

COMMERCIAL POULTRY FEED IN SERBIA – CALCIUM AND PHOSPHORUS CONTENT SURVEY

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Abstract: Calcium and phosphorus represent very important nutrients when it comes to poultry diet formulations. In this paper, we will briefly discuss the relevance and nutritional requirements of these minerals in poultry feedstuffs as well as the average amounts in poultry feed commercially sold in the Serbian market. A total of 1,058 samples of standard complete feed mixtures for broilers and laying hens were collected from the Serbian market, produced by the four major Serbian manufacturers (I-IV) of animal feed over a period of five years (2017-2021). The samples were classified into five groups: broiler starter feed (n = 198) - SF, grower feed (n = 239) - GF, and finisher feed (n = 204) – FF; layers feed 1 (n = 204) – LF1, and layers feed 2 (n = 213) – LF2. This research suggests that the mineral composition of poultry feed is highly variable among manufacturers, but also among the batches of the same manufacturers. All manufacturers for the analyte in focus had values for certain batches that were outside the limits set by the Rulebook. In general, the results of our research indicate that the average content of total phosphorus in feed for broilers and laying hens in Serbia was mostly close to the minimum-to-mid value of the defined (and declared) range of permitted concentrations by the Rulebook, while the calcium content was predominantly close to the maximum-to-middle value. Based on the results of this study, it is recommended that feed manufacturers more frequently conduct an external analysis of samples of feed components and poultry feed products for the composition of these nutrients. Quality control of animal feed could be advised for poultry farms as well in order to make sure that the feed is actually within the parameters given by the manufacturers' declaration.

Key words: broilers, laying hens, feed quality, calcium content, phosphorus content

Introduction

Skeletal abnormalities are of economic importance to the poultry broiler industry as they result in increased mortality, culling, downgrading of carcasses, and trimming of deformed legs at processing. On the other hand, in layers, this is experienced through smaller or misshapen eggs, thinner shells, and changes in color, resulting in downgrades or rejections of the eggs by the industry. Some of these conditions and malformations can be prevented by reducing the growth rate and helping animals through diet modification (*Tablante et al., 2003; Jones et al., 2018*). Taking into account nutritional factors, the attention has to be turned to the two of the major macroelements involved - the amount and ratios intake of calcium and phosphorus which are necessary to ensure bone strength, especially during early life, as well as normal egg production. These elements are the main components of bone mineral structure, where they are present in the form of hydroxyapatite (*Schaible, 1941; Scott, 1971; Lukić, 2008*). In poultry and other animals, the physiological roles of these two macro-minerals are intricately linked (*Rath et al., 2000*).

Calcium metabolism is highly efficient and well regulated in poultry as it represents the most abundant mineral in the body and 99% of it can be found in the skeleton. Thus, the predominant role of Ca provides structural strength and support in bones (*Wilkinson, 2011*). According to *Rath et al. (2000)*, in the case of broiler chickens, deformities of the skeletal system will highly depend on the strength of leg bones (femur, tibia, fibula, and pad skeleton), considering their weight supporting and carrying functions. Bone strength is determined by various factors, such as bird growth rate, age, sex, genotype, and endocrinal metabolism, as well as anthropogenic factors that include feeding systems, quality of feedstuffs (presence of toxins in feed), and bird handling, e.g. mechanical damages caused during catch and slaughter. Severe calcium deficiency in broiler chickens causes bone injuries and poor growth, and it can lead to the development of conditions such as tibial dyschondroplasia and rickets, as well as increased mortality of the young population of poultry and reduced body weight in mature animals. Calcium also take a significant part in many of the biochemical pathways in the body such as muscle and nerve conduction, blood coagulation, eggshell calcification, and control of hormone secretions such as vitamin D3 and parathyroid hormone (*Wilkinson, 2011; Matuszewski et al., 2020; Han et al., 2022*).

