattle and buffaloes raised in the Brazilian pastures, and which must be supplemented, are sodium, phosphorus, copper, cobalt, and selenium [19,20,22,23,41]. Even so, the joint supplementation of all these elements would be a considerable waste of money. There are regions where mineral deficiencies do not occur and where they do occur, most of the time they do not involve all these elements [23,36,41].

On the other hand, paradoxically, there are commercial recommendations that an ideal mineral supplement would contain, in addition to countless other minerals, 6 to 8% P in its composition and that this mixture should be provided throughout the year. Unfortunately, this recommendation has become practically a dogma for cattle and buffalo production in Brazil.

Mineral supplementation is not a cheap practice, especially when P is involved, the deficiency of which is the most important, and its correction the most expensive. Providing additional P to cattle or buffaloes above daily requirements is not financially viable. On the other hand, if there really is a P deficiency in a herd, the correct course of action is supplementation with an appropriate mixture, i.e., based on experimentation.

Mineral supplementation of grazing ruminants should be based on the following principle: diagnose whether or not there is a deficiency of one or more elements in the herd; if so, supplement the deficient mineral(s) with adequate amounts, always systematically monitoring intake and performance, e.g., fertility rate, weight gain, and the clinical aspects of the animals.

Analogously P can be compared with a “growth promoter”: its supplementation will only make nutritional and economic sense if it is used in animals in a phase of accelerated anabolism (i.e., after weaning until puberty, or in the final trimester of pregnancy and after calving, or until about 50 to 60% of the lactation cycle) and, above all, if the diet is sufficient in protein and energy, i.e., forage during the wet season.

It still has to be remembered, especially in relation to P, that there will be no response to its supplementation if its deficiency is not the limiting factor; in the dry season, in farms with adequate forage mass on offer, the main limitation of performance is protein and energy deficiencies. During the dry season, supplementation with P is only useful if the intake of protein and non-structural carbohydrates was previously met or corrected; during this time, although the forage is naturally low in P, it is not the main limiting factor for animal productivity. In the dry season, in situations where there is no adequate forage mass on offer, supplementation with P, nitrogen, and energy produces null or imperceptible results.

Furthermore, it is necessary to remember and interpret the biocatalyst function of minerals as that which can only be effective if there are within the cells (i.e., the tissues) the other dietary substrates, e.g., water, carbohydrates, amino acids, fatty acids and vitamins, for metabolism to occur to its fullness. In Figure 16, it can be clearly seen that it is useless to provide minerals, via supplementation, if there is not adequate intake of forage, especially if this has low N and energy content; this condition worsens if there is a restriction in water intake and/or if the forage is of low quality, i.e., contaminated with faeces, urine, algae. During decades of research, we have seen countless herds submitted to quantitative and qualitative restriction of forage mass on offer and receiving commercial mineral supplementation; they often had limited water supply or only had access to water of low physico-chemical and microbiological quality.

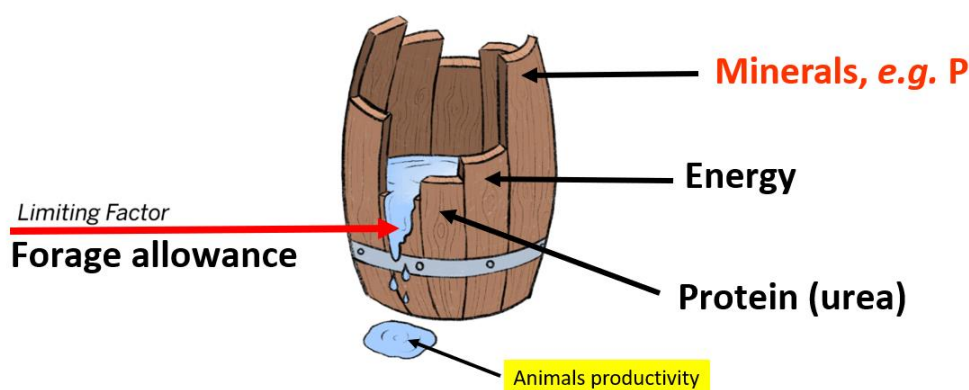


Figure 16. The law of the minimum applied to a ruminant production system in the dry season, in which the limiting variable (lowest stave of the barrel) is the forage mass on offer.

Another recommendation that finds support, even by academics who teach ruminant nutrition, is that the preparation of a mineral supplements should not be executed on the farm. There is no justification for this claim because any professional with good knowledge of ruminant nutrition can perfectly formulate and prepare a mineral supplement on the farm. However, some precautions must be taken when purchasing the ingredients, storing them, and mixing them with sodium chloride.

