



# Negative Digestibility: A Theoretical Perspective

Shozo H. Sugiura

Graduate School of Environmental Sciences, The University of Shiga Prefecture, Hikone-City 522-0057, Shiga, Japan; sugiura@ses.usp.ac.jp

## Abstract

Digestibility is a fundamental concept in animal nutrition and is widely used to evaluate feed ingredients. Although digestibility values are generally expected to be positive, negative values are occasionally reported. Negative values are not analytical or calculation errors; rather, they often convey critical information about feed ingredients. Negative digestibility is most commonly observed for minerals, which readily interact with other components in compound feeds and within the gut lumen. A proper understanding of negative digestibility is therefore essential for formulating nutritionally complete diets. This paper introduces Mellanby's toxamin theory to illustrate the mechanisms underlying negative digestibility. It also explains the rationale behind digestibility calculations, which sometimes yield mathematically extreme and biologically unrealistic negative values. Finally, the paper proposes *in vivo* mineral-binding capacity as an alternative metric for expressing ingredient interactions, as negative digestibility values themselves can lead to confusion and misinterpretation. By reinterpreting the biological significance of negative digestibility, this paper seeks to translate an often-confusing observation into a more practical and informative nutritional index.

**Keywords:** aquafeed; compound feed; antinutritional factors; apparent digestibility; digestibility equations; difference method; mineral interactions; mineral bioavailability; negative digestibility; *in vivo* mineral-binding capacity

## 1. Introduction

Negative digestibility!? How can digestibility possibly be negative? Zero digestibility is easy to grasp—it is what we would expect after consuming something entirely indigestible, like stones. But a value below zero seems to violate basic intuition. Yet negative digestibility values appear frequently when digestibility coefficients are calculated, particularly for minerals e.g., [1–4]. Negative values are often dismissed as analytical errors or attributed to inadequate methodology. But what if they are real? What biological processes could produce such paradoxical outcomes, and what do they actually reveal about nutrient utilization?

This paper reexamines the concept of negative digestibility, arguing that these values are not merely analytical or mathematical anomalies but nutritionally meaningful data. Understanding their true interpretation is essential for accurately evaluating feed ingredients and formulating nutritionally adequate diets.

Over 30 years ago, I began my doctoral studies at the University of Washington. My professors asked me to conduct a digestibility experiment using coho salmon and 12 feed ingredients, providing a feed composition table and all necessary materials.

Academic Editor: Aires Oliva-Teles

Received: 4 May 2026

Revised: 28 June 2026

Accepted: 29 June 2026

Published: 30 June 2026

**Copyright:** © 2026 by the author.

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.

After completing the feeding trial and fecal analysis, I obtained a large dataset in Excel. My next task was to calculate the digestibility of dry matter, protein, and individual minerals for the 12 ingredients. At the time, however, I was unaware that a standard digestibility equation already existed. So, I derived the calculation logically from first principles, without consulting any sources. Very soon, I noticed that many calculated values were negative or exceeded 100%—numbers that appeared very strange. “That can’t be right. How could these values possibly be correct?”

Puzzled, I examined several digestibility papers published up to that time, and found only “normal” values. Looking more closely, I realized that their calculation formula differed from mine, and was incorrect! Even more puzzled, I emailed my professors (on 9 May 1996) under the title “Errors Found!” The following Monday, we held a brief meeting. The professors just listened without comment. Only at the end did one of them ask, “Did you derive this formula yourself?” I replied, “Oh, yes, of course.” The meeting ended there.

It should be noted that my equation (later called the nutrient-contribution formula or simply the contribution formula) was nothing more than a straightforward mass-balance calculation requiring no advanced mathematics. I also soon found that this equation, or its underlying concept (often referred to as the difference method, digestibility by difference, indirect digestibility, or partial digestibility), had already been described by earlier researchers e.g., [5–8]. So, I quickly lost interest in this issue. Nevertheless, my professors encouraged me to present it at the upcoming conference, as it was still necessary to justify our digestibility values, many of which were indeed negative!

The presentation was made at the VII International Symposium on Nutrition and Feeding of Fish, held in Texas, USA, on 12 August 1996. The title was “Apparent digestibility coefficients of macro and micro elements and protein in various feed materials determined using coho salmon, *Oncorhynchus kisutch*.” In retrospect, it was a very unusual presentation. In front of a full conference hall, I switched from PowerPoint to an overhead projector and began to “lecture” on the simple versus nutrient-contribution formulae using transparency and a red marker as I spoke.

Nearly two years later, this work was published [2]. The long delay resulted from rejection of the first submission and a slow review process. The rejected manuscript contained a detailed justification of the nutrient-contribution formula. However, in the resubmitted manuscript, I removed all such explanations and simply presented the contribution formula along with plenty of digestibility data. And the manuscript was accepted.

Since publication, the paper has received considerable attention. Nevertheless, the simple formula continues to be used, despite repeated warnings against its use e.g., [9–11]. The reason for this persistence is unclear, but the use of the simple formula lacks a sound theoretical basis, even though the two formulae often produce similar apparent digestibility coefficients (ADCs).

In the present paper, therefore, I first introduce Mellanby’s toxamin theory as a conceptual framework for interpreting negative digestibility. Second, I provide a rational derivation of the nutrient-contribution formula, which often produces seemingly irrational negative values. Third, I discuss the proper interpretation of negative digestibility and suggest alternative approaches for its practical application. Throughout the paper, data from Sugiura et al. (1998) [2] are used because the original raw data were available to the author, enabling the results to be recalculated and reevaluated from multiple perspectives.

## 2. Mellanby’s Toxamin

In the latter part of the 19th century and the early 20th century, large numbers of children in major cities across Europe and North America suffered to varying degrees

from rickets. Early reports also noted a close association between rickets and dental abnormalities e.g., [12,13]. The diets of these populations consisted largely of bread, with insufficient intake of calcium (Ca)-rich foods.

May Mellanby, a researcher of dental disease, was among the first to suggest that certain cereals had adverse effects on tooth development. She wrote, “Our diet, particularly that of the poor, is now more than ever made up of specially prepared cereals, such as wheat, rice, oats, etc. ... There is no doubt that our modern dietary [sic] is harmful as far as the teeth are concerned” [14]. Building on related concerns, her husband, Edward Mellanby, formulated standard diets containing bread or cereals that consistently produced rickets in puppies. He then added various test substances to these diets to determine their effects on rickets development [15].

Subsequently, Mellanby (1922) demonstrated that the rachitogenic, or rickets-producing effects of cereals vary greatly: oatmeal was the most potent, followed by brown flour, barley, rice, and white flour [16]. Later, Mellanby (1925) reported that oatmeal and maize possess a particularly strong rickets-producing action and argued that “In view of the evidence of interaction and balance among food constituents provided by this investigation, the value of the expression ‘optimum Ca content of a diet’, so commonly used nowadays, must be doubted. The optimum varies every time the other elements of diet are changed.” [17].

In 1926, Mellanby discovered that the anticalcifying potency of cereals such as oats could be reduced through simple chemical treatments—specifically, (1) boiling with acid and (2) malting followed by heating. These findings suggest that cereals contain a distinct anticalcifying substance whose action could be modified either by adding vitamin D or Ca salts or by destroying the putative toxic factor through heat and acid treatment. Mellanby (1926, 1934) referred to this unidentified harmful component as “toxamins.” According to his studies, toxamins impaired Ca utilization, and this toxic action was antagonized by the anti-rachitic vitamin, i.e., vitamin D [18,19].

Supporting this view, Holst (1927) wrote, “If oatmeal is extracted with hydrochloric acid and the extract is given in addition to the starch, the animals develop rickets. The rickets-producing factor of oatmeal must, therefore, be ascribed to some toxic substance.” [20]. He further noted that rickets induced by cereal feeding could be prevented by Ca salts but not by phosphates.

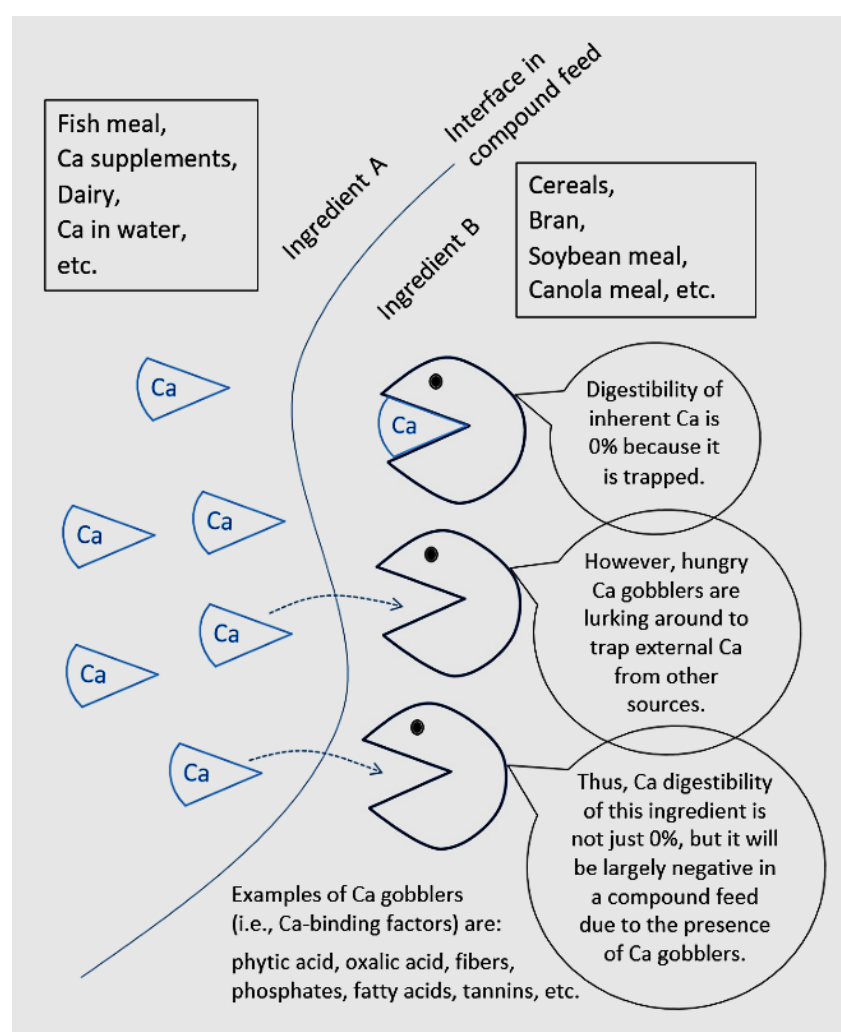
Templin and Steenbock (1933) showed that the loss of rachitogenic activity after acid-boiling was accompanied by the conversion of organic P to inorganic P, suggesting that organic P may be less bioavailable [21]. Soon afterward, Bruce and Callow (1934) demonstrated that phytic acid forms insoluble Ca salts and interferes with Ca absorption in rats [22]. This work was validated by Lowe and Steenbock (1936) [23,24] and Krieger and Steenbock (1940) [25] in rats, and by McCance and Widdowson (1935, 1942) [26–28] in humans. The latter reported that subjects who consumed white bread supplemented with sodium phytate showed a strongly negative Ca balance, and that Ca and P absorption from brown bread increased once it was dephytinized.

