Effects of a reduction of dietary levels of calcium and phosphorus on performance, bone minerals and mineral excretion of turkey breeder hens in the rearing and laying period

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I Abbreviations

av. P	available phosphorus
Ca	calcium
CaO	calcium oxide
EU	European Union
GfE	German Association for Nutrition Physiology *(Gesellschaft für Ernährungs-physiologie)*
IU	international unit
LW	week of live
ME	metabolizable energy
N	sample size
NPP	nonphytate phosphorus
NRC	National Research Council
P	phosphorus
P_2O_5	phosphate (as phosphorus pentoxide)
PAN	Polish Academy of Sciences (*Polska Akademia Nauk*)
pcdP	prececal digestibility of phosphorus
PW	production week
SD	standard deviation
USA	United States of America
U	unit
WPSA	World's Poultry Science Association
XP	crude protein

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1 Introduction

Phosphorus (P) is a crucial resource that is required in many stages of agriculture. In addition to that, it is a finite resource, and therefore, it will grow scarcer and probably increasingly expensive in the coming years. Potchanakorn and Potter (1987) reported that P "represents the third most expensive nutrient following energy and protein" (p. 505), and Amundson et al. (2015) demonstrated that the cost of P per ton (united states) in 2015 was more than eight times as high as in 1961. However, this mineral may also cause or support huge environmental damage, such as eutrophication, when excessive amounts enter water bodies (Huber, 2008; Lan, Y. et al. (2022).

The aforementioned issues lead to the conclusion that the use of P must be reduced along the whole nutrient circle of agriculture. In animal production, the most critical approach may concern animal feed. Regarding turkeys in particular, literature about P requirements is very rare. Only a few studies have reported low but adequate dietary P levels for the fattening of turkeys; almost no recent and robust data are available for breeders.

Because experts assume a great potential for the reduction of P in all poultry species (Rodehutscord, 2001), the aim of the present study was to obtain the first results regarding this potential in turkey breeder hens. Therefore, the scientific method used in this study followed a basic factorial approach to the P requirements for turkey breeder hens using all available data (mostly adapted from other poultry) to build new resilient data for these hens. Because P requirements in animals are strongly linked to calcium (Ca) requirements, the same factorial approach was also applied for Ca. The outcomes of these approaches were embedded into a practical feeding program and compared with a standard diet in a field trial with rearing as well as laying turkey breeder hens. Possible effects of the different feeding regimes were studied along two rearing periods (trial I and trial III) and two laying periods (trial II and trial IV) using several factors of performance and health.

2 Literature Review

2.1 Metabolism of calcium and phosphorus in poultry

2.1.1 Function of calcium and phosphorus in poultry

Ca and P are two of the major minerals in the body of poultry. Their metabolisms are closely linked with each other as well as with the metabolism of hormones such as vitamin D_3. The interactions between these three mostly regulate the formation and mineralization of bones as hydroxyapatite is a crystal built from Ca and P. Hydroxyapatite is the major component of bone mineral. The amount of bone mineral itself is an indicator of bone stability and thus fewer breaking incidents in poultry (Robison & Karcher, 2019; Veum, 2010; Zhang & Coon, 1997).

Approximately 98% of Ca in the body of poultry occurs in the skeleton. The remaining amount is present in the extracellular fluid and within the cells and is involved in blood clotting, muscle contraction, and the conduction of nerves. Moreover, Ca plays an essential role in the formation of eggshell in laying birds (Veum, 2010).

In addition, P has a major quantitative function as a component of bones. Across all animals, approximately 80% of P can be found in the bones. The other 20% occurs as a component of many parts of nucleic acids as well as in energy-saving and membrane-building molecules. Therefore, the healthy growth of poultry without skeletal damage is only possible with optimal supplies of Ca and P (France et al., 2010; Günther, 1966; Jeroch et al., 2019, pp. 38-39; Veum, 2010).

Furthermore, Ca and P can be stored in bones for a long time, meaning that the birds can compensate for a temporary undersupply of one or both of these minerals through resorbing the required amount from their bones. Such use of stored Ca and P does not lead to physiological disturbances as long as the minerals are replaced shortly after (France et al., 2010; Jeroch et al., 2019, pp. 40-42).

Derived from the interactions of Ca and P in the bodies of poultry, an optimum supply level exists for each element. If an animal receives less than the optimum supply for a long period, this will lead to undersupply, whereas a supply above that optimum causes oversupply. The undersupply of Ca and P can lead to weak bones, bone fraction, and lameness in birds. An oversupply of Ca leads to a lower utilization of P and possibly also lower feed intake. Due to an oversupply of P, however, more Ca is mobilized from the bones, which could also lead to weak bones (Jeroch et al., 2019, pp. 42-43).

Related to a long-term insufficient supply of Ca, P, and vitamin D_3, various skeletal disorders may occur in the rearing or laying period. Particularly relevant for turkey breeders are rickets, osteomalacia, and osteoporosis (Thorp, 1994; Whitehead & Fleming, 2000). The occurrence of these sydromes are described as follows.

Rickets is usually observed in young animals with rapid growth. A deficiency of vitamin D_3 or available P (av. P) or imbalances of Ca and P in the feed lead to poor mineralization in bones. This results in weak bones with high flexibility and bone deformations, causing lameness (Thorp, 1994; Wise, 1975).

Osteomalacia particularly occurs in laying flocks in high production, which have high Ca requirements for eggshell formation. Due to an unsuitable supply of Ca, P, and vitamin $D_{3,}$ Ca may not be metabolized for the shell in sufficient amounts. This leads to a switch from the production of structural bone to a greater production of medullary bone mass, leading to a reduction in bone stability and an increase in bone fractures (Jeroch et al., 2019, p. 56; Whitehead & Fleming, 2000).

Osteoporosis is a metabolic disorder and can be observed in all types of laying poultry, even with an adequate mineral supply. Due to a defect, Ca for egg production is not resorbed from the medullary part of bones, which serves as Ca storage, but rather from the structural part of bones, which is responsible for bone stability. Osteoporosis may also result in weak bones and an increase in bone fractures, but it is not related to the concentration of Ca or P in the feed (Thorp, 1994; Whitehead, 2004).

Many pathologic analyses have demonstrated that these skeletal changes often start at a young age and may be diagnosed by the bone quality of culled animals (Thorp, 1994). Roberson (2004) found the tibia to be the most sensitive bone to mineral changes. Shastak et al. (2012b) even concluded that the retention of P in the tibia is a suitable criterion for determining the P retention of the whole body.

2.1.2 Intake and absorption of calcium and phosphorus in poultry

Because of the interaction between Ca and P in animals, the absorption of one of these minerals is always affected by the level of the other. Therefore, it is crucial to observe the levels of both minerals simultaneously when optimizing feed for animals (France et al., 2010; Jeroch et al., 2019, pp. 38-42). The optimal Ca:P ratio for poultry is derived from the optimal dietary levels of each element. The National Research Council (NRC, 1994) reported that the optimal ratio is in a range of 1:1–2:1 for broiler chickens and turkeys for fattening and up to 4:1 for laying poultry, considering a ratio of Ca:total P. Later recommendations have been made based on the ratio of Ca:digestible P; for example, a range of 1.8–2.2:1 was set as a standard in practical diets for fattening turkeys (Angel, 2013). By contrast, Driver et al. (2005) observed no negative effect on the body weight gain of broiler chickens between 0.94 and 1.25:1 Ca:total P. Angel (2013) even suggested comparing available Ca to av. P as a new consideration of the optimal value.

The absorption of Ca and P occurs in the small intestine. When phosphate contacts the intestinal mucosa, it must be in the soluble form of phosphate ions. Any insoluble complex of phosphate would decrease the absorption of P. Large amounts of Ca, for example, may result in the formation of insoluble salts, which would reduce the absorption of P compared with the same amount of sole P present in a solution (France et al., 2010).

Usually, P in grains is present as phytate, the salt of phytic acid (50%–80% of total P) (Jeroch et al., 2019, pp. 40-41). Phytic acid is an essential component of all seeds that is known to form complex bindings with minerals such as Ca, magnesium, iron, and zinc. These complexes can be split by the enzyme phytase, which occurs in many raw materials of feed in different amounts. This enzyme is barely present in poultry's intestinal mucosa. The availability of P in general varies between 20% and 40% in raw materials with low phytase activity and between 40% and 55% with higher phytase activity in the material itself. Availability may be raised through adding exogenous phytase to the feed (Jeroch et al., 2019, pp. 40-41).

Ca often occurs in a chemical combination with phytic acid called Ca phytate. In this complex, the availability of Ca (as well as P) is lowered, which leads to reduced absorption. In general, this phenomenon can be observed whenever the internal P level of the raw material is high. The Ca level in feed being higher than the requirement may lead to low P absorption as well as general declines in feed intake and growth rate, as it has been observed in broiler chickens (Kornegay, 2001; Sebastian et al., 1996).

The homeostasis of Ca and P in plasma is controlled by a feedback mechanism involving parathyroid hormone, activated vitamin D_3 (calcitriol), calcitonin, and the hormone FGF23 (fibroblast growth factor 23). Receptors for these hormones are present in the small intestine, bones, and kidneys. Whenever the concentration of Ca^{2+} and/or PO_4^- (less impact) in plasma is too low, parathyroid hormone is released from the parathyroid gland. If the concentration remains low for a sustained period of time, parathyroid hormone stimulates the conversion of vitamin D_3 to its active form $1,25(OH)_2D_3$ (calcitriol). Calcitriol stimulates Ca and P absorption in the small intestine as well as the solution from Ca and P out of the bones. Then, this additional amout of Ca and P increases the plasma levels of those minerals. Another function of calcitriol is to reduce the excretion of Ca and/or P by the kidneys. If the concentrations of Ca and P in plasma are too high, the hormone calcitonin is released in the C cells of the thyroid glands. The high level of calcitonin causes a decrease in the resorption of Ca and/or P in the small intestine and from the bone storage and then increases excretion through the kidneys. Furthermore, the hormone FGF23 is produced in the bones and its excretion inhibits the absorption of P and stimulates the absorption of Ca in the kidneys (Erben, 2019; Veum, 2010).