Under certain physiological conditions, the need for calcium is greatly increased. For example, laying hens have a much higher calcium demand during reproduction than other vertebrates, as this mineral is necessary for maintaining the integrity of the skeleton as well as the eggshell formation. This can be seen from the mere fact that the laying hen must excrete about 2 - 2.5 g of calcium almost every day during the laying period, and 60 – 75% of the eggshell calcium can be provided by the feed (*Fleming, 2007; Lukić, 2008; Wilkinson, 2011*). Calcium

deficit in laying hens, as the short term occurrence (e.g. overnight during the process of eggshell formation) or a long term one (during a part of the production cycle), increases the transport of calcium from the bone depots in order to maintain normal eggs production, which can negatively reflect on the quality of bones, depending on the level and duration of deficit (*Lukić et al., 2011*). Research conducted by *Lukić et al., 2009* showed that the use of diets with 2.5 – 3.0 % calcium in the nutrition in young layers, regardless of the form of calcium incorporated in feed, can have a negative impact on, not only the productivity of layers, but also on and eggshell quality, with an increased incidence of defected eggs. On the other hand, excessive dietary calcium can lead to a decrease in body weight gain (BWG) and feed intake of broilers (*Han et al., 2022*). An issue that is recognized industry-wide is that diets high in calcium concentrations used to achieve optimal bone density also result in a significantly wetter litter thus, there is an additional cost of excess calcium to be considered, particularly in winter or in humid environments (*Bedford et al., 2017*). Excess dietary calcium interferes with the bioavailability of other minerals such as phosphorus, magnesium, manganese, and zinc (*Selle et al., 2009*). In addition, high dietary calcium concentrations may reduce the energy value of the diet through the chelation of lipids (*Li et al., 2016*).

The second most prevalent mineral critical for the normal functioning of the poultry body is phosphorus. Almost 80% of it can be found in bones where is involved, along with calcium, in the bone mineralization process and normal growth performance. As an essential component of normal physiological functions, phosphorus is very important as it is used in energy pathways (e.g, phosphorus-containing compound is adenosine 5'-triphosphate (ATP)) and during the synthesis of phospholipids which are the building blocks of cell membranes. It also represents a major component of the phosphate buffer system which helps maintain osmotic and acid-base balance. Phosphorus cannot be synthesized in avian organisms, so it must be obtained from different dietary sources (*Wilkinson, 2011; Li et al., 2016; NRC, 1994*). The deficit in levels of phosphorus in poultry leads to similar outcomes as calcium deficiency which includes loss of skeletal integrity, changes in poultry appetite, subnormal growth, and weight loss in older animals (*Wilkinson, 2011*). On the other hand, according to *Li et al. (2016)*, studies on the effects of dietary phosphorus levels on animal growth and bone development showed that high phosphorus intake can negatively impact calcium metabolism and bone properties, whereas low phosphorus diets will limit the growth of the animals. Research in poultry has concentrated on the amount of phosphorus used in feed to optimize its utilization because of its high cost and potential as an environmental pollutant (*Li et al., 2017*).

In order to meet calcium and phosphorus requirements, the diet is usually enriched with limestone, inorganic phosphorus supplements such as dicalcium phosphate, and, where allowed, meat and bone meal (*Han et al., 2022*). Since only a few of the known plants are rich in calcium, with extracted rapeseed meals being

one of them, calcium in its inorganic form has to be added as a supplement in poultry feed. It has been reported that approximately 20–30% of calcium found in plants occurs in the form of oxalates which birds cannot absorb. However, quite considerable amounts of phosphorus (around 50 – 90%) are present in plant-sourced feed ingredients. These forms of phosphorus, if present in plants, can be digested by poultry; however, such digestible forms usually account for only 30 – 40 % of the total phosphorus. The remaining phosphorus is present as phytate-bound in a complex that is only partially, and variably, available to the avian family (NRC, 1994; Selle et al., 2009; Matuszewski et al., 2020). Only about 10 percent of the phytate phosphorus in corn and wheat is digested by poultry (NRC, 1994). This led to the necessity of the incorporation of inorganic phosphates (mono and di-calcium phosphates) and external enzyme phytase in poultry diets. And since these mineral sources are rather expensive, coming from non-renewable sources, and partly responsible for the increased phosphorus load in the environment, they are less and less acceptable (Elwinger et al., 2016). Limestone is low-cost and most frequently used very good source of calcium consisting of ~97% calcium carbonate (CaCO₃). It has a smooth structure and occurs in two forms: fine-grained and coarse-grained (most often used in laying hen feeds).