Human studies further showed that Ca absorption from milk was significantly reduced when milk was consumed together with oatmeal (a rich source of phytate), whereas no such impairment occurred when the two foods were consumed separately at different times of the day (Cruickshank et al., 1945 [29]). Similar results have been reported with wheat bran (Weaver et al., 1991 [30]). Thus, when attempting to avoid unfavorable nutrient interactions, using two diets of different composition may be effective, provided that they are consumed separately—either at different times of the day or on alternating days. (This principle applies to both daily diets and compound feed formulation.)

Harrison and Mellanby (1939) showed that commercial phytin (Ca–magnesium phytate) is not rachitogenic, whereas sodium phytate or phytic acid prepared from commercial

phytin or oatmeal is distinctly so [31]. They concluded that “phytic acid in a rachitogenic cereal like oatmeal immobilizes almost all of the Ca contained in the cereal by converting it into an insoluble, relatively unavailable Ca phytate, and further, that the excess of phytic acid (over and above that required to precipitate the Ca of the cereal) can exert an additional anticalcifying effect by precipitating other Ca present in the non-cereal part of the diet.” [31] (Cf. Figure 1)

Remarkably, decades earlier, William Jenner (1860) wrote “And even the frequency of rickets in London has been supposed to depend on adulterations of the bread whereby its lime-salts (i.e., Ca) are deprived of their solubility.” [32]. Jenner—best known for distinguishing typhoid from typhus—appears to have intuited the importance of nutrient interactions long before the underlying mechanisms were understood.



**Figure 1.** Mellanby’s “toxamin” is illustrated. A distinguished British nutritionist, Edward Mellanby, hypothesized, based on his experimental observations, that cereals contained an anti-calcifying factor that could be counteracted by vitamin D. He named this factor “toxamin” (Mellanby, 1926, 1934), which was later identified as phytic acid [18,19].

Mellanby’s toxamin highlights the importance of mineral interactions and their influence on bioavailability. The concept remains highly relevant to modern nutrition, including human nutrition, as an estimated one-half to two-thirds of the global population is deficient in Ca, Fe, Zn, and/or Mg, with phytates from cereals and legumes recognized as major factors limiting their bioavailability [33,34].

### 3. Rationale for Digestibility Calculation

#### 3.1. Concept of Digestibility

Digestibility can be determined using either *in vitro* or *in vivo* methods. *In vitro* methods digest test materials in a vessel containing simulated digestive fluids, whereas *in vivo* methods involve feeding live animals and collecting feces or digesta to estimate nutrient absorption. *In vivo* methods can be further divided into direct and indicator methods. The direct method requires quantitative collection of all excreta, whereas the indicator method uses an indigestible, inert marker mixed into the test diet and estimates digestibility from partial excreta collection.

In addition, *in vivo* measurements can yield either apparent or true digestibility values e.g., [5,6,35]. The present paper focuses on the most commonly used approach in fish feed research: apparent digestibility determined by the indicator method.

Suppose, for example, that a man consumes only Onigiri, or Japanese rice balls, without restriction. Each rice ball contains a small stone instead of an edible filling. Because the stone is indigestible, it is excreted unchanged in the feces. On one occasion, the man defecates and examines his feces, finding that it contains five stones. This indicates that the feces correspond to the undigested residue of five rice balls. Accordingly, the digestibility of the rice balls (i.e., dry matter digestibility) can be calculated as follows:

$$\text{Digestibility (\%)} = \frac{\text{rice ball} - (\text{feces} / 5)}{\text{rice ball}} \times 100$$

This is the simplest representation of *in vivo* digestibility calculation. However, the same underlying concept applies to more complex calculations, as discussed below. *In vivo* apparent digestibility is often referred to as the apparent digestibility coefficient, or simply digestibility, and is expressed as a percentage.

#### 3.2. Calculating the Nutrient Digestibility of Diets

The first step in calculating nutrient digestibility of a diet is to determine the concentration factor: CF [36], which is obtained as follows:

CF = Indicator concentration (%) in feces/Indicator concentration (%) in the diet.

CF indicates the amount of feces that corresponds to a given amount of diet. Thus, the nutrient concentration in feces is divided by CF to obtain the amount of nutrients excreted in feces per corresponding amount of diet. Nutrient digestibility in the diet is then calculated as follows:

$$\text{ADC}_D = \frac{(\text{Nutr}_D - (\text{Nutr}_F / \text{CF}))}{\text{Nutr}_D} \times 100 \quad (1)$$

where

ADC<sub>D</sub>: Apparent digestibility coefficient (%) of a nutrient in the diet.

Nutr<sub>D</sub>: Nutrient concentration (% or ppm) in the diet.

(for dry matter digestibility, this value is 100%).

Nutr<sub>F</sub>: Nutrient concentration (% or ppm) in feces.

(for dry matter digestibility, this value is 100%).

CF: Concentration factor (as described above).

In the rice ball example, if the rice ball weighs 100g and the feces containing 5 stones also weigh 100 g, the CF will be 5 and the dry matter digestibility (ADC<sub>D</sub>) will be 80%. If the feces containing 5 stones weigh only 10 g (assuming stone weight is negligible), the CF will be 50 and the dry matter digestibility will be 98%. At the opposite extreme, if the feces containing 5 stones weigh 500 g, the CF will be 1 and the dry matter digestibility will be 0%.

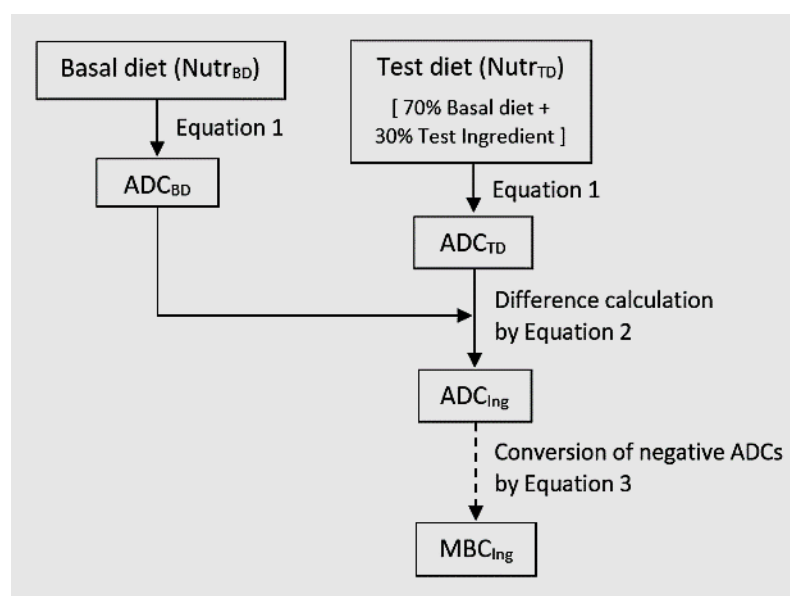
### 3.3. Measuring the Digestibility of Individual Feed Ingredients

Fish feeds are formulated from multiple ingredients; therefore, accurate knowledge of nutrient digestibility in each ingredient is essential for appropriate ingredient selection. Consequently, digestibility assessment has long been a cornerstone of fish nutrition research. For example, Migita et al. (1937) reported the digestibility of fish-flesh protein in carp [37], while Nose (1962) compiled protein digestibility data for 36 feed ingredients across five fish species. Three principal approaches have been used to determine ingredient digestibility [38].

The first approach involves feeding a single ingredient, often by force-feeding e.g., [39,40]. Because the “diet” is nutritionally unbalanced, sometimes toxic, and unrepresentative of practical feeds, this method is now rarely used. The second approach uses a formulated diet in which the nutrient of interest is supplied exclusively by the test ingredient, while other dietary components (e.g., starch, cellulose, lipids, and purified ingredients) do not contribute to that nutrient e.g., [1]. This approach is more reliable because the diet can be formulated to be nutritionally more balanced.