2.1.3 Influences of phytases on the availability of phosphorus and other nutrients

The availability of P from feed varies according to the amount of phytate P and the activity of intrinsic phytase within the ingredients. Since phytate P must be split by phytase, a higher percentage of phytate and a lower activity of intrinsic phytase lead to a worse availability of P; by contrast, a lower percentage of phytate P and a higher activity of intrinsic phytase lead to a better availability of P. Wheat, for example, exhibits a lower percentage of phytate P and a higher activity of intrinsic phytase (U/kg) compared with maize. Therefore, very roughly, the availability of P is better for wheat then for maize (Kornegay, 2001).

Phytase activity within the gastrointestinal tract of poultry is rare. As a result, P can be released only very poorly from the phytate binding if the activity of intrinsic phytase from the feed is low (Humer et al., 2015). However, a phytic acid molecule contains 28.2% P, which can be released by adding an exogenous phytase to the feed. The origins of these commercial phytases include yeast, bacteria, and fungi (Vats et al., 2005).

In general, all phytases belong to the “family of histidine acid phosphatases, a sub-class of phosphatases” (Vats et al., 2005, p. 471). They catalyze the hydrolysis of phytic acid in a step-wise manner, thereby lowering the amount of inositol phosphates, myo-inositol, and inorganic phosphate. The activity of phytase can be defined as the amount of enzyme that exempts 1 µmol of inorganic P from a 5.1 mmol solution of sodium phytate within 1 min. The solution requires 37°C and pH 5.5. The determined amount equals one unit (U) (Kornegay, 2001; Vats et al., 2005).

The effect of 500 U/kg on the availability of P in feed has been estimated to be an extra of 0.037% of available P for poultry. The exact amount varies according to the source and total amount of P in the feed. This results in a net reduction of P excreted of between 20% and 50% (Kornegay, 2001; Ravindran et al., 1995).

Phytate is known to form complex bindings with Ca^{2+} as well as ions of copper, zinc, nickel, cobalt, magnesium, and iron. In addition, complex bindings may also occur with amino acids and starch. Since exogenous phytase in feed releases those bindings, the use of phytase may also improve the availability of the aforementioned ions, protein, and the energy contained in the feed. Thus, amounts of those elements in excreted material may be reduced by adding phytase to feed (Kornegay, 2001; Vohra et al., 1965).

Regarding Ca, the extra usable amount created by 500 U/kg phytase is 0.46–0.87 g. Specifically, Schoner et al. (1994, as cited in Kornegay, 2001) reported 0.46 g in trials with broiler chickens, whereas Kornegay et al. (1996, as cited in Kornegay, 2001) reported 0.87 g from studies on turkey poults. The effect was equal for ratios from 1.1:1 to 1.4:1 (Ca:total P) for broiler chickens and turkeys (Kornegay, 2001).

The absolute force of phytase is influenced by the level of phytase used, level of P in the diet, level of Ca, and ratio of Ca and P. It is also influenced by the intrinsic level of phytase in ingredients and the overall processing and pelleting methods (Kornegay, 2001).

2.2 Requirement data of calcium and phosphorus in turkey parent stock hens

2.2.1 Evaluation systems

Most recommendations regarding the optimal mineral supply for poultry are based on total daily consumption. For example, this approach is currently used for magnesium, sodium, and Ca (Jeroch et al., 2019, p. 243).

To evaluate P requirements, however, the system of total amounts is unsuitable. As poultry exhibit rare endogenous phytase activity in their gastrointestinal tract, their absorption of P is related to the way it is linked with other elements in raw materials. In many forage plants, P occurs as phytate P. This source of P is only usable by turkeys if it is released by a phytase (contained in the raw material, added to the feed or endogenous). As many phytases fluctuate in efficiency due to external factors, such as pH level and temperature, it is difficult and unreliable to estimate the amount of P that may be used by the animals. To avoid this problem, recommendations for P over the last decades have been provided at the level of "available P," which in this context mostly means digestible P (see below), or "non-phytate P" (NPP) (Jeroch et al., 2019, p. 243; Kornegay, 2001; Rodehutscord, 2001).

Available P means the proportion of dietary total P that, at a marginal level of P supply, may be utilized from the animal to cover their requirement of P. In general, the share of av. P from the total amount of P may be determined using several methods, which can be summarized as qualitative measurements, quantitative measurements, and *in vitro* tests (Shastak & Rodehutscord, 2013, 2015; World's Poultry Science Association Working Group No. 2, 2013).

A qualitative measurement may be used only for the determination of relative differences between two or more sources of P on the resulting criteria. Therefore, the determination of the availability of P through qualitative measurements first requires criteria that are measurable and react on different levels of av. P. The fed diet must provide a deficiency of P and the added levels of P may not exceed the requirement of the animal. To obtain resilient data, the standard that must be used for comparison with the tested P source must be suitable and preferably commonly known and accepted. In studies with bone ash, Gillis et al. (1954) first described beta-tricalcium phosphate as a reference source with a relative availability of 100 (Shastak & Rodehutscord, 2013).

Qualitative measurements with bones may be performed by determining the amount of bone ash, mineral density, and breaking strength of the appropriate bones. A review by Shastak and

Rodehutscord (2013), however, considered bone breaking strength an uncertain parameter as it has been observed not to be as sensitive to the share of av. P as bone ash. Orban et al. (1993) also highlighted that all kinds of measurements with bones may be affected by the type of instruments, preparation method, and other physical and mechanical properties of the bones used for measurement.

Another qualitative measurement focuses on the blood of the animal. Gardiner (1962) found that the level of plasma inorganic P correlates with differences in the availability of P in the diet. The benefit of blood measurements is that the tested animals do not need to be killed for the test, and hence, blood measurements may be repeated with the same animals. On the other hand, the level of plasma P is affected by many other parameters, so a differing level of plasma P may also be caused by a previous feed intake.

The measurement of growth rate as a qualitative measure was proclaimed by Vandepopuliere et al. (1961) to be as good as bone ash for determining the relative availability of P. A few years later, however, Nelson and Walker (1964) found that growth rate is a less sensitive criterion than bone ash. Moreover, Shastak et al. (2012a) concluded that both blood serum and body weight gain are unsuitable for evaluating P availability.

A quantitative measurement, however, could be performed focusing on P retention. The retention of P may be determined by collecting the complete excreta and calculate the difference between the intake of P and its excretion. Another method for classifying P retention may be performed with the help of an indigestible marker (Shastak & Rodehutscord, 2013).

In principle, P retention may also be determined through whole body analysis. However, studies using this method are rare. A problem with whole body analysis is the preparation of the sample as it is difficult to receive a homogenous and representative sample (Shastak & Rodehutscord, 2013). Wolynetz and Sibbald (1985) also found high variations between individuals even when they were of the same age.

Another quantitative method involves the use of digestibility. Prececal digestibility is a method that is already used to determine protein quality and may be transferred to P. Digestibility may be calculated using the results of animal trials with a measurement of P intake and P excretion with faeces or studies with indigestible markers. The advantage is that the determined level is not affected by post-ileal microbial activity and that any urinary excretions are excluded (Shastak & Rodehutscord, 2013).

For *in vitro* tests of P solubility to determine the availability of P, conflicting results have been published regarding their success (Waldroup, 1999). From those results, Shastak and Rodehutscord (2013) inferred that no reliable method exists that uses the solubility of phosphate sources to determine the availability of P, and consequently, that *in vitro* solubility is a poor indicator of *in vivo* bioavailability.

In general, all resilient methods exhibit a high correlation and similar results for the availability of P. However, as there is a need for quantitative results to optimize diets, Shastak and Rodehutscord (2013) suggested focusing on retention and prececal digestibility as the preferred methods for determining the availability of P for poultry.

To improve the comparability of trials concerning the digestibility of P, the *World's Poultry Science Association* (WPSA) published a proposal to determine the availability of P based on prececal digestibility in 2013. This protocol contains agreements about the animals to be used, the experimental diets, and other details of the trial. For example, the calculated level of av. P in the basal diet should not exceed 0.15%, which equals approximately 0.30% of total P. The ratio of total Ca to total P may vary between 1.3:1 and 1.4:1. As there are still other known and unknown factors that influence the availability of P, this protocol must be viewed as a guideline that needs to be proven and improved through several trials. It is open for additions and changes. Nevertheless, this protocol provides a guideline for standardizing the determination of digestible P, which will probably lead to a better comparison and worldwide harmonization of several trials concerning different dietary levels of P (Shastak & Rodehutscord, 2015; WPSA Working Group No. 2, 2013).

Shastak and Rodehutscord (2015) attempted to combine data from quantitative and relative measurements of P availability to make them comparable. They used relative bioavailability data from older studies that examined various raw materials to recalculate digestibility. The recalculated data exhibited a lower variation than the original quantitative data, while the slope was only 0.45 and the intercept differed from zero. Consequently, the authors concluded that a recalculation of relative bioavailability data into quantitative digestibility data is not possible. This led them to suggest accessing as much digestibility data as possible using the standard protocol from the WPSA.

In 2017, the quality of said protocol was tested with a global ring test, including research stations in Europe and North America. The aim was to determine the prececal P digestibility (pcdP) of soybean meal in broiler chickens. To achieve this target, each of the 17 research stations followed the standard protocol of the WPSA. A comparison of the results indicated that the pcdP of soybean meal varied widely between 19% and 51%. An evaluation of the procedure revealed that some differences existed among the stations in, for example, the diet and management conditions during the pre-experimental period. It was concluded that the standardizations in the protocol must be improved and should also include these factors. Until a more standardized protocol is established, pcdP data from different stations must still be compared carefully (Rodehutscord et al., 2017).

2.2.2 Requirement recommendations

In general, dietary allowances for poultry and all nutrients are, for example, provided by the German Society of Nutrition Physiology (*Gesellschaft für Ernährungsphysiologie;* GfE) or the WPSA. These recommendations are based on calculations of the factorial approached requirement data (Jeroch et al., 2019, p. 243). For turkey parent stock, no recommendations have been published for Ca and P, which indicates a poor database of requirements for maintenance and growth for turkey breeders (see also Section 3.2.2). Other concentration norms for minerals are estimated based on evaluations of dose and response. This system is used by the NRC (1994), the Polish Academy of Sciences (*Polska Akademia Nauk* [PAN], 2005 as cited in Jeroch et al., 2019, p. 343) and in trials by Leeson and Summers (2005). All these publications focused on heavy breeds only (Table 1).