The nutritional requirements of the poultry animals are essential for good performance, and the poultry industry relies on the supply of commercially available ready-to-use feed. There are many commercial producers of poultry feed in Serbia and raw materials for the production of this feed are of different origins and quality, as well as final products. Also, the regulations in Serbia provide the ranges of required quantities for calcium and total phosphorus for different categories of poultry feed, which are very wide, but generally in line with very precise recommendations given by poultry hybrid breeder companies. All this in practice implies that different feed producers in Serbia may have different concentrations in the same poultry feed category.

Therefore, the present study aimed to investigate the concentrations of calcium and phosphorus in poultry feed available on the Serbian market. The established calcium and phosphorus levels in analyzed poultry feeds were, then, also compared to allowed ranges described in the Rulebook on the quality of animal feed (*Official Gazette R.S. No. 4/10, 113/12, 27/14, 25/15, 39/16, and 54/17*), by categories.

Material and Methods

Sample collection

A total of 1,058 samples of different poultry feeds were collected from the Serbian market from four different major Serbian manufacturers of animal feed over five years (from the beginning of January 2017 to the end of December 2021).

The samples were classified into five groups: Broiler Starter Feed, standard commercial feed mixture intended for hybrid broiler chickens aged 0-21 days (n = 198) – SF; Grower Feed intended for hybrid broiler chickens aged 22-35 days (n = 239) - GF, Finisher feed for broilers aged 36-42 days (n = 204) – FF; Layers feed 1, standard commercial feed mixture for hybrid laying hens aged 20-50 weeks (n = 204) – LF1; Layers feed 2 for hybrid laying hens over 50 weeks of age (n = 213) – LF2. After collection, feeds were labeled in accordance with laboratory practice and stored in polyethylene bags until analysis.

Sample preparation and measurement of calcium and phosphorus concentrations

Prior to analysis, all samples were homogenized and ground in a laboratory mill (ϕ 1 mm). Determination of calcium levels was performed using Atomic Absorption Spectrophotometer Varian AA-175 according to the official method SRPS ISO 27085:2008. Phosphorus concentration was determined using the spectrophotometry method, on spectrophotometer SPECOL 1300 (Analytik Jena, Germany) in accordance with official method SRPS ISO 6491:2002. Total calcium and phosphorus levels were expressed as % of the calcium/phosphorus of the feed sample. Each sample was carried out in triplicate for both, calcium and phosphorus levels.

Statistical analysis

The results of our research were statistically processed (Statsoft Inc. Statistics for Windows, Version 5.0) and presented in tables as the arithmetic mean (\bar{X}), the standard deviation (SD), coefficient of variation (CV), and the variation interval (minimum-maximum).

Results and Discussion

Table 1 shows the results of total calcium (%) content in samples collected from four major manufacturers of animal feed available on the Serbian market.