The third approach uses a basal (reference) diet with known nutrient composition and digestibility. The basal diet is mixed with a test ingredient at a predetermined ratio—typically 70% basal diet and 30% test ingredient—to produce a test diet. By measuring the digestibility of the test diet and that of the basal diet, the nutrient digestibility of the test ingredient can be calculated by difference (subtraction) using Equation (2). The use of a nutritionally adequate basal diet helps buffer large differences in nutrient composition among test ingredients, enabling digestibility measurements without disrupting normal digestive or physiological functions [5–7]. Hence, this method is most widely used for measuring the digestibility of individual ingredients. The following discussion focuses exclusively on this method, a schematic representation of which is provided in Figure 2.

A key assumption underlying the third approach is that the digestibility of the basal diet remains unchanged when mixed with the test ingredient. However, this assumption does not hold when strong nutrient interactions occur between the basal diet and the test ingredient, as illustrated earlier in Mellanby’s toxamin example. Thus, the third approach effectively captures interactive effects of ingredients. For minerals, such interactive properties cannot be ignored when evaluating their digestibility.



**Figure 2.** Schematic diagram illustrating the determination of apparent digestibility coefficients (ADCs) using the difference method and the proposed conversion of negative ADC values into mineral-binding capacity (MBC). Abbreviations are defined in Equation (2).

### 3.4. Calculating Digestibility of Test Ingredients

Using the third approach (70% basal diet + 30% test ingredient), the digestibility of nutrients in test ingredients can be calculated as follows [2]:

$$ADC_{Ing} = \frac{(Nutr_{TD} \times ADC_{TD} - 0.7 \times Nutr_{BD} \times ADC_{BD})}{0.3 \times Nutr_{Ing}} \quad (2)$$

where

$ADC_{Ing}$ : Apparent digestibility coefficient of the test ingredient.

$Nutr_{TD}$ : Nutrient concentration (% or ppm) in the test diet.

$ADC_{TD}$ : Apparent digestibility coefficient of the test diet.

(Note:  $Nutr_{TD} \times ADC_{TD}$  = Net absorption from the test diet).

$Nutr_{BD}$ : Nutrient concentration (% or ppm) in the basal diet.

$ADC_{BD}$ : Apparent digestibility coefficient of the basal diet.

(Note:  $Nutr_{BD} \times ADC_{BD}$  = Net absorption from the basal diet).

$Nutr_{Ing}$ : Nutrient concentration (% or ppm) in the test ingredient.

(Note:  $0.3 \times Nutr_{Ing}$  =  $Nutr_{TD} - 0.7 \times Nutr_{BD}$ ).

In Equation (2), the first term in the numerator represents the amount of nutrients absorbed from the test diet. The second term reflects the amount absorbed from the basal-diet portion of that test diet. Thus, the entire numerator represents the total amount of nutrient absorbed specifically from the test-ingredient portion of the test diet. The denominator represents the amount of that nutrient contributed by the test ingredient within the test diet.

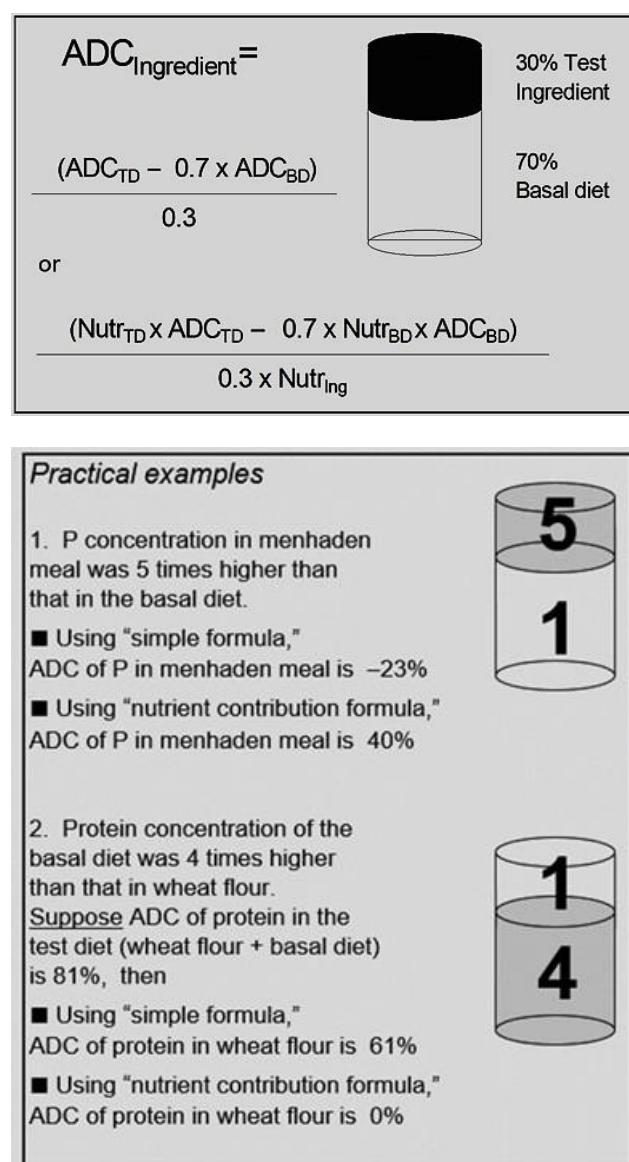
The coefficient 0.7 (70%) in Equation (2) represents the dry-matter ratio, that is, the proportion of the basal diet in the test diet on a dry-matter basis. Hence, the dry-matter contents of the basal diet and each test ingredient must be determined before formulating the test diets. In practice, however, when an indicator (e.g.,  $Cr_2O_3$ ,  $Y_2O_3$ ,  $TiO_2$ ) is premixed into the basal diet prior to blending with a test ingredient, the dry-matter ratio can be determined conveniently and accurately by analyzing indicator concentrations in both the basal and test diets [36]. Although this approach may result in a slight deviation from the nominal 70:30 ratio, such a deviation poses no problem as long as the actual ratio is used in ADC calculations.

Although the ratio itself is not critical for digestibility measurement, when the inclusion level of the test ingredient is low (e.g., 90:10 ratio), the resulting digestibility estimate will be more sensitive to experimental error. A similar issue arises when the nutrient concentration in the test ingredient is low relative to that in the basal diet: small differences in the measured digestibility of the test diet must then account for a small nutrient contribution from the test ingredient, which amplifies error e.g., [41].

Conversely, a high concentration of the nutrient of interest in the test ingredient relative to the basal diet allows for more precise estimation of ADCs, or so it seems. When the nutrient in the test diet is supplied predominantly by the test ingredient, the digestibility of the test diet closely approximates that of the test ingredient, regardless of the basal diet's digestibility, because the basal diet contributes little of that nutrient. In this situation, however, the total nutrient level in the diet could far exceed the fish's requirement, and the fish's homeostatic regulation can distort ADC estimates, particularly for minerals.

Therefore, the optimal inclusion level of a test ingredient depends on its nutrient contribution relative to that of the basal diet within the test diet. Ideally, the concentration of each nutrient in both the basal and test diets should be close to, or slightly below, the requirement level to minimize physiological up- or down-regulation of nutrient transport e.g., [42]. However, when ADCs of multiple minerals are determined simultaneously, such adjustment becomes difficult because each ingredient is invariably high in some minerals and low in others. This limitation represents an inherent weakness of the method.

Notably, when nutrient concentrations in the basal diet and test ingredient differ greatly, the two calculation formulae may yield markedly different ADC values, as illustrated in Figure 3. The two formulae become mathematically equivalent only when the concentration of the nutrient of interest is identical in both components. In that case, the terms  $\text{Nutr}_{\text{BD}}$ ,  $\text{Nutr}_{\text{TD}}$ , and  $\text{Nutr}_{\text{Ing}}$  in Equation (2) cancel, reducing the equation to the simple formula. Such conditions, however, are rarely encountered in practice.



**Figure 3.** Apparent digestibility coefficients (ADCs) calculated using the simple formula (NRC, 1993) [43] and the nutrient-contribution formula. PowerPoint slide (**top**) and transparency (**bottom**) presented at the VII International Symposium on Nutrition and Feeding of Fish, College Station, Texas, USA, 12 August 1996 [44]. Abbreviations are defined in Equation (2).

## 4. Interpretation of Negative Digestibility

### 4.1. Chemical Interactions in the GI Environment

Negative ADCs have been reported by several researchers, including those cited in the Introduction. However, these studies examined different ingredients, species, and methodologies under varying experimental conditions. Consequently, the reported val-

ues are not directly comparable. Rather than attempting a systematic review or quantitative comparison of these values, the present paper focuses on the general mechanisms underlying negative ADCs.

As shown in Table 1, both the simple and nutrient-contribution formulae can yield negative ADCs. However, the contribution formula tends to produce more extreme values, sometimes reaching negative hundreds or even thousands of percent. Negative ADCs imply that (1) the net contribution of the nutrient supplied by the test ingredient is negligible or negative, and (2) one or more factors in the test ingredient inhibit the digestion or absorption of the same nutrient supplied by the basal diet portion (e.g., the Ca-gobblers in Figure 1). Additional mechanisms may also contribute to negative ADCs, including diet-induced endogenous GI secretions, reduced reabsorption of endogenous nutrients, altered chyme transit, dietary toxins that disrupt digestive physiology, and changes in the GI microbiota. Collectively, these effects can result in a negative calculated ADC.

Major factors causing negative digestibility are chemical interactions, such as chelation, precipitation, and co-precipitation e.g., [45]. Mellanby's toxamin focused primarily on interference with Ca absorption via chelation by phytates. However, current knowledge clearly shows that phytates also affect the absorption of many other minerals, both directly (chelation) and indirectly (co-precipitation) e.g., [46]. Thus, the concepts described above apply broadly to several minerals.