However, those recommendations are partly based on the standards of earlier research and breeding improved turkey growth rates in the years prior to the NRC's publication, which suggests transferring the recommended levels to earlier ages to ensure optimal supply. Since the publication of those estimations, the growth rates of turkeys for fattening have increased again by approximately 24% (1993: 124 g/day, 2020: 154 g/day; Meyer, 2020). Reliable weight data for parent stock are not available, but as the production of fattening turkeys is based on the parent stock, the general development might be quite similar. Following the argumentation of the NRC (1994), this leads to the assumption that the levels or the relation of levels to age must be adjusted again. Additionally, breeding objectives have changed over the last two decades. For example, the influence of leg stability on the breeding goal has increased since 2006. These changes in the body weight and breeding of animals suggest that concentration norms from many years ago probably no longer fit the parent stock hens that are currently producing poults.

The recommendations of the NRC, for example, are based on trials from 1948 to 1962 for Ca and from 1954 to 1989 for P (Meyer, 2020).

Table 1: Recommendations for Ca, av. P, and nonphytate P (NPP) for heavy turkey breeder hens

	Age (weeks)	Ca (g/kg)	Av. P (g/kg)	NPP (g/kg)
Leeson and Summers (2005)	0 – 3	1.40	0.80	
	4–7	1.30	0.70	
	8–11	1.10	0.60	
	12–14	1.00	0.50	
	15–lighting	0.90	0.45	
	Breeder	2.60–2.80	0.35–0.40	
NRC (1994)	0–3	1.20		0.60
	4–7	1.00		0.50
	8–10	0.85		0.42
	11–13	0.75		0.38
	14–16	0.65		0.32
	17–30	0.50		0.25
	Breeder	2.25		0.35
PAN[1] (2005)	0–3	1.35	0.75	0.60
	4–6	1.25	0.72	0.50
	7–11	1.10	0.65	0.42
	12–15	1.00	0.55	0.38
	16–28	0.90	0.45	0.32
	29–30	2.50–3.20	0.45–0.50	
	Breeder	2.50–3.20	0.45–0.50	

[1] As cited in Jeroch et al. (2019, pp. 343, 345)

All of these recommendations have now existed for more than 15 years, and since then only a few research studies have proven those levels' practical suitability. For example, Godwin et al. (2005) reported no negative effect on the performance of Large White turkey breeder hens fed with 0.17% av. P (0.3% total P) between weeks 31 and 62 compared with hens fed with 0.35% av. P (0.5% total P). Other studies with white laying hens have also suggested a possible reduction of up to 0.15% av. P (0.34% total P) and 0.09% NPP (0.22% av. P) for broiler breeder hens to have no negative effects on health and performance, while 0.10% NPP for laying hens led to severe negative effects on egg production (Jing et al., 2018; Plumstead et al., 2007; Snow et al., 2005).

Different systems for P evaluation as well as different systems for calculating the availability of P lead to a lack of comparability. For example, the NRC used the terms “NPP” and “available P” interchangeably in their 1994 publication. However, this system does not consider that a certain amount of phytate P may also be available and that NPP for poultry is only available at approximately 70%. France, for example, uses an alternative system, while the Netherlands uses a third system. This incomparability of trials concluded over the last decades has resulted in numerous gaps in the knowledge of real P requirements. To avoid undersupply and negative effects, most poultry diets contain a surplus of P as a safety margin, which leads to the assumption that many present diets contain levels of P that are too high (Plumstead et al., 2007; Rodehutscord, 2001).

Because Ca levels are highly related to P levels, it can be assumed that the levels of Ca are highly unreliable and probably also too high in present poultry diets (Angel, 2013).

2.2.3 Target levels defined by breeding companies

Aviagen Turkeys Ltd. and *Hybrid Turkeys* are the two main breeding companies for turkeys in the world. Both provide parent stock animals to their customer companies, which sell turkeys for fattening as well as hatching eggs (Stevens, 2016). On their homepages, the companies publish guidelines for the management of parent stock turkeys, which include nutrient recommendations for feeding. A closer examination of the general feeding concept and its levels of Ca and av. P reveals many similarities but also some differences between the companies (Table 2).

The largest difference in the recommended concentration of Ca appears in the laying period. From 30 weeks of age up to the end of production, the level of Ca in the diet of *Hybrid Turkeys* is 0.2% higher than that in the diet of *Aviagen Turkeys Ltd.* In addition, the differences fluctuate between *Hybrid Turkeys* and *Aviagen Turkeys Ltd.* by +0.05% and −0.10% (Figure 1).

The largest difference for the concentration of av. P also appears in the laying period, during which the diet of *Hybrid Turkeys* is 0.1% higher in av. P than that of *Aviagen Turkeys Ltd.* In addition, all deviations fluctuate between *Hybrid Turkeys* and *Aviagen Turkeys Ltd.* by +0.07% and −0.04% (Figure 2).

All assertions in this explanation focus on the standard diet. Both companies also describe special diets for particular situations, such as hot or cold weather or if the hens' weight is below or above the target weight. For example, *Hybrid Turkeys* suggests a pre-layer diet with a higher concentration of Ca, which can be fed for two weeks before the start of the lighting program for the hens if their weight is below the target. However, the feeding program from *Aviagen Turkeys Ltd*. does not have any comparable feeding phases.

Table 2: Recommended levels of Ca (%) and av. P in the standard feeding program for turkey breeder hens from Aviagen Turkeys and Hybrid Turkeys (own summary based on Aviagen Turkeys Ltd, 2019; Hybrid Turkeys, 2016a, 2016b)

Aviagen				**Hybrid**			
Weeks	Feed	Level of Ca (%)	Level of av. P (%)	Weeks	Feed	Level of Ca (%)	Level of av. P (%)
0 – 3	Starter	1.45	0.73	0 – 2	Starter 1	1.40	0.75
3 – 6	Rearer	1.35	0.68	2 – 4	Starter 2	1.40	0.75
6 – 10	Grower 1	1.25	0.62	4 – 7	Grower 1	1.30	0.65
10 – 12	Grower 2	1.10	0.55	7 – 12	Grower 2	1.15	0.58
12 – 29	Grower 3	1.00	0.50	12 – 28	Holding 1	1.00	0.50
From 30	Standard Breeder	2.80	0.38	From 30	Female layer	2.90 – 3.10	0.45 – 0.50

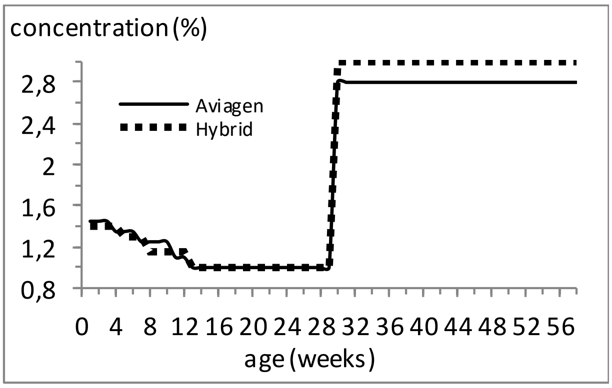

Figure 1: Concentration of Ca (%) in the standard feeding program from Aviagen Turkeys Ltd. and Hybrid Turkeys (own diagram based on Aviagen Turkeys Ltd, 2019; Hybrid Turkeys, 2016a, 2016b)

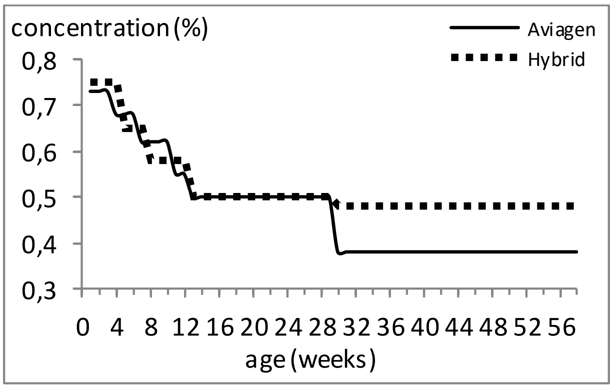

Figure 2: Concentration of av. P (%) in the standard feeding program from Aviagen Turkeys Ltd. and Hybrid Turkeys (own diagram based on Aviagen Turkeys Ltd, 2019; Hybrid Turkeys, 2016a, 2016b)

2.3 Effects of different dietary levels of calcium and phosphorus on the performance of hens

The performance of turkey breeder hens may be described by monitoring different factors. Commonly used factors include laying performance (also called laying percentage), fertility and hatchability, egg weight, and egg shell thickness. Of course, all may be affected by each other as well as by the dietary levels of Ca and P.

For laying hens, Austic and Keshavarz (1988) determined that dietary Ca levels of 2.0%, 2.8%, and 3.5% did not affect laying performance or egg weight. The same result was observed in a study by Clunies et al. (1992) with dietary Ca levels of 2.5%, 3.5%, and 4.5%. In addition, the authors found that higher Ca levels in the feed led to less shell deformation and increasing shell weight, although no effect on the percentage of Ca in the shell could be verified. In a trial from 1992 with dietary Ca levels between 2.5% and 5.0% (increments of 0.5%), however, it was found that increasing Ca levels indeed increased laying performance but again had no effect on egg weight (Roland et al., 1996).

Similar results from 1994 even indicated no negative effects from higher dietary levels of Ca but negative effects from feed with a low dietary Ca level of 2.5% (e.g., reduced egg-specific gravity). This led the authors to recommend not feeding any marginal dietary Ca levels to laying hens (Roland & Bryant, 1994).

Concerning the level of dietary P, Keshavarz and Nakajima (1993) found that av. P levels between 0.2% and 0.4% with a constant level of Ca (3.5%) had no effect on shell quality. Scott et al. (1999), however, found optimal shell quality with both high and low levels of Ca and P; therefore, the ratio between Ca and av. P seems to be the critical factor.