Table 1. Calcium content (%) in poultry feed by categories

Manufacturer	I (n = 267)	II (n = 222)	III (n = 272)	IV (n = 257)	Rulebook on the quality of animal feed*
Feed sample	$\bar{x} \pm SD$ (minimum; maximum) (number of samples) (CV, %)				
SF	1.07 ± 0.12 (0.88; 1.40) (n = 65) (CV=11.59)	0.93 ± 0.11 (0.68; 1.08) (n = 48) (CV=11.90)	0.88 ± 0.08 (0.73; 1.01) (n = 52) (CV=8.54)	0.96 ± 0.09 (0.82; 1.10) (n = 33) (CV=9.34)	0.9-1.10
GF	1.05 ± 0.12 (0.78; 1.34) (n = 65) (CV=11.68)	0.85 ± 0.15 (0.48; 1.14) (n = 57) (CV=17.98)	0.86 ± 0.13 (0.65; 1.15) (n = 57) (CV=6.09)	0.96 ± 0.06 (0.84; 1.06) (n = 60) (CV=6.71)	0.80-1.00
FF	0.99 ± 0.23 (0.79; 1.27) (n = 60) (CV=23.08)	0.80 ± 0.16 (0.43; 0.99) (n = 42) (CV=19.60)	0.84 ± 0.07 (0.64; 0.90) (n = 53) (CV=8.18)	1.02 ± 0.05 (0.96; 1.18) (n = 49) (CV=4.98)	0.70-0.90
LF1	3.61 ± 0.27 (3.22; 4.00) (n = 58) (CV=7.60)	3.33 ± 0.19 (3.00; 3.66) (n = 38) (CV=5.71)	3.27 ± 0.16 (2.98; 3.48) (n = 56) (CV=4.86)	3.54 ± 0.23 (3.22; 3.83) (n = 52) (CV=6.38)	3.20-4.00
LF2	3.60 ± 0.25 (3.40; 3.87) (n = 59) (CV=6.97)	3.38 ± 0.18 (3.17; 3.65) (n = 37) (CV=5.19)	3.52 ± 0.21 (3.11; 3.76) (n = 54) (CV=6.09)	3.57 ± 0.24 (3.04; 3.84) (n = 63) (CV=6.71)	

*Rulebook on the quality of animal feed (*Official Gazette R.S." No.27/14, 25/15, 39/16, and 54/17*); I, II, III, IV – manufacturers in the Serbian market; n – number of samples; SD – standard deviation; \bar{x} - arithmetic mean; CV - coefficient of variation SF – Starter feed; GF – Grower feed; FF – Finisher feed; LF1 – Layer feed 1; LF2 – Layer feed 2

While observing, we can see that the contents of calcium varied not only between the manufacturers but also within the different batches of the same manufacturers over five years.

The arithmetic mean was somewhat higher for the groups SF, GF, and FF from the manufacturer I as well as the FF group from manufacturer IV than the limits given by the Rulebook on the quality of animal feed, while this value is slightly lower for the SF group from the manufacturer III. However, in the Rulebook on the quality of animal feed, there is section 105. that is referring to feed also meeting the quality requirements if the chemical quality analysis reveals deviations within the limits set by the Rulebook if they meet allowed tolerances for mixtures: if the calcium content is lower than 1%, the tolerance equals 0.15 % of the absolute value of the result (e.g. result of the arithmetic mean for the group SF from manufacturer III is 0.88 ± 0.15 , which now when calculated, can be considered acceptable); if the calcium content is in range of 1 – 6 %, the tolerance equals to 15 % of the results relative value (e.g. result of the arithmetic mean for

the group GF manufacturer I is 1.13 ± 0.17 , which when calculated may be considered within the acceptable ranges). This tolerance gives a rather wide range of results that would be acceptable. When this principle is applied to all of the arithmetic means from every feed group from each manufacturer it can be concluded that all of the mixtures were within the ranges described by the Rulebook. Special attention has to be turned to several batches from each manufacturer that, even when tolerance by the Rulebook applied, were not acceptable in quality, as their calcium content was higher than allowed for this poultry feed mixtures.

Taking into consideration maximum levels of total calcium content prescribed by the Rulebook, the research showed that all four of the manufacturers had values for certain batches that were outside the limits, with the exception of manufacturer III for feed LF1. The same manufacturer had calcium results for the FF feed (100% of them) above the maximum of the defined ranges. With the ranges calculated to adhere to allowed tolerances for each poultry feed, as described by the Rulebook, the results for total calcium for mixtures SF, GF, and FF for the manufacturer I had several batches results above permitted (7.69%, 15.38%, and 36.84% respectively, of all samples). Also, the portion of samples that were above the specified values, with applied tolerance by the Rulebook, for manufacturer II for the same three poultry feed categories were 8.33%, 7.02%, and 9.52%, respectively. For manufacturer III for the feed FF, 22.45% of samples were above the ranges with applied tolerances, and in manufacturer IV for feed SF that share was 5.77%. These batches are considered to be unacceptable in quality, by the Rulebook.