**Table 1.** Apparent digestibility coefficients (ADC, %) of feed ingredients calculated using two different formulae.

|                   | Protein | Ca   | P   | Mg | K  | Na    | Fe  | Cu  | Mn  | Sr   | Zn  |
|-------------------|---------|------|-----|----|----|-------|-----|-----|-----|------|-----|
| Menhaden meal     | 87      | −47  | −24 | 32 | 96 | 86    | −78 | 86  | −82 | −11  | 14  |
|                   | 88      | 12   | 40  | 42 | 96 | 84    | −3  | 89  | −38 | 11   | 26  |
| Feather meal      | 76      | 35   | 75  | 77 | 94 | 61    | 80  | 7   | −28 | 77   | 20  |
|                   | 80      | 38   | 75  | 57 | 91 | 64    | 55  | 46  | −2  | 41   | 42  |
| Soybean meal      | 93      | −52  | 27  | 65 | 99 | −58   | −19 | 112 | −23 | −71  | 8   |
|                   | 93      | −65  | 28  | 74 | 98 | −2225 | 11  | 96  | −9  | −16  | −33 |
| Wheat middlings   | 93      | −95  | 23  | 32 | 90 | −24   | −6  | 52  | −85 | −129 | 16  |
|                   | 86      | −329 | 41  | 59 | 92 | −878  | 1   | 63  | −14 | −70  | 16  |
| Casein basal diet | 98      | 42   | 91  | 92 | 97 | 81    | 39  | 81  | 40  | 22   | 81  |

Values in the upper rows (italic font) indicate ADC (%) based on the simple formula (Figure 3; NRC, 1993 [43]). Values in the lower rows (normal font) indicate ADC (%) based on the nutrient-contribution formula (Figure 3; NRC, 2011 [11]). Negative values are indicated in boxes and are recalculated as shown in Tables 2 and 3. Nutrient concentrations in the casein basal diet (dry basis) were as follows: 58.6% crude protein; 4.00% ash; 0.497% Ca; 4.93 ppm Cu; 98.0 ppm Fe; 0.743% K; 0.227% Mg; 34.9 ppm Mn; 0.412% Na; 0.741% P; 3.37 ppm Sr; and 123 ppm Zn. Data in Tables 1–3 were derived from [2], whose original raw data were available to the author for recalculation.

Furthermore, minerals are influenced by numerous interactions unrelated to phytates, which can significantly alter their absorption, potentially producing negative or over 100% digestibility values [47–50]. Such interactions may occur during feed processing and storage, as well as in the GI lumen, where minerals from dietary, endogenous, branchial, and environmental sources coexist and interact.

Suppose, for example, a basal diet contains 10 mg of available nutrient A per 100 g and is mixed with a test ingredient at a 7:3 ratio. The mixed diet should therefore provide at least 7 mg of available A per 100 g, even if nutrient A in the test ingredient is completely unavailable. If net absorption from the mixed diet falls below 7 mg/100 g, this indicates that the test ingredient contains substances that can bind or otherwise inhibit absorption

of nutrient A supplied by the basal diet. Under such conditions, the calculated digestibility of A in the test ingredient becomes negative.

Under otherwise identical conditions, if the basal diet contains only 1 mg of available A per 100 g, the calculated digestibility will be only slightly negative. In this case, the small amount of A can be bound by inhibitory factors in the test ingredient, leaving much of the binding capacity unutilized (or bind other minerals instead). Consequently, the calculated ADC depends on the basal diet composition, the presence of other minerals, the level of binding factors in the test ingredient, and the ingredient's inclusion level.

Because negative digestibility values are counterintuitive, it may be more informative to assume zero digestibility for nutrient A in the test ingredient and then recalculate the digestibility of nutrient A supplied by the basal diet portion (Table 2). This provides a clearer indication of the magnitude of antagonism than directly reporting the negative value (Cf. Section 4.3 for further discussion).

**Table 2.** Apparent digestibility coefficients (ADC, %) of the basal diet portion, recalculated assuming zero digestibility of the test ingredient.

|                 | Protein | Ca  | P   | Mg  | K   | Na  | Fe  | Cu  | Mn | Sr  | Zn  |
|-----------------|---------|-----|-----|-----|-----|-----|-----|-----|----|-----|-----|
| Menhaden meal   | ---     | --- | --- | --- | --- | --- | 28  | --- | 10 | --- | --- |
| Feather meal    | ---     | --- | --- | --- | --- | --- | --- | --- | 38 | --- | --- |
| Soybean meal    | ---     | 21  | --- | --- | --- | 47  | --- | --- | 35 | −14 | 73  |
| Wheat middlings | ---     | 5   | --- | --- | --- | 63  | --- | --- | 15 | −38 | --- |

Values indicate the net absorption of dietary nutrients from the casein basal diet portion (70% of the diet), assuming that the nutrients contributed by the test ingredient (30% of the diet) are completely unavailable. This can be compared with the ADC of the basal diet given in Table 1. Dashed lines indicate absorption of nutrients from the test ingredient (i.e., ADC is not negative; Cf. Table 1). Original data were derived from [2].

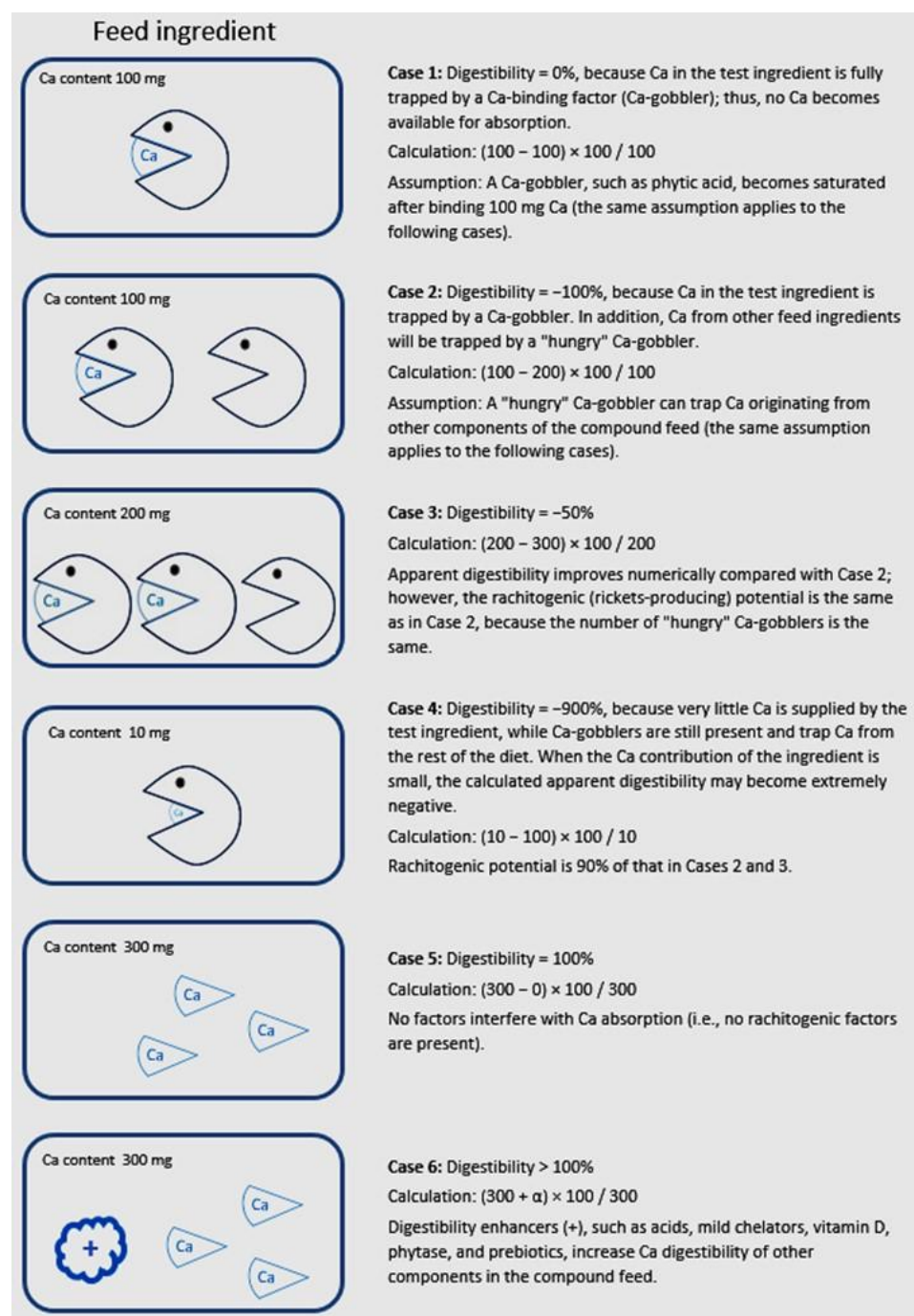
Substances responsible for reducing nutrient A absorption may include phytates, polyphenols, oxalate, fiber, saponins, various minerals (e.g., Ca, P, Mg, Fe), or other dietary constituents with binding, precipitating, inhibiting, or competitive properties [51]. Table 2 shows that the absorption of several trace elements—especially Fe, Mn, and Zn—is markedly reduced by many feed ingredients compared with their absorption from a casein basal diet.

Dietary Ca appears to depress the absorption of multiple minerals, as its concentration (as well as that of P, ash, and bone) is inversely correlated with apparent mineral absorption [2]. Tri-Ca phosphate reduces the absorption of Zn and/or Mn in carp and rainbow trout [52,53]. Various organic and inorganic acids markedly enhance the availability of P and some other minerals in fish meal [44]. Ascorbic acid increases absorption of inorganic Fe [54]. Numerous mineral–mineral and mineral–diet interactions have been documented across species [49,55].