An older trial with young pullets focused on the fertility and hatchability of eggs with dietary Ca levels between 0.5% and 2.0% in the rearing diet and between 1.75% and 3.25% in the laying diet. The fertility of eggs was not affected by the Ca level in the rearing or laying period, while the hatchability of fertile eggs decreased as the level of Ca became higher in the laying diet (Berg et al., 1962).

For broiler breeder chickens, Wilson et al. (1980) postulated that excess P intake (1.42% total P) with a dietary Ca level of 2.85% may reduce shell quality and therefore indirectly decrease hatchability. Recent trials with a combined reduction of 10% or 20% of Ca and P indicated an increased embryonic mortality with diets containing reduced Ca and P levels (Kazemi-Fard et al., 2018).

In 1974, a trial with turkey breeders was conducted with dietary Ca levels between 0.99% and 3.33% and total P levels between 0.64% and 0.82%. Egg weight and fertility did now exhibit any variation concerning the different dietary levels. Egg production was influenced by the dietary level of Ca (groups with the lowest dietary Ca level produced fewer eggs), which indicated that the dietary Ca level should be raised to 2.0%, or rather 2.5%, if the rate of egg production is high. Groups with lower levels of Ca also exhibited a lower hatchability of fertile eggs and a lower shell weight as a percentage of egg weight. Concerning the dietary P level, all measured factors in the trial were unaffected by different P levels (Potter et al., 1974).

A more recent trial by Godwin et al. (2005) with Large White turkey breeder hens focused on the effect of different dietary P and phytase levels on the reproductive performance of the animals. In the experiment, three different levels of av. P (0.55%, 0.35%, and 0.17%) were fed to the hens; each level with and without an addition of 11.27 phytase units per g (Ca level = 2.90% for each diet). No negative effect was found for a lower (or higher) level of P on several parameters, such as laying performance, mortality, fertility, hatchability, egg weight, egg shell thickness, and weight of the progeny.

Overall, the results of these trials indicate that Ca should not be lower than 2.0% to avoid negative impacts on the performance of laying poultry. A dietary level of Ca higher than 2.5% might introduce negative impacts on performance. For P, negative effects on the performance of laying poultry might occur with a high dietary level of total P (1.42%). No negative effects were found in the aforementioned trials from lowering dietary P levels to 0.2% (available P) or 0.64% (total P).

2.4 Environmental impacts of phosphorus in agriculture along the production chain

The stocks of mineral P on Earth are finite, which means that P cannot be acquired from stores forever. Estimations on a particular end date differ widely. For example, Werner (1999) published different scenarios which suggested an end of P stores between 2060 and 2130 depending on the management of P in the 21st century. A more recent paper by Egle et al. (2016) suggested a durability of up to 300 years since new stores have been discovered.

Stores of P are located in a few countries only. Egle et al. (2016) suggested that 95% of all reserves are owned by five states (Morocco, China, Jordan, South Africa, and the USA). Other states with deposits of P include Brazil, Russia, Israel, Egypt, Syria, and Tunisia. As many of these states have an insecure geopolitical and economic situation, P stores might be held for strategic reasons. This leads to a difficult purchase situation for other countries, which have no P stores themselves and depend on trade with other states. Europe, for example, does not have any P deposits beside small stores in Finland. Therefore, all states in Europe must import P from other countries if their P needs exceed the amount of P they are able to recycle from circuits domestically (Egle et al., 2016).

P is an essential resource for all types of animals and plants. Thus, agriculture is one of the largest users of phosphates followed by industry. Egle et al. (2016) even estimated that approximately 80%–90% of mined P resources are used for mineral fertilizers. On the other hand, surpluses of P are not often used for recycling and may be translocated to lakes and rivers through erosion, where they can support the eutrophication and pollution of waters. As there is a surplus of approximately 10 kg of P per hectare per year, agriculture has a large potential to reduce the outcome of unused P entering nature (Egle et al., 2016; European Commission, 2013; Huber, 2008).

Reducing P in livestock farming could be achieved by reducing its use in animal diets. As the use of P is reduced in the diets of animals, they will excrete less P, meaning that the manure used for fertilization in crop production contains less P. Particularly in regions with a high concentration of livestock farming, the oversupply of manure has led to a gradual increase of the P content in soil. When this P from soil reaches lakes or rivers, eutrophication occurs, causing an increase of cyanobacterial blooms and thus a lack of oxygen, death of aquatic animals, and higher production of nitrous oxide, which has a strong impact as a greenhouse gas (European Commission, 2013; Vats et al., 2005).

Another factor supporting the necessity of reducing P in livestock farming is the legal situation in Germany. The fertilizer ordinance prescribes that all outputs of nitrogen and P from livestock farming must be used for fertilization in crop production. Surpluses of the nutrients can lead to official regulations and penalty payments for farmers. Furthermore, the restrictions became more severe with the latest amendment to the fertilizer ordinance, meaning that all livestock farmers must be mindful of their nutrient balance for nitrogen and P (Hahne & Hessel, 2019).

Furthermore, other countries of the European Union (EU) have started to support research into reducing P usage in general and increasing the recycling of used P. Many countries also have national guidelines and laws about the application of mineral fertilizers. Sweden for example published national targets about the recycling of P in wastewater. The Netherlands put in place an agreement among stakeholders to use a certain percentage of recycled P in their processes. Switzerland ruled that P should be recycled from waste as much as technically possible. Austria, however, does not have national guidelines for recycling P but supports resources in this direction (Egle et al., 2016; European Commission, 2013).

The global challenge for the EU is to minimize the waste of P in agriculture as well as industry to reduce the import of P, thus becoming independent from the insecure geopolitical stores and protecting nature and biodiversity in flora and fauna (European Commission, 2013).

2.5 Conclusions from the literature relevant to the present work

Knowledge of the actual requirements of Ca and P for turkeys, especially turkey breeders, is scarce. Existing recommendations and target levels were published many years ago and have not been updated since then. Moreover, the resources of P are finite, politically insecure, and potentially environmentally harmful, which will lead to high feeding costs in the near future. Therefore, the reduction of P in the diets of turkey breeder hens can make a critical contribution in this direction. The aim is to reduce the use of P in feed without any negative impacts on the animals or their progeny.

This study focused on turkey breeder hens in the rearing and laying and attempted to answer the question of the possible effects (negative or positive) of a reduction of the dietary levels of P as well as Ca down to assumed optimum levels. These optimums were calculated using available data on the requirements of turkey breeder hens.

3 Materials and methods

3.1 Structure of this work

Due to the limited data concerning the requirements of Ca and P for turkey breeders in the rearing and laying phases, the dietary levels used for this work were calculated beforehand using a factorial approach. The calculated dietary levels were used to create a new feeding plan called "Ca/P reduced feed." This feeding system was compared with a standard feeding regime for turkey breeding hens during rearing and laying in four different trials (trials I–IV) concerning performance and health of the animals and effects on the composition of manure.

Trials I and III focused on the rearing period while trials II and IV investigated the laying period. The first trial (trial I) started in November 2019 and ended in May 2020. Afterwards, the hens were moved to the laying farm and used in trial II, which was conducted from May to November 2020. Trials III and IV were conducted independently of each other from February to July 2021 (trial III) or June 2021 (trial IV).

3.2 Factorial approach to calculating requirements for turkey breeder hens

3.2.1 Method of the factorial approach

The mineral requirements of animals consist of the requirements for maintenance and the requirements for performance, such as growth and egg production. For the performance of rearing turkeys, only daily weight gain is relevant. Derived from that, the factorial approach for requirements consists of a part for maintenance and another part for weight gain (GfE, 1999; Jeroch et al., 2011).

For turkey breeder hens in the laying phase, the part for the growth of animals may be disregarded because weight gain is concluded at the approximate age of 30 weeks (Crouch et al., 2002). Therefore, the factorial approach for laying turkey hens consists of a part for the maintenance of the animals and another part for egg production (GfE, 1999).

The factorial approach is a standard procedure for evaluating the requirements of metabolizable energy (ME) of broiler chickens and laying hens. For broilers, laying hens, and poultry for fattening, this method is also used for the requirements of protein and major minerals if enough data for calculation are available for the respective mineral (GfE, 1999, 2004; Jeroch et al., 2011; Majewska et al., 2011).

In general, the calculation of the requirements of Ca and P for laying birds follows the method described for calculating the requirements of ME (GfE, 1999). Adapted for the requirements of Ca and P, the calculation includes a basal amount for maintenance. That amount of Ca/P is required for the maintenance of the body, feed intake, movement, and homeostasis and is mostly determined by the metabolic body size (Jeroch et al., 2011). The additional amounts needed for daily gain follow the weight gain on the respective day. This varies along the growth phase as animals do not grow linearly but rather follow a certain growth curve (Hoffmann, 1994). The additional amounts needed for egg production are related not only to the egg weight but also to the composition of the egg. To calculate the exact needs for egg production, requirements should be split into needs for the egg shell, egg yolk, and albumen as they have different structures and compositions. Since data for this part are not available, requirements have to be rounded for the whole egg. For both performance factors (daily gain and egg production), the sum of needs must be divided by a utilization factor, which estimates how much of the P ingested by the hen is utilisable for those performance factors. The requirements for maintenance and performance may be summed to obtain the total amounts of Ca/P requirements per day. To determine the necessary concentration in the feed, the amount of required P must subsequently be divided by the actual feed intake of the hens (GfE, 1999).

3.2.2 Database and calculation of the factorial approach of Ca and P requirements for turkey breeder hens during rearing and laying

The GfE (2004) determined that the database for the requirements of minerals in turkeys is not resilient enough to calculate the required concentrations with the factorial approach. Due to that, requirement recommendations for minerals are mostly given based on the results of dose–response trials.

Nevertheless, the target values in the trials of the present study were based on the factorial approach for the requirements of Ca and P. Therefore, the calculation followed the factorial approach system introduced by the GfE (1999). The goal was to predict the optimal concentrations of Ca and P for turkey breeder hens during rearing and laying.