As indicated above in Figure 16, if there is an adequate forage mass on offer, the next limitation will be the intake of N, which should be guaranteed via supplementation of

urea or a true protein source. Supplementing with N, energy, or minerals brings little to no success when the forage supply is not primarily corrected. Unfortunately, the non-observance and compliance with the law of the minimum is responsible for the fact that the vast majority of cattle and buffalo supplementation programs in Brazil neither result in benefits for animals nor economic returns for producers. The extensionist recommending supplementation of ruminants needs to know this scientific concept; otherwise, if it is based only on the commercial aspect, there is a huge risk of not solving problems and/or causing economic losses to people under their advice.

5. Conclusions

In this review we describe the main aspects of the use of P for cattle and buffaloes in Brazil, which can be summarized below.

Under Brazilian conditions, there is a need for the rational use of P, since this mineral resource is expensive, exhaustible, and has heavy external dependence.

Buffaloes are much more sensitive to clinical P deficiency than cattle; however, the clinical signs of affected buffaloes are similar to those of clinically deficient cattle.

The appropriate professional conduct when assisting livestock producers starts by the establishment of a diagnosis through careful and detailed history taking, followed by a detailed examination of the herd and the use of ancillary diagnostic tests. Remembering that ancillary tests are complementary to the diagnosis; they should neither be the first nor the most important step in conducting a professional diagnosis.

The basis for success in any supplementation scheme for ruminants (whether mineral or not) is the complete understanding of the principles of the law of the minimum, which clearly state that there is no productive response if the limiting variable is not recognized and promptly addressed. In a farm with no adequate forage mass on offer, supplementation with N, energy and, above all, with different minerals (including P) does not generate positive results. Unfortunately, from our experience, the vast majority of mineral supplementation strategies recommended by the industry for cattle and buffaloes in Brazil appear to fail in this respect.

Author Contributions: Conceptualization, P.M., V.C.d.S. and D.F.A.C.; methodology, P.M., J.D.B. and M.F.B.; validation, P.M.; formal analysis, J.D.B. and M.F.B.; investigation, P.M., J.D.B. and M.F.B.; data curation, P.M.; writing—original draft preparation, P.M.; writing—review and editing, D.F.A.C. and V.C.d.S.; visualization, P.M.; supervision, P.M., J.D.B. and M.F.B.; project administration, P.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data supporting reported results can be shared upon request directly to the first author, Prof. Pedro Malafaia.

Acknowledgments: We dedicate this work to C.H. Tokarnia (*in memoriam*) who ignited the spark that culminated in decades of research on mineral deficiencies in ruminants raised on pastures in Brazil. His great effort generated much wealth for the country and, above all, a vast team of disciples.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Theiler, A.; Green, H.H.; Dutoit, P.J. Studies in mineral metabolism (III). Building of cattle on phosphorus deficient pasture. *J. Agr. Sci.* **1928**, *18*, 369–371. [[CrossRef](#)]
2. Theiler, A.; Green, H.H. Aphosphorosis in ruminants. *Nutr. Abstr. Rev.* **1932**, *1*, 359–385.
3. Eckles, C.H.; Palmers, L.S.; Gullickson, T.W.; Fitch, C.P.; Boyd, W.L.; Bishop, L.; Nelson, J.W. Effects of uncomplicated phosphorus deficiency on estrous cycle, reproduction and composition of tissues of mature dairy cows. *Cornell Vet.* **1935**, *25*, 22–43.
4. Turner, A.W.; Kelley, R.B.; Dann, A.T. Peg-leg of cattle in north Queensland. *J. Counc. Sci. Ind. Res.* **1935**, *8*, 120–124.
5. Rose, A.L. Osteomalacia in the Northern Territory. *Austr. Vet. J.* **1954**, *30*, 172–177. [[CrossRef](#)]