Feed ingredients exert two opposing effects: they supply minerals, and they supply substances that modulate mineral absorption (Figure 4). In addition, ingredients differ in their effects on endogenous fluxes. The net balance of these processes determines mineral availability, i.e., net absorption or apparent digestibility. Consequently, the available mineral content of an ingredient may be negative (reflecting net inhibition of absorption) or exceed the total mineral content (>100% ADC). When formulating feeds, the interactive properties of each ingredient must therefore be considered, particularly antagonistic effects associated with ingredients showing negative digestibility (Table 1).

What about macronutrients, such as proteins, fats, and carbohydrates? Compared with minerals, macronutrients exhibit fewer interactions with other dietary components;

therefore, their digestibility values hardly ever become negative or exceed 100%. Nonetheless, several well-known interactions still occur. Protein digestibility can be reduced by phytates and various antinutritional factors commonly found in plant ingredients, including protease inhibitors, lectins, tannins, and others [56,57]. Fat digestibility can be lowered through soap formation with soluble Ca and Mg in the intestine [58–60], and by inhibitors such as saponins and polyphenols [61]. Carbohydrate digestibility can be reduced by amylase inhibitors present in many plant sources [62]. High-fiber ingredients can depress the digestibility of both macro- and micronutrients [63]. When plant feed ingredients contain substantial quantities of these inhibitors, significant nutrient interactions can be expected [56].



**Figure 4.** Illustrative examples of negative and >100% apparent digestibility. These values can arise from interactions with Ca binders or digestibility enhancers present in feed ingredients. Ca and other minerals can easily produce negative digestibility or over-100% digestibility due to numerous

interactions in the compound feed. Note (Cases 2–4): If Ca-binding factors (“Ca gobblers”) are excreted unbound due to the absence of available dietary Ca, apparent Ca digestibility remains 0% (not negative; non-rachitogenic), highlighting the importance of the diet matrix.

Apparent digestibility reflects both incomplete absorption of dietary nutrients and net endogenous losses. These components primarily capture ingredient-specific effects, such as nutrient binding and the stimulation of endogenous secretions. Accordingly, when measured under well-defined and standardized conditions, ADC can serve as a useful comparative index of feed ingredients. However, both components are also sensitive to methodological and biological variation (e.g., diet composition, rearing conditions, and physiological status). Consequently, ADC values are inherently protocol-sensitive, and their use as reference values requires careful standardization of experimental conditions as discussed below.

#### 4.2. Reducing Experimental Noise

##### 4.2.1. Mineral Solubility as a Prerequisite for Absorption

Mineral absorption depends primarily on their solubility; i.e., insoluble forms are generally unavailable for GI uptake. Therefore, in many species of fish, the absorption of Ca, P, and other minerals from poorly soluble sources (e.g., hydroxyapatite in fish meal) depends on gastric acidity, which promotes their dissolution. Diets containing high levels of bone may exceed the solubilizing capacity of gastric acid, thereby reducing mineral digestibility. Similarly, high levels of antacids, such as  $\text{CaCO}_3$  or shell-rich ingredients, can impair mineral solubilization and consequently decrease digestibility [64]. However, solubilization alone does not guarantee absorption. Once dissolved, minerals become available for uptake, but their actual absorption depends on the mineral species, luminal concentration, and the transport mechanisms operating in the GI tract, as discussed below.

##### 4.2.2. Mineral-Specific Absorption Mechanisms

Soluble P (e.g.,  $\text{NaH}_2\text{PO}_4$  and acid-solubilized hydroxyapatite) is readily absorbed with relatively little regulation, predominantly via the paracellular pathway, while excess absorbed P is eliminated renally. Because P exists as negatively charged ions ( $\text{H}_2\text{PO}_4^-$  and  $\text{HPO}_4^{2-}$ ), its passive diffusion may be aided by favorable electrochemical gradients and solvent drag associated with water absorption [65,66].

Soluble Ca and Mg are also absorbed through both transcellular and paracellular pathways. Unlike P, however, these divalent cations experience an opposing electrical force. Also, their permeability is strongly influenced by tight-junction selectivity, hydration characteristics, and luminal concentration gradients. At low luminal concentrations, transcellular transport becomes important, whereas paracellular transport predominates at higher concentrations. Under normal physiological conditions, Mg absorption occurs mostly via the paracellular route. However, because  $\text{Mg}^{2+}$  is strongly hydrated, its paracellular permeability is relatively low, limiting overall absorption efficiency [67,68].

In contrast, trace minerals such as Fe, Zn, Mn, and Cu are absorbed primarily through saturable transporter-mediated transcellular pathways. Such regulation is necessary because excess-absorbed trace minerals cannot be readily eliminated. Also, apical uptake of some trace minerals is competitively inhibited by shared transporters, e.g., divalent metal transporter-1 (DMT1) and Zrt/Irt-like protein (ZIP) transporters. Consequently, fractional absorption declines as luminal concentrations increase, reducing ADC. In addition, excess trace minerals may be returned to the intestinal lumen via bile, digestive fluids, or sloughed epithelial cells. These endogenous losses can further decrease measured ADC [48,50].

#### 4.2.3. Homeostatic Regulation and Endogenous Excretion

For many micronutrients, transporter expression increases in response to deficient intake, thereby upregulating absorption efficiency e.g., [69]. Consequently, at the whole-diet level, negative digestibility (i.e., a negative balance) rarely occurs, except in cases of carry-over excretion or high intake of antinutritional factors that chelate or precipitate trace minerals secreted into GI fluids. Carry-over excretion, defined as mineral excretion originating from previous intakes, can produce an apparent negative balance until homeostasis is re-established [70–72]. Thus, diet history can have major effects on net absorption, especially of trace minerals.

In ruminants, substantial quantities of minerals are secreted into the digestive fluid. Also, minerals absorbed in excess of requirements are excreted into distal regions of the GI tract. These processes greatly limit the usefulness of ADC measurements for some minerals e.g., [5–7,35,73,74]. However, in monogastric animals, including fish, such endogenous excretion appears to be smaller or mineral-dependent. In salmonids, net absorption of K, Na, Mg, and protein from various feed ingredients is highly correlated with dietary intake. In contrast, trace minerals show weaker correlations between net absorption and dietary intake, indicating stronger physiological regulation and greater effects of endogenous excretion on ADC [44]. Therefore, when measuring ADC of trace minerals, dietary intake should be carefully controlled at or below requirement levels.

#### 4.2.4. Reducing Physiological Noise

Factors unrelated to the test ingredient should be identified, controlled, and minimized as experimental noise to allow accurate evaluation of ingredient ADCs. Fish species differ in digestive anatomy and physiology, and consequently in their inherent capacity to digest and absorb dietary nutrients e.g., [75,76]. Even within a species, however, physiological state can significantly influence digestibility measurements.

Major sources of physiological noise include: (1) up- or down-regulation of nutrient uptake at the GI epithelium; (2) endogenous excretion of minerals, including carry-over excretion; (3) ontogenetic changes in gut morphology, digestive enzyme activity, transporter expression, and gut microbial activity; (4) branchial uptake of waterborne minerals followed by GI excretion; (5) ingestion of mineral-rich seawater for osmoregulation; and (6) impaired physiological condition caused by prolonged nutrient deficiency or excess, toxin exposure, suboptimal temperature, diseases, or other stressors. Because these factors are largely independent of ingredient-specific digestibility, they should, wherever possible, be quantified separately as background noise and excluded from digestibility calculations, thereby yielding “standardized digestibility values” e.g., [77,78].

#### 4.2.5. Non-Physiological Noise

In addition to physiological factors, non-physiological sources of noise should also be minimized, including (1) differences in feed palatability, leading to different feed intake; (2) fecal leaching losses due to loose fecal consistency or inadequate collection methods; (3) cannibalism, which can contaminate fecal samples with shed scales and reduce feed intake in subordinate fish; (4) contamination of collected feces with suspended solids including uneaten feed; (5) inaccurate sampling and analysis; and (6) inadequate statistical replication or pseudo-replication. These factors are common in digestibility studies and can substantially increase measurement error.

In addition, because the test ingredient typically constitutes only a minor portion of the diet (e.g., ~30%), sampling and analytical errors are magnified when digestibility is expressed on an ingredient basis. As a result, the difference method is inherently sensitive to small measurement errors, often yielding relatively large standard deviations. These

limitations highlight the sensitivity of ADC estimates to experimental conditions and the importance of careful study design and data interpretation.

#### 4.3. Alternative Indices of Mineral Availability

Negative digestibility values often create confusion. When the inherent nutrient content of a test ingredient is low, even a modest inhibitory effect can produce a disproportionately large negative value due to the small denominator in the calculation. For example, many plant ingredients, including soybean meal and wheat middlings, contain very little Na; yet feces from fish fed these ingredients still contain measurable Na. As a result, calculated Na digestibility becomes irrationally negative (Table 1), despite only modest “inhibition” (Table 2; Case 4 in Figure 4). In such cases, negative digestibility misrepresents the magnitude of inhibition, as the numerical value is not proportional to its true strength, thereby preventing meaningful comparisons among ingredients.

How, then, should inhibitory effects be expressed? One pragmatic—albeit awkward—approach is to recalculate the digestibility of the basal-diet portion after assigning zero digestibility to the test ingredient, as described earlier (Table 2; [2]). This method is often more interpretable than directly reporting negative digestibility values. Yet, are there more appropriate indices for expressing the inhibitory effects of ingredients?