Values were taken from fattening turkeys or other species such as laying hens or broiler chickens to compensate for the lack of information. The necessary concentrations of Ca and P in the feed were calculated week by week using the following equations. Data sources for the factors that were used for trials I and II are summarized in Table 3.

$$P - concentration\ in\ feed\ (\%) = \frac{PM + \frac{PG}{PUG}}{FI} * 100$$

$$Ca - concentration\ in\ feed\ (\%) = \frac{CaM + \frac{CaG}{CaUG}}{FI} * 100$$

where PM = P requirement for maintenance (g/d), PG = P retained in growth (g/d), PUG = P utilization factor for growth, CaM = Ca requirement for maintenance (g/d), CaG = Ca retained in growth (g/d), CaUG = Ca utilization factor for growth, and FI = feed intake (g/d).

Table 3: Calculation factors for P and Ca concentrations in the first period (trials I and II)

Abbreviation	Complete name	Unit	Value/calculation	Animal type/source
PM	P requirement for maintenance	g/d	0.08 * body weight (kg)	Broiler chickens (Rodehutscord, 2001)
PG	P retained in growth	g/d	5.1 * daily weight gain (kg)	Fattening turkeys (Deutsche Landwirtschafts-Gesellschaft [DLG], 2014, p. 14)
PUG	P utilization factor for growth	---	0.6	Assumption (see paragraph below the table)
PE	P contained in egg mass	g/d	0.0017 * egg weight (g)	Laying hens, found on the Internet without a citable source
PUE	P utilization factor for egg production	---	0.5	Assumption (see paragraph below the table)

Abbreviation	Complete name	Unit	Value/calculation	Animal type/source
CaM	Ca requirement for maintenance	g/d	0.01 * body weight (kg)	Laying hens (GfE, 1999)
CaG	Ca retained in growth	g/d	10 * daily weight gain (kg)	Fattening turkeys (previous analyses for different trials)
CaUG	Ca utilization factor for growth	---	0.55	Assumption (see paragraph below the table)
CaE	Ca contained in egg mass	g/d	0.037 * egg weight (g)	Laying hens, found on the Internet without a citable source
CaUE	Ca utilization factor for egg production	---	0.55	Assumption (see paragraph below the table)
FI	Feed intake	g/d	empirical	Data were monitored from all breeder flocks of *Moorgut Kartzfehn Turkey Breeder GmbH* from March 2016 to June 2019 (≈ 6500 individual data items per factor)
---	Weight	kg	empirical	
---	Daily gain	kg	empirical	
---	Egg weight	g		Breeder performance objectives (Aviagen Turkeys Ltd, n.d.)

The utilization factors for both Ca and P for growth and egg production were set on a secure low basis. A study on Japanese quail reported mean P utilization values between 71% and 72% and mean Ca utilization values between 61% and 63% but with high individual variation (Beck et al., 2014, 2016).

In trial II, some adjustments were made to optimize the procedure. First, the estimated value for the inevitable losses (see PM) of 0.08 g/kg did not fit the model anymore as the laying turkey breeder hens were larger than broiler chickens. Dänner et al. (2006) and Hempel et al. (2004) proposed inevitable losses of 0.01 g/kg at maximum for fattening turkeys from week 8 to 12 and around 0.05 g/kg for laying hens. Based on this, the new estimated value was set at $0.02 * body\ weight$ for P requirement for maintenance (PM). This level was mostly inspired by the level of turkeys for fattening but was slightly higher because of the higher age and relation to table egg layers.

The second adjustment in trial II was made concerning feed intake. As the level of feed intake had a large impact on the calculated concentrations of Ca and P in the layer feed, the estimations for feed intake in the rearing phase were compared with the feed intake in the rearing phase under a practical condition. Evaluations of feed intake data from 15 parent stock flocks of *Moorgut Kartzfehn Turkey Breeder GmbH* between March 2016 and June 2019 suggested a correlation of 0.7 between feed intake in the rearing period and that in the laying period. The comparison was performed up to week 25 of age as the laying feed had to be planned at this date. The real feed intake in the rearing period turned out to be at a level of approximately 85% compared with the levels assumed beforehand when planning the rearing period. Thus, the level of feed intake for this flock in the laying period was assumed to be at a level of 85% of the values that were calculated with the monitored data.

In general, the equations for the laying period changed as follows:

$$P - concentration\ (\%) = \frac{PM + \frac{PE}{PUE}}{FI} * 100$$

$$Ca - concentration\ (\%) = \frac{CaM + \frac{CaE}{CaUE}}{FI} * 100$$

where PM = P requirement for maintenance (g/d), PE = P contained in egg mass (g/d), PUE = P utilization factor for egg mass, CaM = Ca requirement for maintenance (g/d), CaE = Ca contained in egg mass (g/d), CaUE = Ca utilization factor for egg mass, and FI = feed intake (g/d).

Trials III and IV were planned simultaneously with two different flocks on two different farms to validate the results from trials I and II with one flock that was followed from rearing to the end of production. On both farms, the trial started some weeks after the turkeys were placed in the barns (see Section 3.3); hence, it was possible to predict their approximate level of feed intake. Feed consumption could not be measured on a daily basis on both farms, so the feed intake could only be estimated as an approach using the consumption of feed per barn throughout the deliveries of the feed. For the rearing period, feed intake corresponded to the assumed data (Table 3) quite well, so no adjustment was required. For the laying period, the flock exhibited a slightly smaller feed intake than that calculated at the beginning, so feed intake again was set at 85% of the initial calculated values (the same as in trial II).

Another change concerning trials III and IV was made to the manner of setting the P levels of the Ca/P reduced feed. Differing from trials I and II, the calculated P concentration was set at total P of the feed to avoid possible issues when comparing the output (see Section 5.1.1).

In trial II, the requirements for egg production could only be calculated based on assumptions about egg components. As the results of trial II also included analyses of egg components, those values could be used to improve the estimate in trial IV (see subsection 4.3.5.2). The calculation of P requirements for egg production (PE) changed to $0.0021 * egg\ weight$ and the calculation of Ca requirements for egg production (CaE) changed to $0.033 * egg\ weight$.

3.3 Feeding program

3.3.1 Feeding program in trial I (rearing period)

For the feeding program of the Ca/P reduced feed, the same division into feeding phases as in the standard feed was used (Table 4). The only difference in classification was created in weeks 16 and 17. In this time, the so-called "PA5 plus P-," which should have been fed from weeks 18 to 19, showed a better adaptation to the desired requirements. From the beginning of week 28, all hens were fed the laying diet to separate the change of feed from the change of barns for stress reduction.

From week 1 to 6, all animals were given the identical standard rearing feed to ensure an identical start for both groups. The Ca/P reduced feed did not contain any phytase as it would create difficulty in calculating correct phytase utilization rates at all times, as their efficiency is dependent on, for example, temperature and pH value (Hemme, 2004). This could have distorted the results. The standard feed, however, contained the normal amount of phytase (500 FTU/kg) to have the same standard as usual. The concentrations of av. P and Ca compared with the calculated requirements are plotted in Figure 3 and Figure 4.

Along all feeding phases in rearing, the reduction of av. P varied between 15% and 29% while the Ca target levels exhibited a reduction of between 27% and 44%.

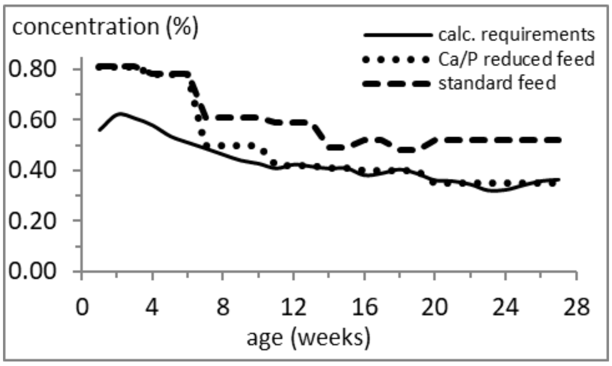

Figure 3: Calculated concentrations of av. P (%) in standard feed and Ca/P reduced feed in relation to the calculated requirements per week (week 1 to 27, trial I)

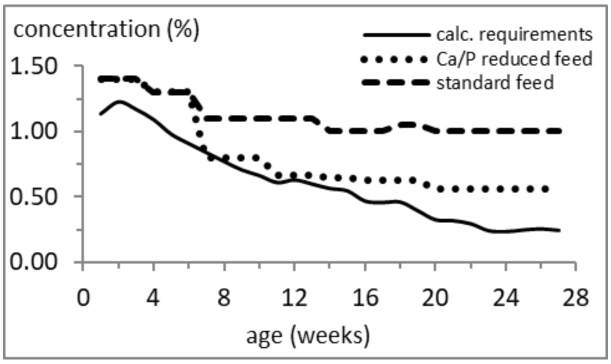

Figure 4: Calculated concentration of Ca (%) in standard feed and Ca/P reduced feed in relation to the calculated requirements per week (week 1 to 27, trial I)

Table 4: Feeding program for standard feed and Ca/P reduced feed with target concentrations of av. P and Ca in trial I (see other nutrient concentrations in Table A.- 1 and ingredients in Table A.- 3)

	Standard feed			**Ca/P reduced feed [1]**		
Week	**Feed name**	**Av. P (%)**	**Ca (%)**	**Feed name**	**Av. P (%)**	**Ca (%)**
1–3	PA1/2	0.81	1.40	PA1/2	0.81	1.40
4–6	PA3	0.78	1.30	PA3	0.78	1.30
7–10	PA4	0.61	1.10	PA4 P-	0.50	0.80
11–13	PA5	0.59	1.10	PA5 P-	0.42	0.67
14–15	JP1	0.49	1.00	JP1 P-	0.41	0.65
16–17	JP2	0.52	1.00	PA5 plus P-	0.40	0.63
18–19	PA5 Plus	0.48	1.05	PA5 plus P-	0.40	0.63
20–27	JP2	0.52	1.00	JP2 P-	0.35	0.56
28–30	*Layer standard*	*0.37*	*2.90*	*Layer P-*	*0.30*	*2.80*

[1] ratio Ca : av. P = 1.6 : 1 (week 1 to 27)

3.3.2 Feeding program in trial II (laying period)

In trial II, “layer standard” feed was given to the group with standard feed for the entire trial duration. For the trial group, however, two different feed types were used. The feed “Layer P-” provided reductions of 17% in av. P and 3% in Ca, while the feed used later (“Layer P- II”) included higher reductions of 33% in av. P and 10% in Ca compared with standard feed (Table 5). Simultaneously to the first rearing period, the standard feed contained phytase (500 FTU), whereas the trial feed did not contain any. Figure 5 and Figure 6 illustrate the concentrations of av. P and Ca for the laying period compared with the calculated requirements.