6. Ternouth, J.H. Phosphorus and beef production in northern Australia. *Trop. Grass.* **1990**, *24*, 159–169.
7. Giovine, N. Estudo clínico da deficiência de fósforo nos bovinos de Minas Gerais. *Arq. Esc. Vet. UFMG.* **1943**, *1*, 17–25.
8. Menicucci Sobrinho, L. Carência de fósforo e cálcio nos bovinos. *Arq. Esc. Vet. UFMG.* **1943**, *1*, 944–949.
9. Costa, D.F.A.; Poppi, D.P.; McLennan, S.R.; Santos, F.A.P. (Eds.) Beef cattle production in northern Australia—Management and supplementation strategies. In Proceedings of the 7th International Congress on Beef Cattle, São Pedro, SP, Brazil, 19–21 December 2012. (FEALQ).
10. Dixon, R.M.; Anderson, S.T.; Kidd, L.J.; Fletcher, M.T. Management of phosphorus nutrition of beef cattle grazing seasonally dry rangelands: A review. *Anim. Prod. Sci.* **2020**, *60*, 863–879. [[CrossRef](#)]
11. Malafaia, P. *Phosphorus for Cattle and Buffaloes in Brazil*; E-book; 2020; ISBN 978-65-0003575-9. Available online: amazon.com.br (accessed on 15 August 2022).
12. Cordell, D.; Drangert, J.O.; White, S. The story of phosphorus: Global food security and food for thought. *Global Environ. Chang.* **2009**, *19*, 292–305. [[CrossRef](#)]
13. Scholz, R.W.; Ulrich, A.E.; Eilittä, M.; Roy, A. Sustainable use of phosphorus: A finite resource. *Sci. Total Environ.* **2013**, *461*–*462*, 799–803. [[CrossRef](#)] [[PubMed](#)]
14. CSIRO. *Nutrient Requirements of Domesticated Ruminants*; CSIRO Publishing: Melbourne, Australia, 2007.
15. BR Corte. *Exigências Nutricionais de Zebuínos Puros e Cruzados*; 3ª edição; Editora UFV: Viçosa, MG, Brazil, 2016.
16. NASEM. National Academies of Sciences, Engineering, and Medicine. In *Nutrient Requirements of Beef Cattle*, 8th ed.; National Academies Press: Washington, DC, USA, 2016.
17. Megale, F. Sobre a incidência da esterilidade em vacas no estado de Minas Gerais. *Arq. Esc. Sup. Vet. Est. Minas Gerais.* **1949**, *2*, 9–19.
18. Tokarnia, C.H.; Canella, C.F.C.; Döbereiner, J.; Langenegger, J. Deficiência de fósforo em bovinos no Piauí. *Pesq. Agropec. Bras.* **1970**, *5*, 483–494.
19. Tokarnia, C.H.; Döbereiner, J. Diseases caused by mineral deficiencies in cattle raised under range conditions in Brazil, a review. *Pesq. Agropec. Bras.* **1973**, *8*, 1–6.
20. Tokarnia, C.H.; Döbereiner, J.; Moraes, S.S. Situação atual e perspectivas da investigação sobre nutrição mineral em bovinos no Brasil. *Pesq. Vet. Bras.* **1988**, *8*, 1–16.
21. Tokarnia, C.H.; Döbereiner, J.; Peixoto, P.V. Deficiências minerais em animais de fazenda, principalmente bovinos. *Pesq. Vet. Bras.* **2000**, *20*, 127–138. [[CrossRef](#)]
22. Barbosa, J.D.; Oliveira, C.M.C.; Duarte, M.D.; Albernaz, T.T.; Júnior, C.A.O.; Correa, F. Phosphorus deficiency in buffaloes in the state of Pará, Northern Brazil. *Italian J. Anim. Sci.* **2007**, *6*, 971–973.
23. Tokarnia, C.H.; Peixoto, P.V.; Barbosa, J.D.; Brito, M.F.; Döbereiner, J. *Deficiências Minerais em Animais de Produção*; Ed. Helianthus: Rio de Janeiro, RJ, Brazil, 2010; pp. 19–42.
24. De Waal, H.O.; Koekemoer, G.J. Blood, rib, bone and rumen fluid as indicators of phosphorus status of grazing beef cows supplemented with different levels of phosphorus at Armoedsvlakte. *S. Afr. J. Anim. Sci.* **1997**, *27*, 76–81. [[CrossRef](#)]
25. Dixon, R.M.; Kidd, L.J.; Coates, D.B.; Anderson, S.T.; Benvenutti, M.A.; Fletcher, M.T.; McNeill, D.M. Utilising mobilisation of body reserves to improve the management of phosphorus nutrition of breeder cows. *Anim. Prod. Sci.* **2017**, *57*, 2280–2290. [[CrossRef](#)]
26. Tokarnia, C.H.; Langenegger, J.; Langenegger, C.L.; Canaibo, E.V. Botulismo em bovinos, no Piauí, Brasil. *Pesq. Agropec. Bras.* **1970**, *5*, 465–472.
27. Döbereiner, J.; Tokarnia, C.H.; Langenegger, J.; Dutra, I.S. Epizootic botulism of cattle in Brazil. *Deutsche Tierärztliche Wochenschrift.* **1992**, *99*, 188–190. [[PubMed](#)]
28. Camargo, W.V.A.; Fernandes, N.S.; Santiago, A.M.H. Estudos de elementos minerais de interesse pecuário em regiões da Amazônia legal. *Arq. Inst. Biol.* **1980**, *47*, 83–111.
29. Sousa, J.C.; Nicodemo, M.L.F.; Darsie, G. Deficiências minerais em bovinos de Roraima, Brasil. V. Cobre e Molibdenio. *Pesq. Agrop. Bras.* **1989**, *24*, 1547–1554.
30. Cavalcanti, A.C.; Partelli, F.L.; Gontijo, I.; Dias, J.R.M.; Freitas, M.S.M.; Carvalho, A.J.C. Establishment of leaf nutrient patterns for the nutritional diagnosis of *Urochloa brizantha* pastures in two seasons. *Acta Sci.* **2021**, *43*, e50359. [[CrossRef](#)]
31. Moir, K.M. Nutrition of grazing cattle. 2. Estimation of phosphorus and calcium in pasture selected by grazing cattle. *Qld. J. Agric. Sci.* **1960**, *17*, 373–383.
32. Dixon, R.M.; Coates, D.B.; Holmes, W.E.; English, B.H.; Rolfe, J.W. Phosphorus nutrition and management—Overcoming constraints to wider adoption. In Proceedings of the NBRUC Conference, Darwin, Australia, 3–4 August 2011.
33. Little, D.A. Bone biopsy in cattle and sheep for studies of phosphorus status. *Aust. Vet. J.* **1972**, *48*, 668–670. [[CrossRef](#)]
34. Malafaia, P.; Granja-Salcedo, Y.T.; Uscategui, R.A.R.; Souza, V.C.; Costa, D.F.A.; Berchielli, T.T. A simple and fast sampling method for chemical analyses and densitometry of bones through rib biopsies in cattle. *Pesq. Vet. Bras.* **2017**, *37*, 31–35. [[CrossRef](#)]
35. Malafaia, P.; Garcia, F.Z.; Lopes, S.P.; Souza, V.C.; Canella Filho, C.F.; Costa, D.F.A.; Veiga, C.C.P. Evaluation of an inexpensive needle test for the diagnosis of phosphorus deficiency and management of phosphorus supplementation for cattle: A multiple case study. *An. Acad. Bras. Ciências.* **2018**, *90*, 3337–3352. [[CrossRef](#)]
36. Peixoto, P.V.; Malafaia, P.; Miranda, L.V.; Canella, C.C.F. Eficiência reprodutiva de matrizes bovinas de corte submetidas a três diferentes tipos de suplementação mineral. *Pesq. Vet. Bras.* **2003**, *23*, 125–130. [[CrossRef](#)]