A more robust approach would be to use indices independent of the nutrient content of the test ingredient. For instance, expressing mineral loss per unit inclusion of an ingredient better reflects its biological impact (Table 3; Figure 5). In this way, inhibitory factors, such as “hungry” Ca gobblers, can be numerically expressed as a mineral-binding capacity or equivalent (mg mineral trapped per kg ingredient). Such indices enable meaningful comparisons among ingredients with widely differing mineral contents and avoid the denominator-driven exaggeration inherent in negative digestibility coefficients.

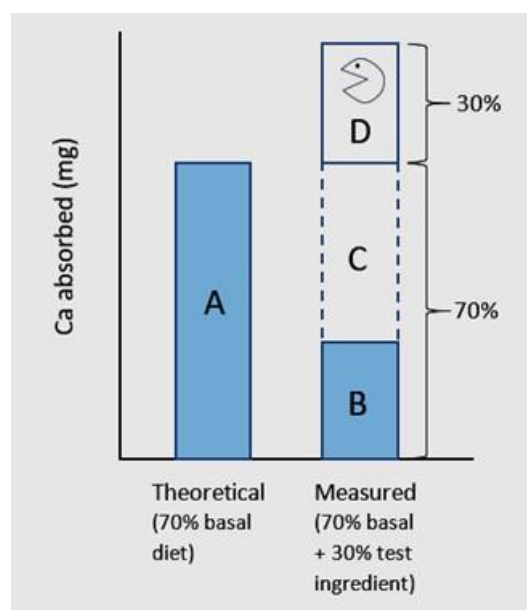
Indeed, mineral-binding capacity (MBC) per se has been extensively studied for a wide range of materials under diverse conditions, predominantly through in vitro experiments or by calculations based on the content of binding substances e.g., [79–83]. Such in vitro approaches enable the collection of reproducible data under well-controlled assay conditions.

However, the in vivo environment within the GI lumen is far more complex, heterogeneous, and dynamic, making it difficult to simulate experimentally [76,84]. Weak binding interactions do not necessarily reduce mineral absorption and may even enhance it (e.g., via organic acids or peptides). Physicochemical interactions within complex diets introduce additional layers of complexity. Consequently, predicting in vivo MBC from in vitro data remains highly challenging [85,86].

**Table 3.** Mineral-binding capacity (MBC) of selected feed ingredients.

|                 | Protein | Ca   | P   | Mg  | K   | Na   | Fe  | Cu  | Mn | Sr  | Zn  |
|-----------------|---------|------|-----|-----|-----|------|-----|-----|----|-----|-----|
| Menhaden meal   | ---     | ---  | --- | --- | --- | ---  | 29  | --- | 27 | --- | --- |
| Feather meal    | ---     | ---  | --- | --- | --- | ---  | --- | --- | 2  | --- | --- |
| Soybean meal    | ---     | 2727 | --- | --- | --- | 4449 | --- | --- | 5  | 3   | 23  |
| Wheat middlings | ---     | 4843 | --- | --- | --- | 4392 | --- | --- | 23 | 5   | --- |

Values indicate the amount of each mineral sequestered by mineral-binding factors in the feed ingredients, expressed as MBC (mg mineral/kg ingredient); Cf. Figures 2 and 5, and text for details. Dashed lines indicate absorption of nutrients from the test ingredient (i.e., ADC is not negative; Cf. Table 1). Original data from [2].



**Figure 5.** Mineral-binding capacity: MBC (mg/kg ingredient). A: Absorbed Ca from the basal diet (mg/0.7 kg diet) = Ca content × Ca digestibility × 0.7. B: Absorbed Ca from the test diet (mg/kg diet) = Ca content × Ca digestibility. C (=A – B): Ca trapped (i.e., not absorbed) due to Ca-binding factors (hungry Ca gobblers) in the test ingredient D. Thus, Ca binding capacity = C/0.3 (mg Ca/kg ingredient). Assumption: Ca digestibility of the test ingredient D is 0%. Cf. Table 3 for calculated values. Example: When Ca digestibility of test ingredient D is calculated to be negative (i.e., hungry Ca gobblers are present), assume 0% digestibility for D, and calculate B accordingly. If A = 7 mg and B = 3 mg, then C = 4 mg, representing trapped (i.e., unabsorbed) Ca. Because C is incurred by hungry Ca-gobblers present in D, and D constitutes 0.3 kg of the diet, the Ca-binding capacity of ingredient D is C/0.3.

Alternatively, in vivo MBC can be estimated from the same dataset used to determine ADC. The corresponding equation is as follows:

$$\text{MBC} = \frac{\text{Nutr}_{\text{BD}} \times \text{ADC}_{\text{BD}} \times 0.7 - \text{Nutr}_{\text{TD}} \times \text{ADC}_{\text{TD}}}{0.3} \quad (3)$$

(Abbreviations are identical to those defined in Equation (2)).

The calculation procedure is outlined in Figure 5, and the resulting MBC values are presented in Table 3. For example, the MBC values for soybean meal shown in Table 3 indicate that supplementation with 2.73 g Ca, 4.45 g Na, 5 mg Mn, and 23 mg Zn per kg of soybean meal would be required to fully offset its MBC (Sr was excluded from calculation because it is not considered an essential mineral for fish).

Therefore, practical mineral requirements may be expressed as follows:

$$\text{Practical requirement} = \text{Basic requirement} + \sum_{i=1}^n (\text{MBC}_i \times \text{Dietary}\%_i) \quad (4)$$

where

Practical requirement is the dietary requirement in practical feeds, Basic requirement is the requirement determined using semi-purified diets (e.g., NRC, 2011 [11]),  $\text{MBC}_i$  is the MBC of ingredient  $i$ ,  $\text{dietary}\%_i$  is the dietary inclusion level of ingredient  $i$ , and  $n$  is the number of ingredients showing measurable MBC (i.e., negative ADC). The equation assumes that the MBC values of individual ingredients are additive within a mixed diet.

Like ADC, MBC may have several inherent limitations. First, because MBC is derived from the same dataset used to estimate ADC, it inherits many of the methodological limitations discussed previously (Section 4.2. Reducing experimental noise). Second, unlike enzymes and transporters, mineral-binding factors such as phytates lack strict substrate specificity. Consequently, their MBC depends strongly on the composition of the chyme (i.e., the diet plus GI secretions). For example, Ca may be preferentially bound in Ca-rich media, whereas other minerals, such as Fe, Zn, and Mg, may occupy available binding sites when Ca concentrations are low e.g., [87,88]. Importantly, such variability reflects the reality of diverse dietary and luminal conditions encountered in vivo and can therefore be regarded as an inherent biological characteristic rather than a methodological limitation.

Third, MBC may be underestimated if the basal diet is low in Ca and other minerals, if its minerals are protected (e.g., chelated), if the test ingredient is included at a high level, or if the test ingredient contains an excess of binding factors. Under these conditions, a substantial proportion of the binding factors may remain unbound and be excreted, leading to an underestimation of the ingredient's MBC. Likewise, the first and second approaches described in Section 3.3 (single-ingredient and semi-purified diets, respectively), as well as mineral bioavailability studies in poultry and livestock [55], are designed to minimize nutrient interactions. Although these approaches are valuable for quantifying the intrinsic availability of nutrients, they are not intended to capture the complex mineral interactions that occur in practical diets.

Fish feeds, most livestock feeds, and human diets are composed of multiple ingredients that interact within the GI tract. Because such interactions are inherent features of practical feeding conditions, the difference method, despite its limitations, may provide the most practically relevant estimates of both mineral availability and MBC.

## 5. Conclusions

One hundred years ago, Mellanby proposed his toxamin theory, a concept that remains highly relevant today, as Ca, Fe, and Zn deficiencies continue to affect a large proportion of the world's population. As Mellanby noted, plant-derived ingredients such as oatmeal and maize possess strong rachitogenic potential, followed by brown flour, barley, rice, and white flour. These properties should be quantified and expressed numerically as Ca-binding capacity, allowing estimation of the amount of Ca that must be fortified per kilogram of ingredient. The same principle applies to other essential minerals.

Adopting such complementary indices in place of negative digestibility would enable more accurate formulation of aquafeeds, animal feeds, and human diets. Such an approach could help prevent ingredient-driven mineral deficiencies while translating the biological significance of negative digestibility into a more practical and nutritionally meaningful index. Future research should therefore focus on quantifying the MBC of major feed and food ingredients and incorporating these values into diet formulation and mineral fortification strategies.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** As this manuscript is a review and does not involve any research on animals or human subjects, ethical approval from an Institutional Review Board was not required.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** No new data were created or analyzed in this study.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## Abbreviations

The following abbreviations are used in this manuscript:

|      |                                    |
|------|------------------------------------|
| ADC  | Apparent digestibility coefficient |
| CF   | Concentration factor               |
| GI   | Gastrointestinal                   |
| DMT1 | Divalent metal transporter-1       |
| ZIP  | Zrt/Irt-like proteins              |
| MBC  | Mineral-binding capacity           |