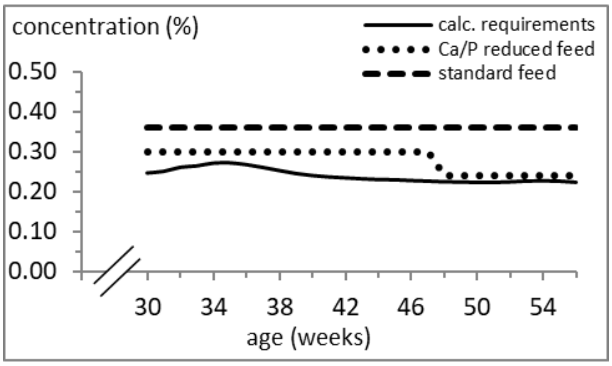

Figure 5: Calculated concentration of av. P (%) in standard feed and Ca/P reduced feed in relation to the calculated requirements per week (week 30 to 56, trial II)

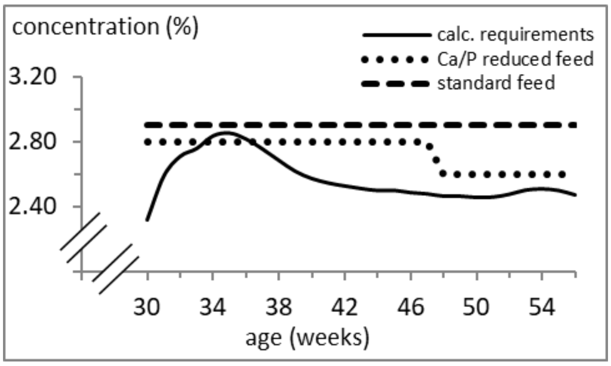

Figure 6: Calculated concentration of Ca (%) in standard feed and Ca/P reduced feed in relation to the calculated requirements per week (week 30 to 56, trial II)

Table 5: Feeding program for standard feed and Ca/P reduced feed with target concentrations of av. P and Ca in trial II (see other nutrient concentrations in Table A.- 1 and ingredients in Table A.- 3)

	Standard feed			Ca/P reduced feed		
Week	**Feed name**	**Av. P (%)**	**Ca (%)**	**Feed name**	**Av. P (%)**	**Ca (%)**
30–47	Layer standard	0.36	2.90	Layer P-	0.30	2.80
48–56	Layer standard	0.36	2.90	Layer P- II	0.24	2.60

3.3.3 Feeding program in trial III (rearing period)

In trial III, the trial period started with the feed “PA5,” which was fed from the beginning of week 11 (Table 7). As the standard feeding schema in rearing was adjusted between trial I and trial III, the feeding phases for both the standard and trial groups followed the new schema now. The difference compared with the schema in trial I was that the feed “JP1” was now used from week 14 to 17, not only from week 14 to 15; the feed “JP2” was used after the feed “PA 5 Plus” (Table 6). For all feed types, the ingredient composition was adjusted, which led to small changes in nutrient concentrations (Table A.- 2).

Table 6: Changes in the feeding phases of the standard feed between trials I and III

	Standard feed (trial I)				Standard feed (trial III)			
Week	**Feed name**	**Av. P (%)**	**Total P (%)**	**Ca (%)**	**Feed name**	**Av. P (%)**	**Total P (%)**	**Ca (%)**
14–15	JP1	0.49	0.70	1.00	JP 1	0.49	0.70	1.00
16–17	JP2	0.52	0.70	1.00	JP 1	0.49	0.70	1.00
18–19	PA5 Plus	0.48	0.70	1.05	PA 5 Plus	0.48	0.70	1.05

In contrast to trials I and II, the feed now contained phytase (500 FTU) for both the standard and the trial feed. The laying feed was fed from the beginning of week 28 to separate feed changes from the change of barns.

The reduction in the Ca/P reduced feed was calculated based on total P (see also Section 3.2.2) and differed between 25% and 29% compared with the standard feed; furthermore, the reduction of Ca compared with the standard feed varied between 35% and 44% (Table 7). Target levels of total P and Ca along the rearing period are plotted in Figure 7 and Figure 8.

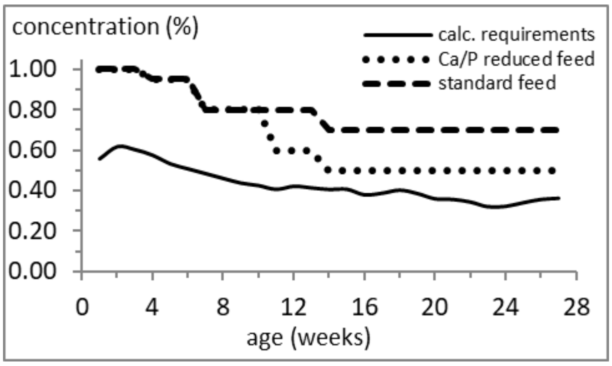

Figure 7: Calculated concentration of total P (%) in standard feed and Ca/P reduced feed in relation to the calculated requirements per week (week 1 to 27, trial III)

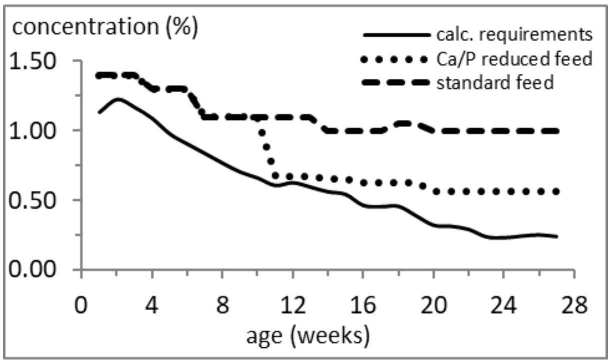

Figure 8: Calculated concentration of Ca (%) in standard feed and Ca/P reduced feed in relation to the calculated requirements per week (week 1 to 27, trial III)

Table 7: Feeding program for standard feed and Ca/P reduced feed with target concentration of total P and Ca in trial III (see other nutrient concentrations in Table A.- 2 and ingredients in Table A.- 4)

	Standard feed			**Ca/P reduced feed**		
Week	**Feed name**	**Total P (%)**	**Ca (%)**	**Feed name**	**Total P (%)**	**Ca (%)**
1–3	PA1/2	1.00	1.40	PA1/2	1.00	1.40
4–6	PA3	0.95	1.30	PA3	0.95	1.30
7–10	PA4	0.80	1.10	PA4	0.80	1.10
11–13	PA5	0.80	1.10	PA5 P-	0.60	0.67
14–17	JP1	0.70	1.00	JP1 P-	0.50	0.65
18–19	PA5 Plus	0.70	1.05	PA5 plus P-	0.50	0.63
20–27	JP2	0.70	1.00	JP2 P-	0.50	0.56
28–30	*Layer standard*	*0.65*	*2.90*	*Layer P-*	*0.50*	*2.80*

3.3.4 Feeding program in trial IV (laying period)

As for the rearing feed (trial III), the ingredient composition of the laying feed was adjusted between trials II and IV. This resulted in small changes in the nutrient concentrations (Table A.- 1, Table A.- 2).

Analogous to trial III, target levels for P were given on a total basis (see also Section 3.2.2). The trial started at week 43 (production week 12) of a flock that was already in production. An earlier start could not be realized due to issues in production and management as well as not every farm in the running progress being suitable for comparing two different feed types. Calculated levels for the requirements of total P and Ca and the target levels are plotted in Figure 9 and Figure 10.

The reduction of total P in the Ca/P reduced feed was 23% compared with the standard feed; the target level of Ca was reduced by 3% (Table 8).

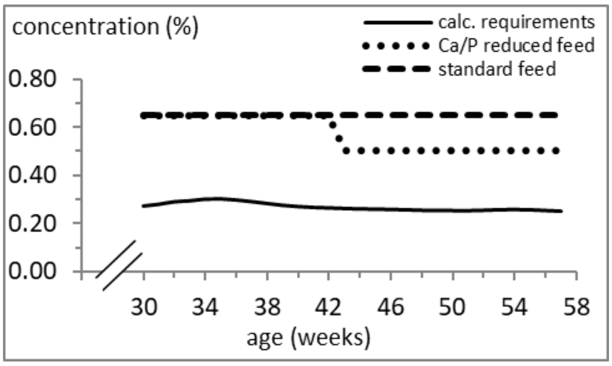

Figure 9: Calculated concentration of total P (%) in standard feed and Ca/P reduced feed in relation to the calculated requirements per week (week 30 to 58, trial IV)

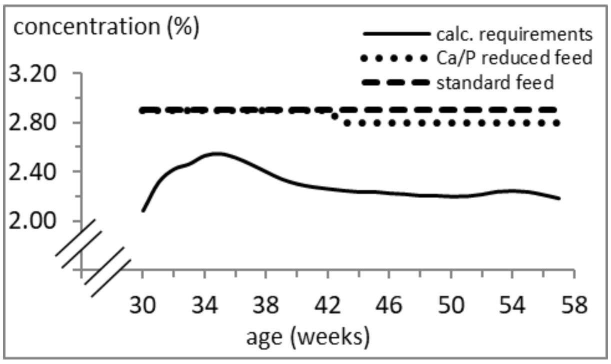

Figure 10: Calculated concentration of Ca (%) in standard feed and Ca/P reduced feed in relation to the calculated requirements per week (week 30 to 58, trial IV)

Table 8: Feeding program for standard feed and Ca/P reduced feed with target concentrations of total P and Ca in trial IV (see other nutrient concentrations in Table A.- 2 and ingredients in Table A.- 4)

	Standard feed			**Ca/P reduced feed**		
Week	**Feed name**	**Total P (%)**	**Ca (%)**	**Feed name**	**Total P (%)**	**Ca (%)**
30–41	Layer standard	0.65	2.90	Layer Standard	0.65	2.90
43–57	Layer standard	0.65	2.90	Layer P-	0.50	2.80

3.3.5 Chemical analysis of the feed

The nutrient concentrations of the feed were analyzed for all deliveries in trials I and II and for at least one sample per feeding phase in trials III and IV. All samples were removed from the silo in the barns before the feed was passed to the feed troughs.