37. Malafaia, P.; Peixoto, P.V.; Gonçalves, J.C.S.; Moreira, A.L. Ganho de peso e custos em bovinos de corte submetidos a dois tipos de suplementos minerais. *Pesq. Vet. Bras.* **2004**, *24*, 160–164. [[CrossRef](#)]
38. Costa, R.M.; Ponsano, E.H.G.; Souza, V.C.; Malafaia, P. Reduction of phosphorus concentration in mineral supplement on fertility rate, maternal ability and costs of beef cows reared in pastures of *Urochloa decumbens*. *Trop. Anim. Health Prod.* **2016**, *48*, 417–422. [[CrossRef](#)] [[PubMed](#)]
39. Lopes, S.P.; Garcia, F.Z.; Souza, V.C.; Costa, D.A.F.; Malafaia, P. Bio-economic evaluation of phosphorus supplementation strategies for a cow-calf system in Brazil: A case study. *Trop. Anim. Health Prod.* **2018**, *50*, 205–208. [[CrossRef](#)] [[PubMed](#)]
40. Lopes, S.P.; Garcia, F.Z.; Cosma, F.; Brito, M.F.; Ubiali, D.G.; Souza, V.C.; Costa, D.F.A.; Malafaia, P. Clinical parameters and calf liveweight of Nellore breeders supplemented or not with phosphorus, during pregnancy and lactation—A case study. *Arq. Bras. Med. Vet. Zootec.* **2022**, *74*, 1101–1110. [[CrossRef](#)]
41. Malafaia, P.; Costa, R.M.; Brito, M.F.; Peixoto, P.V.; Barbosa, J.D.; Tokarnia, C.H.; Döbereiner, J. Equívocos arraigados no meio pecuário sobre deficiências e suplementação minerais em bovinos no Brasil. *Pesq. Vet. Bras.* **2014**, *34*, 244–249. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.