## References

1. Yamamoto, T.; Ikeda, K.; Unuma, T.; Akiyama, T. Apparent availabilities of amino acids and minerals from several protein sources for fingerling rainbow trout. *Fish. Sci.* **1997**, *63*, 995–1001.
2. Sugiura, S.H.; Dong, F.M.; Rathbone, C.K.; Hardy, R.W. Apparent protein digestibility and mineral availabilities in various feed ingredients for salmonid feeds. *Aquaculture* **1998**, *159*, 177–202.
3. Guimarães, I.G.; Pezzato, L.E.; Barros, M.M.; Fernandes, R.D.N. Apparent nutrient digestibility and mineral availability of protein-rich ingredients in extruded diets for Nile tilapia. *Rev. Bras. Zootec.* **2012**, *41*, 1801–1808.
4. Araújo, J.G.; Nebo, C.; Pádua, D.M.; Souto, C.N.; Guimarães, I.G. Apparent digestibility of minerals from several ingredients for Tambaqui, *Colossoma macropomum*, juveniles. *J. World Aquac. Soc.* **2018**, *49*, 1026–1038.
5. Henry, W.A. *Feeds and Feeding, a Hand-Book for the Student and Stockman*; Published by the author; also later editions; The Morrison Publishing Company: Madison, WI, USA, 1898.
6. Morrison, F.B. *Feeds and Feeding*, 20th ed.; Morrison Publishing Co.: Ithaca, NY, USA, 1945.
7. Crampton, E.W. *Applied Animal Nutrition*, 2nd ed.; W.H. Freeman and Company: San Francisco, CA, USA, 1956; pp. 96–121. (also the 2nd edition).
8. Nengas, I.; Alexis, M.N.; Davies, S.J.; Petichakis, G. Investigation to determine digestibility coefficients of various raw materials in diets for gilthead sea bream, *Sparus auratus* L. *Aquac. Res.* **1995**, *26*, 185–194.
9. Forster, I. A note on the method of calculating digestibility coefficients of nutrients provided by single ingredients to feeds of aquatic animals. *Aquac. Nutr.* **1999**, *5*, 143–145.
10. Bureau, D.P.; Hua, K. Letter to the editor of aquaculture. *Aquaculture* **2006**, *252*, 103–105.
11. NRC. *Nutrient Requirements of Fish and Shrimp*; National Academies Press: Washington, DC, USA, 2011.
12. Fleischmann, L. Kieferrachitis. *Dtsch. Vjschr. Zahnheilk.* **1878**, *18*, 180–185. (cited in: Seki, S.; Tsuchida, S.; Komoribayashi, N.; Sekiyama, S.; Nitana, A. Oral manifestations of rickets. *Dent. J. Iwate Med. Univ.* **1976**, *1*, 143–149).
13. Dick, J.L. The teeth in rickets. *Proc. R. Soc. Med.* **1916**, *9*, 83–91.
14. Mellanby, M. An Experimental Study of the influence of diet on teeth formation. *Lancet* **1918**, *192*, 767–770.
15. Mellanby, E. The part played by an “accessory factor” in the production of experimental rickets. *Proc. Physiol. Soc. Jan.* **1918**, *26*, xi–xii.
16. Mellanby, E. Discussion on the etiology of rickets. *Br. Med. J.* **1922**, *ii*, 849–853.
17. Mellanby, E. *Experimental Rickets: The Effect of Cereals and Their Interaction with Other Factors of Diet and Environment in Producing Rickets*; No. 93; Special Report Series; Medical Research Council: London, UK, 1925. (cited in Mellanby, E. *A Story of Nutritional Research: The Effect of Some Dietary Factors on Bones and the Nervous System*; Williams & Wilkins: Baltimore, MD, USA, 1950; p. 219).
18. Mellanby, E. The presence in foodstuffs of substances having specific harmful effects under certain conditions. *J. Physiol.* **1926**, *61*, xxiv–xxvi.
19. Mellanby, E. *Nutrition and Disease: The Interaction of Clinical and Experimental Work*; Oliver & Boyd, Ltd.: Edinburgh, UK, 1934; pp. 1–14.
20. Holst, P.M. Experimental rickets. *J. Hyg.* **1927**, *26*, 437–440.
21. Templin, V.M.; Steenbock, H. Cereals and rickets. V. The effect of germination and autolysis on the rachitogenic properties of the maize kernel. *Biochem. J.* **1933**, *27*, 2061–2068.
22. Bruce, H.M.; Callow, R.K. Cereals and rickets. The role of inositolhexaphosphoric acid. *Biochem. J.* **1934**, *28*, 517–528.
23. Lowe, J.T.; Steenbock, H. Cereals and rickets. VIII. The hydrolysis of phytin in the intestine. *Biochem. J.* **1936**, *30*, 1991–1995.

24. Lowe, J.T.; Steenbock, H. Cereals and rickets. VII. The role of inorganic phosphorus in calcification on cereal diets. *Biochem. J.* **1936**, *30*, 1126–1134.
25. Krieger, C.H.; Steenbock, H. Cereals and rickets. XII. The effect of calcium and vitamin D on the availability of phosphorus. *J. Nutr.* **1940**, *20*, 125–132.
26. McCance, R.A.; Widdowson, E.M. Phytin in human nutrition. *Biochem. J.* **1935**, *29*, 2694–2699.
27. McCance, R.A.; Widdowson, E.M. Mineral metabolism of healthy adults on white and brown bread dietaries. *J. Physiol.* **1942**, *101*, 44–85.
28. McCance, R.A.; Widdowson, E.M. Mineral metabolism on dephytinized bread. *J. Physiol.* **1942**, *101*, 304–313.
29. Cruickshank, E.W.H.; Duckworth, J.; Kosterlitz, H.W.; Warnock, G.M. The digestibility of the phytate-P of oatmeal in adult man. *J. Physiol.* **1945**, *104*, 41–46.
30. Weaver, C.M.; Heaney, R.P.; Martin, B.R.; Fitzsimmons, M.L. Human calcium absorption from whole wheat products. *J. Nutr.* **1991**, *121*, 1769–1775.
31. Harrison, D.C.; Mellanby, E. Phytic acid and the rickets-producing action of cereals. *Biochem. J.* **1939**, *33*, 1660–1680.
32. Jenner, W.M. A series of three lectures on rickets, delivered at the Hospital for Sick Children, in December 1859 and January 1860. (Lecture III). *Med. Times Gaz.* **1860**, *I*, 465–467.
33. Beal, T.; Massiot, E.; Arsenault, J.E.; Smith, M.R.; Hijmans, R.J. Global trends in dietary micronutrient supplies and estimated prevalence of inadequate intakes. *PLoS ONE* **2017**, *12*, e0175554.
34. Passarelli, S.; Free, C.M.; Shepon, A.; Beal, T.; Batis, C.; Golden, C.D. Global estimation of dietary micronutrient inadequacies: A modelling analysis. *Lancet Glob. Health* **2024**, *12*, e1590–e1599.
35. McDonald, P.; Edwards, R.A.; Greenhalgh, J.F.D. *Animal Nutrition*; Oliver and Boyd: London, UK, 1966; pp. 144–155.
36. Sugiura, S.H. Digestibility. In *Encyclopedia of Aquaculture*; John Wiley & Sons: New York, NY, USA, 2000; pp. 209–218.
37. Migita, M.; Hanaoka, T.; Tsuzuki, K. *Studies on Plant-Based Artificial Feeds for Fish Culture*; Report of the Fisheries Experiment Station, No. 8; Imperial Fisheries Experimental Station: Tokyo, Japan, 1937; pp. 99–177. (cited in Nose, 1962). (In Japanese)
38. Nose, T. Digestion and absorption in fishes. *Bull. Japan. Soc. Sci. Fish.* **1962**, *28*, 1033–1042. (In Japanese)
39. Smith, R.R. A method for measuring digestibility and metabolizable energy of fish feeds. *Progress. Fish-Cult.* **1971**, *33*, 132–134.
40. Smith, R.R.; Winfree, R.A.; Rumsey, G.W.; Allred, A.; Peterson, M. Apparent digestion coefficients and metabolizable energy of feed ingredients for rainbow trout *Oncorhynchus mykiss*. *J. World Aquac. Soc.* **1995**, *26*, 432–437.
41. Glencross, B.; Muñoz-Lopez, P.; Matthew, C.; MacKenzie, S.; Powell, A.; Longshaw, M.; LeBlanc, A. Digestibility of bacterial protein by Atlantic salmon (*Salmo salar*) is affected by both inclusion level and acclimation time. *Aquaculture* **2023**, *565*, 739137.
42. Pimentel-Rodrigues, A.; Oliva-Teles, A. Phosphorus availability of inorganic phosphates and fish meals in European sea bass (*Dicentrarchus labrax* L.) juveniles. *Aquaculture* **2007**, *267*, 300–307.
43. NRC. *Nutrient Requirements of Fish*; National Academy Press: Washington, DC, USA, 1993.
44. Sugiura, S.H. Development of Low-Pollution Feeds for Sustainable Aquaculture. Ph.D. Thesis, University of Washington, Seattle, WA, USA, 1998; p. 252.
45. Ashmead, H.D.; Zunino, H. Factors which affect the intestinal absorption of minerals. In *The Roles of Amino Acid Chelates in Animal Nutrition*; Noyes Publications: Westwood, NJ, USA, 1993; pp. 21–46.
46. Chondrou, T.; Adamidi, N.; Lygouras, D.; Hirota, S.A.; Androutsos, O.; Svolos, V. Dietary phytic acid, dephytinization, and phytase supplementation alter trace element bioavailability—A narrative review of human interventions. *Nutrients* **2024**, *16*, 4069.
47. Goff, J.P. Invited review: Mineral absorption mechanisms, mineral interactions that affect acid–base and antioxidant status, and diet considerations to improve mineral status. *J. Dairy Sci.* **2018**, *101*, 2763–2813.
48. Sugiura, S.; Satoh, S. Minerals. In *Nutrition and Physiology of Fish and Shellfish*; Academic Press: Cambridge, MA, USA, 2025; pp. 493–559.
49. Richards, J.D.; Cori, H.; Rahn, M.; Finn, K.; Bárcena, J.; Kanellopoulos, A.K.; Péter, S.; Spooren, A. Micronutrient bioavailability: Concepts, influencing factors, and strategies for improvement. *Front. Nutr.* **2025**, *12*, 1646750.
50. Rolić, T.; Yazdani, M.; Mandić, S.; Distant, S. Iron metabolism, calcium, magnesium and trace elements: A review. *Biol. Trace Elem. Res.* **2025**, *203*, 2216–2225.
51. Popova, A.; Mihaylova, D. Antinutrients in plant-based foods: A review. *Open Biotechnol. J.* **2019**, *13*, 68–76.
52. Hardy, R.W.; Shearer, K.D. Effect of dietary calcium phosphate and zinc supplementation on whole body zinc concentration of rainbow trout (*Salmo gairdneri*). *Can. J. Fish. Aquat. Sci.* **1985**, *42*, 181–184.