The analyses of crude protein, crude fat, starch, and sugar and the calculation of metabolizable energy were performed by the *LUFA Nord-West* laboratory in Oldenburg, Germany, following the methods book from the Association of German Agricultural Analytics and Research Institutes (*Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten* [VDLUFA], 2012) in the 3rd edition.

3.4 Turkeys and housing

The study was conducted at two rearing and two laying farms for turkey breeders (BUT 6) belonging to the company *Moorgut Kartzfehn Turkey Breeder GmbH & Co. KG.* All farms were located in Brandenburg, Germany.

The farm for trial I consisted of three houses, all connected by an in-house service passage. For the trial, all houses were divided into two single barns (A + B) with identical numbers of equipment (food/water) and identical space. First, 9,000-day-old BUT 6 parent stock turkey hens were initially hatched in house 2. At the end of week 6, the remaining birds were randomly allocated to four experimental groups in four barns (2A/2B/3A/3B). Both barns in house 2 (2A/2B) were provided with standard feed, while both in house 3 (3A/3B) were fed with the Ca/P reduced feed (Table 9). House 1 contained the males and was not included in the trial. For all hens, feed and water were provided *ad libitum.* The trial started at the beginning of week 7 and lasted until the end of the rearing period (up to week 30). Afterwards, the trial continued with the same animals on the laying farm (trial II).

The laying farm for trial II consisted of four barns (barns 1–4) for the hens and one barn for the toms (no trial with males). All barns were connected by an in-house service passage. The trial was conducted with 8,219 of the parent stock hens from trial I. The animals changed farm within their group so that all hens from the group with standard feed also received the standard diet on the laying farm (barns 1 and 2) and all hens from the group with Ca/P reduced feed received the Ca/P reduced diet again (barns 3 and 4) (Table 9). Every hen barn was of an identical size with identical numbers of equipment (nests/food/water); feed and water were provided *ad libitum.* For the laying period, the age of the flock was described by production weeks (PW). For this laying period, PW 1 equaled week 33 of life.

Trial III started with 10,763 hens in two solitary barns on the same farm. From week 11, one barn was fed with standard feed (barn 2) while the other barn received the Ca/P reduced feed (barn 3) (Table 9). Barn 1 contained the males outside of the trial. Both experimental barns were of identical size with identical numbers of equipment (food/water); feed and water were provided *ad libitum.*

Trial IV started independently and simultaneously to trial III. The farm consisted of four solitary barns. Two barns with a total of 3,405 hens were chosen for the trial. From PW 12, barn 1 was fed the Ca/P reduced feed, while barn 4 received the standard diet. Barns 2 and 3 were not part of the trial. Both experimental barns were of identical size with identical numbers of equipment (nests/food/water); feed and water were provided *ad libitum*. In trial IV, PW 1 equaled week 32 of life.

All animals were hatched by a hatchery owned by *Moorgut Kartzfehn Turkey Breeder GmbH & Co. KG*, and the eggs were delivered by *Aviagen Turkeys Ltd*, UK.

Table 9: Distribution of standard feed and Ca/P reduced feed and the number of animals at trial start

Group/ Trial	Standard feed		Ca/P reduced feed	
	Barn	Number of animals	Barn	Number of animals
Trial I	2A	2,178	3A	2,176
	2B	2,176	3B	2178
Trial II	1	1,933	3	2,096
	2	2,094	4	2,096
Trial III	2	5,381	3	5,382
Trial IV	4	1,742	1	1,663

3.5 Data recording from the farm along the production process

Hen weights were recorded at both rearing and both laying periods through hand-weighing randomly selected hens weekly or biweekly. The number of all weighed hens per barn and their intervals is presented in Table 10. For weighing a part of the flock, which had approximately the approbiate number, was fixed in the front left corner of the barn using a barrier. Afterwards, each hen was caught and weighed using a hanging scale for poultry (Bröring BIT PS 3.0, accuracy 0.01 kg). Afterwards, the hens were placed outside the barrier again.

Feed consumption was measured automatically per barn every single day during trials I and II. Therefore, the integrated feed scale weighed 20 kg of one feed type and passed it into the stable if the feed was empty in the last feed trough. The portions were counted by the scale and summed per day to obtain the feed consumption per barn per day. To obtain the feed consumption per hen per day, this value was subsequently divided by the actual number of animals per day. To avoid frequent daily fluctuations, further analyses used the weekly averages. As no weighing technology for feed was present on the farms during trials III and IV, the feed intake for these periods could be compared using the delivered amount of feed per feeding phase only.

Laying performance was determined as the percentage of collected eggs per housed hen for every single day using the total number of laid eggs divided by the actual number of hens per barn. Afterwards, the weekly average was used for further evaluations. Furthermore, the number of culled eggs was monitored daily and classified into causes: cracked eggs, dirty eggs, double-yolked eggs, misshapen eggs, thin eggs (meaning eggs with a thin shell), and eggs that were too small for the brooding process. For evaluation again the weekly average was used, or rather the total average along the whole trial period.

Table 10: Overview of the recorded data (and their intervals) from the farms

Trial	**Hens weight**		**Feed intake**
	Number per barn	Interval	kg/bird/day
Trial I (rearing period)	25	weekly	daily
Trial II (laying period)	50	weekly	daily
Trial III (rearing period)	36	weekly	---
Trial IV (laying period)	30	biweekly	---

From the beginning of week 18 up to the end of rearing, tibias were collected from fallen or culled hens in trial I to analyze the bone mineralization. Therefore, these animals were collected and stored in the cadaver barrel at the farm. All lost animals were brought for dissection weekly (marked with the day of their loss and the barn). After a visual inspection for disease, one tibia per hen was dissected and stored in a freezer. At the end of the rearing period, the collected bones were sent to the *University of Veterinary Medicine Hanover* for an analysis of bone ash and the proportions of Ca and P.

If available, target values from *Moorgut Kartzfehn Turkey Breeder GmbH* (Table A.- 5) were applied for all results of the production; otherwise, target values were lent from breeder objectives (Aviagen Turkeys Ltd, n.d.) or, if also unavailable from there, left out.

3.6 Collected data from the hatcheries

The collected eggs from the laying farm were transported to one of the two different hatcheries from *Moorgut Kartzfehn Turkey Breeder GmbH & Co. KG*. After delivery, the egg weight of 150 randomly chosen eggs per barn was determined by hand using a kitchen scale with an accuracy of 0.1 g.

For the brooding process, the eggs were divided into a trial group (Ca/P reduced feed) and a group with standard feed regardless of the different barns. Fertility was determined through a visible screening of the eggs at 10–12 days after the start of brooding. Unfertilized eggs and eggs with dead embryos were excluded from the hatching process at this time. The hatchability reflects the percentages of hatched eggs from the total number of eggs that were put into the brooding process at the beginning (hatchability of all eggs – including unfertile eggs).

The weight of the hatchlings was determined on the day of hatching. Therefore, 200 poults in trial II and 100 progeny in trial IV were weighted by hand shortly after hatching before delivery to customers using a kitchen scale (accuracy 0.01 g).

In trial II, 10 eggs per barn were removed from the production process in PW 21. Those eggs were sent to *LUFA Nord-West* in Oldenburg to determine the respective percentages of Ca and P from the albumen, egg yolk, and shell following the German Guideline ASU F 0042, 2010-09 (Deutsches Institut für Normung, 2017). For the shell, the percentage of water was determined additionally following the method described in annex III, part A by European Regulation (Amtsblatt der Europäischen Union, 2009).

The weights of these different parts of the eggs were determined using a kitchen scale (accuracy 0.1 g). Combining both values allowed the total concentrations of Ca and P in the egg to be calculated as a weighted summation of the single shares (including and excluding the egg shell).

Even though trial IV started in PW 12, the collection of some data out of the hatchery started later in PW 14. The reason for this shift was access restrictions due to the COVID-19 pandemic and avian influenza.

If available, target values from *Moorgut Kartzfehn Turkey Breeder GmbH* (Table A.- 6) were applied for all results out of the hatchery; otherwise, target values were lent from breeder objectives (Aviagen Turkeys Ltd, n.d.) or, if also unavailable from there, left out.

3.7 Collection of manure samples

To determine the mineral output from the barn with manure, manure samples were collected on the day after the exit of the animals. A wheel loader took one shovel load (approximately 1 m^3) of the manure at some widely distributed places in each barn. If possible and visible in the manure, the areas around feed and water lines were left out.

Afterwards, each load was mixed by the wheel loader and later by hand using a pitchfork. Out of that mixture, a sample of approximately 1 kg was placed in a plastic bag. In total, nine samples per barn were collected in trial I, 20 samples per barn in trial II, 10 samples per barn in trial III, and 10 samples per barn in trial IV.

All samples were sent to *LUFA Nord-West* in Hameln for analyses of dry matter and concentrations of Ca and P. The analysis also included the proportion of other nutrients (e.g., nitrogen, nitrogen out of ammonium, available nitrogen, and organic matter), which were not used in this trial and are not provided here. The levels of P and Ca in the manure were determined based on the chemical bounded forms of phosphate (P_2O_5) and Ca oxide (CaO). Afterwards, those values were recalculated to P and Ca using the following equations based on the conversion table published by the *LUFA Nord-West* (2021):

$$P\ (\%) = 0.4364 * P_2O_5\ (\%)$$

$$Ca\ (\%) = 0.7147 * CaO\ (\%)$$

3.8 Calculation of nutrient balance

To evaluate the results from the manure analyses, the nutrient loads were calculated for Ca and P. Therefore, the complete input of the nutrient (consisting of the number of placed birds and the amount of given feed) was compared with the complete output of that nutrient (consisting of the amount in the removed animals at the end of rearing and for the trials in a laying period as well as the amount in eggs).