The research results of the analyzed feeds for broilers and laying hens in Serbia indicate that the average calcium content was predominantly close to the maximum-to-middle value of the defined (and declared) range of permitted concentrations according to the Rulebook. With that in mind, the research showed relatively high variability of calcium concentration in poultry feed, especially in broiler feed, both between different manufacturers and among different batches of the same product from the same manufacturer.

Table 2. Total phosphorus content (%) in poultry feed by categories

Manufacturer	I (n = 267)	II (n = 222)	III (n = 272)	IV (n = 257)	Rulebook on the quality of animal feed*
Feed sample	$\bar{x} \pm SD$ (minimum; maximum) (number of samples) (CV, %)				
SF	0.77 ± 0.11 (0.56; 1.0) (n = 65) (CV=13.92)	0.60 ± 0.04 (0.52; 0.68) (n = 48) (CV=6.18)	0.74 ± 0.10 (0.56; 0.86) (n = 52) (CV=13.99)	0.64 ± 0.07 (0.54; 0.76) (n = 33) (CV=11.66)	0.65-0.85
GF	0.74 ± 0.09 (0.58; 0.95) (n = 65) (CV=11.68)	0.55 ± 0.06 (0.42; 0.64) (n = 57) (CV=11.14)	0.66 ± 0.06 (0.57; 0.77) (n = 57) (CV=8.76)	0.60 ± 0.04 (0.52; 0.66) (n = 60) (CV=5.81)	0.60-0.80
FF	0.65 ± 0.08 (0.47; 0.82) (n = 60) (CV=11.66)	0.47 ± 0.06 (0.36; 0.54) (n = 42) (CV=11.85)	0.62 ± 0.07 (0.53; 0.74) (n = 53) (CV=10.89)	0.63 ± 0.03 (0.57; 0.70) (n = 49) (CV=5.47)	0.50-0.70
LF1	0.73 ± 0.08 (0.52; 0.85) (n = 58) (CV=10.41)	0.56 ± 0.04 (0.51; 0.62) (n = 38) (CV=6.35)	0.69 ± 0.06 (0.62; 0.82) (n = 56) (CV=8.83)	0.61 ± 0.07 (0.52; 0.77) (n = 52) (CV=11.40)	0.65-0.85
LF2	0.74 ± 0.06 (0.57; 0.83) (n = 59) (CV=7.70)	0.53 ± 0.03 (0.46; 0.58) (n = 37) (CV=6.28)	0.59 ± 0.05 (0.52; 0.68) (n = 54) (CV=8.43)	0.62 ± 0.06 (0.54; 0.84) (n = 63) (CV=10.42)	0.60-0.80

*Rulebook on the quality of animal feed (*Official Gazette R.S." No.27/14, 25/15, 39/16, and 54/17*); I, II, III, IV – manufacturers in the Serbian market; n – number of samples; SD – standard deviation; \bar{x} – arithmetic mean; CV - coefficient of variation SF – Starter feed; GF – Grower feed; FF – Finisher feed; LF1 – Layer feed 1; LF2 – Layer feed 2

Table 2 shows the results of total phosphorus (%) content in samples of the same feed category. The same tolerance rules are applied to the total phosphorus content, as the one applied to the calcium content. The average value for the contents of this element differed, as manufacturer II showed the lowest results for all of the poultry feed mixtures, lower than prescribed (and declared). When taking into consideration the tolerance of 0.15 % from the absolute value of the results given as calculated arithmetic mean, these values could be regarded as acceptable, as there is a chance of them being in the ranges given by the Rulebook. However, it has to be mentioned that some of the results of the certain batches for the categories GF and FF sampled by manufacturer II, in regards to total phosphorus content were lower even with the tolerance level by the Rulebook applied.

Based on the ranges for the content of total phosphorus, set by the Rulebook, all manufacturers for the analyte in focus had values for certain batches that were outside the limits, with the exception of manufacturer III for the feed FF who had all of the samples within the ranges. Manufacturer II had results for all of

the mixtures from the group LF1 and LF2 below the minimum given by the Rulebook. But when we take into account the tolerance allowed by the Rulebook, the total phosphorus content was acceptable for almost all manufacturers with the exception of the manufacturer I, who had results of total phosphorus for the mixtures SF, GF, and FF (6.15%, 15.38%, and 18.33% respectively, of all samples) that were lower even with the allowed tolerances for mixtures applied. Also, manufacturer II for the GF mixture had 10.53% of the total samples that were lower than the ranges calculated to adhere to the Rulebook by applying the tolerance. Hence, these batches could be considered unacceptable.