53. Satoh, S.; Izume, K.; Takeuchi, T.; Watanabe, T. Effects of supplemental tricalcium phosphate on zinc and manganese availability to common carp. *Nippon. Suisan Gakk.* **1992**, *58*, 539–545.
54. Lynch, S.R.; Cook, J.D. Interaction of vitamin C and iron. *Ann. N. Y. Acad. Sci.* **1980**, *355*, 32–44.
55. Ammerman, C.B.; Baker, D.H.; Lewis, A.J. *Bioavailability of Nutrients for Animals*; Academic Press: San Diego, CA, USA, 1995; p. 441.
56. Francis, G.; Makkar, H.P.; Becker, K. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture* **2001**, *199*, 197–227.
57. Gilani, G.S.; Xiao, C.W.; Cockell, K.A. Impact of antinutritional factors in food proteins on the digestibility of protein and the bioavailability of amino acids and on protein quality. *Br. J. Nutr.* **2012**, *108*, S315–S332.
58. Keller, A. Phosphorstoffwechsel im Saeuglingsalter. *Ztchr. f. klin. Med.* **1899**, *36*, 49. (cited in Hess, A.F. *Rickets Including Osteomalacia and Tetany*; Lea & Febiger: Philadelphia, PA, USA, 1929; p. 159).
59. Keller, A. Phospho und Stickstoff im Saeuglingsorganismus. *Archiv. f. Kinderheilk.* **1900**, *29*, 1. (cited in Hess, A.F. *Rickets Including Osteomalacia and Tetany*; Lea & Febiger: Philadelphia, PA, USA, 1929; p. 159).
60. Kawamoto, K.; Sakuma, M.; Tanaka, S.; Masuda, M.; Nakao-Muraoka, M.; Niida, Y.; Nakamatsu, Y.; Ito, M.; Taketani, Y.; Arai, H. High-fat diets provoke phosphorus absorption from the small intestine in rats. *Nutrition* **2020**, *72*, 110694.
61. Birari, R.B.; Bhutani, K.K. Pancreatic lipase inhibitors from natural sources: Unexplored potential. *Drug Discov. Today* **2007**, *12*, 879–889.
62. Sales, P.M.; Souza, P.M.; Simeoni, L.A.; Magalhães, P.O.; Silveira, D.  $\alpha$ -Amylase inhibitors: A review of raw material and isolated compounds from plant source. *J. Pharm. Pharm. Sci.* **2012**, *15*, 141–183.
63. Jha, R.; Berrocoso, J.D. Dietary fiber utilization and its effects on physiological functions and gut health of swine. *Animal* **2015**, *9*, 1441–1452.
64. Sugiura, S.H. Digestion and absorption of dietary phosphorus in fish. *Fishes* **2024**, *9*, 324.
65. Sugiura, S.H.; McDaniel, N.K.; Ferraris, R.P. In vivo fractional Pi absorption and NaPi-II mRNA expression in rainbow trout are upregulated by dietary P restriction. *Am. J. Physiol.* **2003**, *285*, R770–R781.
66. Knöpfel, T.; Himmerkus, N.; Günzel, D.; Bleich, M.; Hernando, N.; Wagner, C.A. Paracellular transport of phosphate along the intestine. *Am. J. Physiol.* **2019**, *317*, G233–G241.
67. Ross, A.C.; Taylor, C.L.; Yaktine, A.L.; Del Valle, H.B. *Dietary Reference Intakes for Calcium and Vitamin D*; Institute of Medicine, National Academies Press: Washington, DC, USA, 2011.
68. Houillier, P.; Lievre, L.; Hureauux, M.; Prot-Bertoye, C. Mechanisms of paracellular transport of magnesium in intestinal and renal epithelia. *Ann. N. Y. Acad. Sci.* **2023**, *1521*, 14–31.
69. Diamond, J.M.; Karasov, W.H. Adaptive regulation of intestinal nutrient transporters. *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 2242–2245.
70. Walker, A.R.P.; Fox, F.W.; Irving, J.T. Studies in human mineral metabolism 1. The effect of bread rich in phytate phosphorus on the metabolism of certain mineral salts with special reference to calcium. *Biochem. J.* **1948**, *42*, 452–462.
71. Turnlund, J.R.; King, J.C. Assessment of the bioavailability of dietary zinc in humans using the stable isotopes  $^{70}\text{Zn}$  and  $^{67}\text{Zn}$ . In *Nutritional Bioavailability of Zinc*; Inglett, G.E., Ed.; American Chemical Society: Washington, DC, USA, 1983; pp. 31–40.
72. Schwartz, R.; Apgar, B.J.; Wien, E.M. Apparent absorption and retention of Ca, Cu, Mg, Mn, and Zn from a diet containing bran. *Am. J. Clin. Nutr.* **1986**, *43*, 444–455.
73. Wildt, E. Ueber die Resorption und Secretion der. Nahrungsbestandtheile im Verdauungskanal des. Schafes. *J. Für Landwirthschaft* **1874**, *22*, 1–34. (cited in Forbes, E.B.; Keith, M.H. A Review of the Literature of Phosphorus Compounds in Animal Metabolism. *Ohio Agric. Exp. Stn. Tech. Bull.* **1914**, *6*, 182).
74. Underwood, E.J.; Suttle, N.F. *Mineral Nutrition of Livestock*, 3rd ed.; CABI Publishing: New York, NY, USA, 1999.
75. Bakke, A.M.; Glover, C.; Kroghdahl, Å. Feeding, digestion and absorption of nutrients. In *Fish Physiology*; Academic Press: San Diego, CA/Cambridge, MA, USA, 2010; Volume 30, pp. 57–110.
76. Karasov, W.H.; Douglas, A.E. Comparative digestive physiology. *Compr. Physiol.* **2013**, *3*, 741–783.
77. Stein, H.H.; Fuller, M.F.; Moughan, P.J.; Sève, B.; Mosenthin, R.; Jansman, A.J.M.; Fernández, J.; De Lange, C.F.M. Definition of apparent, true, and standardized ileal digestibility of amino acids in pigs. *Livest. Sci.* **2007**, *109*, 282–285.
78. Zhang, F.; Adeola, O. Techniques for evaluating digestibility of energy, amino acids, phosphorus, and calcium in feed ingredients for pigs. *Anim. Nutr.* **2017**, *3*, 344–352.
79. Platt, S.R.; Clydesdale, F.M. Mineral binding characteristics of lignin, guar gum, cellulose, pectin and neutral detergent fiber under simulated duodenal pH conditions. *J. Food Sci.* **1987**, *52*, 1414–1419.

80. Weber, C.W.; Kohlhepp, E.A.; Idouraine, A.; Ochoa, L.J. Binding capacity of 18 fiber sources for calcium. *J. Agric. Food Chem.* **1993**, *41*, 1931–1935.
81. Claye, S.S.; Idouraine, A.; Weber, C.W. In-vitro mineral binding capacity of five fiber sources and their insoluble components for magnesium and calcium1. *Food Chem.* **1998**, *61*, 333–338.
82. Charrier, M.J.; Savage, G.P.; Vanhanen, L. Oxalate content and calcium binding capacity of tea and herbal teas. *Asia Pac. J. Clin. Nutr.* **2002**, *11*, 298–301.
83. Yaguchi, A.; Akahane, K.; Tsuchioka, K.; Yonekubo, S.; Yamamoto, S.; Tamai, Y.; Tatemichi, S.; Takeda, H. A comparison between the combined effect of calcium carbonate with sucroferic oxyhydroxide and other phosphate binders: An in vitro and in vivo experimental study. *BMC Nephrol.* **2019**, *20*, 465.
84. Hurrell, R.; Egli, I. Iron bioavailability and dietary reference values. *Am. J. Clin. Nutr.* **2010**, *91*, 1461S–1467S.
85. Moustafa, M.; Lehrner, L.; Al-Saghir, F.; Smith, M.; Goyal, S.; Dillon, M.; Hunter, J.; Holmes-Farley, R. A randomized, double-blind, placebo-controlled, dose-ranging study using Genz-644470 and sevelamer carbonate in hyperphosphatemic chronic kidney disease patients on hemodialysis. *Int. J. Nephrol. Renov. Dis.* **2014**, *7*, 141–152.
86. Davidson, J.P.; King, A.J.; Kumaraswamy, P.; Caldwell, J.S.; Korner, P.; Blanks, R.C.; Jacobs, J.W. Evaluation of the pharmacodynamic effects of the potassium binder RDX7675 in mice. *J. Cardiovasc. Pharmacol. Ther.* **2018**, *23*, 244–253.
87. Platt, S.R.; Clydesdale, F.M. Effects of iron alone and in combination with calcium, zinc and copper on the mineral-binding capacity of wheat bran. *J. Food Prot.* **1986**, *49*, 37–41.
88. Idouraine, A.; Khan, M.J.; Weber, C.W. In vitro binding capacity of wheat bran, rice bran, and oat fiber for Ca, Mg, Cu, and Zn alone and in different combinations. *J. Agric. Food Chem.* **1996**, *44*, 2067–2072.

**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.