In general, the results of our research indicate that the average content of total phosphorus in feed for broilers and laying hens in Serbia is close to the minimum-to-mid value of the defined (and declared) range of permitted concentrations by the Rulebook. The reason can be found in the fact that phosphorus sources are among the most expensive components for the production of poultry feed, as well as the established practice of adding phytase enzymes to commercial poultry feed (*Li et al., 2017*).

The research results also suggest that Serbian commercial food producers are relatively more precise in formulating total phosphorus than calcium in poultry feed (the average coefficient of variation in all producers was 9.03% for total phosphorus and 13.36% for calcium in broiler feed, and 6.72 % for calcium in food for laying hens). The source of these variations may be different, but it is usually associated with the variability in the quality of raw materials for production and the efficiency of the quality control system. For this reason, manufacturers are recommended to work on more efficient and frequent quality control, both raw materials that go into food mixtures and finished products.

Conclusion

This research suggests that the composition of two main minerals in poultry feed is highly variable among manufacturers. The variation was noticeable even between the batches of the same manufacturers, and results found in several batches were unacceptable and mislabeled. Based on the results of this study it may be advisable to conduct an external analysis of poultry feed samples more frequently for the composition of these nutrients as well as the components that go into these mixtures prior to production. Quality control of animal feed could be advised for poultry producers as well in order to make sure that the feed is actually within the parameters given by the manufacturers' declaration.

Komercijalna hrana za živinu u Srbiji – analiza sadržaja kalcijuma i fosfora

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Rezime

Kalcijum i fosfor predstavljaju važne mikronutrijente u hrani za živinu. U ovom radu ćemo ukratko govoriti o značaju i nutritivnim potrebama ovih minerala u ishrani živine, kao i o prosečnim količinama ovih nutrijenata u hrani živine koja se može komercijalno naći na tržištu Srbije. Sa tržišta Srbije prikupljeno je ukupno 1.058 uzoraka od četiri velika srpska proizvođača stočne hrane u periodu od pet godina - od januara 2017. do decembra 2021. Uzorci su klasifikovani u četiri grupe: Potpune smeše za tov pilića I (n = 198) - SF, Potpune smeše za tov pilića II (n = 239) - GF, Potpune smeše za tov pilića III (n = 204) - FF, Potpune smeše za nosilje jaja za konzum I (n = 204) – LF1, i Potpune smeše za nosilje jaja za konzum II (n = 213) – LF2. Ovo istraživanje ukazuje na to da je mineralni sastav hrane za živinu veoma različit među proizvođačima, ali i među šaržama istog proizvođača. Nekoliko šarži proizvođača I (kod grupa SF, GF, FF) i IV (kod FF grupe), čak i kada se primene pravila za dozvoljena odstupanja za smeše iz Pravilnika o kvalitetu hrane za životinje, nisu bile prihvatljive po kvalitetu, jer je njihov sadržaj kalcijuma bio veći od dozvoljenog za analiziranu smešu hraniva. U pogledu sadržaja ukupnog fosfora, rezultati pojedinih šarži za kategorije GF i FF proizvođača II bili su niži i po primeni računice za dozvoljena odstupanja za smeše prema Pravilniku, pa se kao takve, smatraju neprihvatljivim. Na osnovu rezultata ove studije može se preporučiti da se češće vrše eksterne analize uzoraka hrane za živinu na sastav ovih nutrijenata, kao i komponenta koje ulaze u ove smeše. Kontrola kvaliteta stočne hrane može se savetovati i uzgajivačima živine kako bi se uverili da je hrana koju daju životinjama zaista u okviru parametara datih u deklaraciji proizvođača.

Ključne reči: brojleri, nosilje, kvalitet hraniva, sadržaj kalcijuma, sadržaj ukupnog fosfora

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