For all parts of the trial, the nutrient loads were calculated along the trial period only. This included the following weeks: week 7 to 29 in trial I; PW 1 to 24 in trial II; week 12 to 29 in trial III; and PW 12 to 26 in trial IV.

To determine the total input of Ca or P from the placement of the turkeys, the actual weight of the animals was multiplied by the number of placed turkeys to obtain the total placed weight. Afterwards, the total placed weight was multiplied by the share of Ca or P in turkeys (see also Table 3).

For the input of Ca and P from the feed, the weekly average feed intake (kg/bird/day) was multiplied by 7 days and the actual number of animals in the barn to obtain the total amount of feed used for the respective week. This weekly amount of feed for the particular barn was multiplied by the concentration of Ca or P in the respective feed to obtain the total amount of Ca or P given through the feed. Afterwards, the amounts of Ca or P per week were summed along all weeks of the trial period to obtain the total amount of Ca or P provided by the feed.

For the output of Ca or P from the animals, the total number of turkeys lost per week was multiplied by the actual weight per week to obtain the sum of lost weight per week. This sum was multiplied by the share of Ca or P in turkeys (see also Table 3) to obtain the total amount of lost Ca or P per week. Afterwards, these amounts were summed along all weeks in the trial to obtain the total amount of output Ca or P from the animals. For the last week, all remaining animals were considered lost animals as they left the stable for the change to the laying period (trials I and III) or for slaughtering (trials II and IV).

In trials II and IV, the output of Ca and P through the eggs was added to the formula. Therefore, one egg per hen per day was assumed. The actual egg weight as a weekly mean (kg) was multiplied by 7 days and the actual number of animals in the barn to obtain the total egg mass per barn per week. This weekly amount was multiplied by the average concentration of Ca or P in the respective group (results from trial II). Afterwards, the amounts of Ca or P per week were summed along all weeks in the trial period to obtain the total amount of Ca or P exported with eggs.

Since the management and amount of litter were identical for all barns within one trial, this input factor was left out. On that basis, the input of the nutrient from the animals plus the input from the feed minus the output referred to the animals resulted in the total amount of the nutrient in manure (see the equations below).

Considering the calculated nutrient amounts in manure, it is important to note that the manure also contained Ca or P from the litter itself. Therefore, the nutrient loads could only be used for comparisons between the barns and between both treatments within the same trial and for relations to the analyzed concentrations in manure (without using absolute numbers).

$$amount\ of\ Ca\ in\ manure^{1} = input\ of\ Ca\ from\ animals + input\ of\ Ca\ from\ feed - output\ of\ Ca\ from\ animals - output\ of\ Ca\ from\ eggs^{2}$$

[1] Manure also contains Ca from the litter itself

[2] Only in trials II and IV

$$amount\ of\ P\ in\ manure^{3} = input\ of\ P\ from\ animals + input\ of\ P\ from\ feed - output\ of\ P\ from\ animals^{4} - output\ of\ P\ from\ eggs$$

[3] Manure also contains P from the litter itself

[4] Only in trials II and IV

3.9 Statistics

All statistical analyses were conducted with the statistics software R (R Core Team, 2020). A significance level of $P < 0.05$ was applied at all times.

Differences in hen weights, egg weights, and egg components per barn were analyzed using analysis of variance (ANOVA) and the post-hoc Tukey-HSD test. Those factors per group as well as the group differences for concentrations in manure and poult weights were compared using Welch's t-test. In trials III and IV, the animals in one barn equaled one group; therefore, Welch's t-test was used for comparisons at this point. To compare the weekly difference between the feed intake of both treatments in trials I and II, the values per day per barn were used (N = 14 per group). A statistical comparison followed Welch's t-test.

Welch's t-test was also used to compare the differences between both treatments for laying performance, fertility, and hatchability in trials II and IV. Therefore, the mean value throughout the laying period was used. In trial II, PW 1 was excluded because the values in that week differed widely from the mean of the values; for the performance of the laying turkeys in trial II, the values of both barns per group were used (N = 46 per group for laying performance, N = 23 per group for fertility and hatchability in trial II). In trial IV, the barns equaled the group, which lowered the values for laying performance (N = 15 per group for laying performance in trial IV). For the fertility and hatchability in trial IV, only weeks with a clear distinction between both groups were considered (N = 6 per group) (see also subsections 4.5.6.1 and 4.5.6.2).

For both statistical tests, normal distribution was assumed without testing as the ANOVA and Welch's t-test exhibited high robustness when comparing the same sample sizes (Blanca et al., 2017; Hedderich & Sachs, 2020).

For the group differences concerning bone mineralization, however, sample sizes were different in both groups. The test on normal distribution (Shapiro–Wilk test) verified a non-normal distribution. Therefore, significant group differences were tested using the Mann–Whitney U-test. To analyze the relationship between age and the concentration of bone ash, Ca, or P in the lost bones, the correlation was calculated according to the Pearson correlation coefficient.

The variation coefficient was calculated as a percentage of the standard deviation (SD) to the mean. This calculation was used to determine the variation of nutrient concentrations in manure.

Where boxplots were used for the results of this study, the parameters consisted of the minimum (lowest horizontal line), 1st quartile (lower border of the gray box), median (thick horizontal line), 3rd quartile (upper border of the gray box), and maximum (highest horizontal line) of the data. Outliers are marked by dots.

4 Results

4.1 Results of the chemical analyses of feed

The differences of the analyzed Ca concentrations from the target values expressed in percentages varied between −36% and +140% for each delivery. The means of the analyzed concentrations are presented in Table 11. The differences between the analyzed Ca concentrations and the target values (expressed as means per feeding phase, both absolute and as a percentage) can be found in the Appendix (Table A.- 7). For more than a quarter of all delvieries (26%) the analyzed Ca level was higher than the calculated level. For only eight of a total of 81 deliveries, the analyzed Ca concentration was lower than the calculated Ca concentration. For 64% of all feed deliveries, the analyzed Ca concentration was within the range of ±0.2 around the calculated level, which represents the analytical and technical latitude from the laboratory (Regulation (EC) 767/2009).

The analyzed Ca concentration turned out to be within the range for 88% of all deliveries of standard feed; 7% of deliveries were above this calculated latitude while 5% were below it. For the Ca/P reduced feed, the majority of the analyzed Ca concentrations of all feed deliveries were above the calculated concentration and its analytical latitude (45%); 15% of all deliveries were below this latitude and 40% were within the range. The mean value of all deviations was + 7% for both the standard feed and the Ca/P reduced feed.

For P, the differences between the analyzed and calculated concentrations varied between –13% and +44% for each delivery. The means of the analyzed concentrations are presented in Table 11, while differences between the analyzed P concentrations and the target values (expressed as means per feeding phase, both absolute and as a percentage) can be found in the Appendix (Table A.- 7). For nearly all of the feed samples (98%), these differences were within the range of ±0.2 around the calculated concentrations, which represents the analytical latitude (Regulation (EC) 767/2009). For 2%, the analyzed P level was above that range, while no deliverie exhibited an analyzed P level below that range.

The analyses of standard feed revealed that all feed deliveries were within the range around the optimum for the concentration of P. For the Ca/P reduced feed, 95% of all deliveries turned out to be within the range, while 5% were above and no deliverie below the analytical latitude. The mean value of all deviations was –1% for the standard feed and +1% for the Ca/P reduced feed.

Overall, the analyzed Ca concentration in the rearing periods (trials I and III) tended to be above the calculated level (mean deviation in trial I: +19%, mean deviation in trial III: +30%). The analyzed Ca level in the laying periods (trials II and IV), however, tended to be below the calculated level (mean deviation in trial II: −9%, mean deviation in trial IV: −8%). The analyzed level of P in the feed deliveries did not differ widely from the calculated levels (mean deviation in trial II: −3%, mean deviation in trial III: +5%, mean deviation in trials I and IV: 0%).

Tables containing information about the mean values of the analyzed concentrations of energy (ME) and protein and their differences from the calculated levels can be found in the Appendix (Table A.- 8, Table A.- 9).

Table 11: Analyzed levels of Ca and total P in the feed samples (trials I–IV)

Trial	Feed	N	Ca (g/kg)	Total P (g/kg)	Trial	Feed	N	Ca (g/kg)	Total P (g/kg)
Trial I	PA 4	5	12.1	8.3	**Trial III**	PA 4	-	---	---
	PA4 P-	4	10.5	8.2		PA4 P-	-	---	---
	PA 5	3	11.5	8.0		PA 5	1	21.7	6.7
	PA5 P-	3	10.0	7.3		PA5 P-	1	11.0	5.2
	JP 1	2	9.5	7.4		JP 1	1	10.1	5.6
	JP1 P-	2	13.0	7.0		JP1 P-	1	10.7	4.6
	PA5 Plus	2	9.2	7.1		PA5 Plus	1	12.3	6.9
	PA5 Plus P-	5	9.6	7.4		PA5 Plus P-	1	11.5	7.2
	JP2	15	11.5	6.9		JP2	1	10.6	5.2
	JP 2 P-	11	8.6	7.0		JP 2 P-	1	7.0	7.1
Trial II	Layer standard	10	28.0	6.2	**Trial IV**	Layer standard	1	26.0	5.9
	Layer P-	9	24.5	5.7		Layer P-	1	26.8	5.7

4.2 Results of trial I

4.2.1 Body weight development

In general, the mean body weight of both groups showed a positive trend from 1.5 kg in the group with Ca/P reduced feed and the group with standard feed in week 7 up to 13.7 kg and 13.2 kg in week 30, respectively. Significant differences between treatments occurred in weeks

10, 11, 13, 25, and 28 (higher mean weight in the group with standard feed) and in weeks 18, 19, and 30 (higher mean weight in the group with Ca/P reduced feed). Those weeks are marked with * on the x-axis (Figure 11). All mean weights and their SD can be found in the Appendix (Table A.- 10). Significant differences between the barns may also be found in the Appendix (Table A.- 10). For weeks 18 and 29, the results of the ANOVA revealed a significant difference between the barns, but the post-hoc Tukey-HSD test was nonsignificant for the difference between any two.

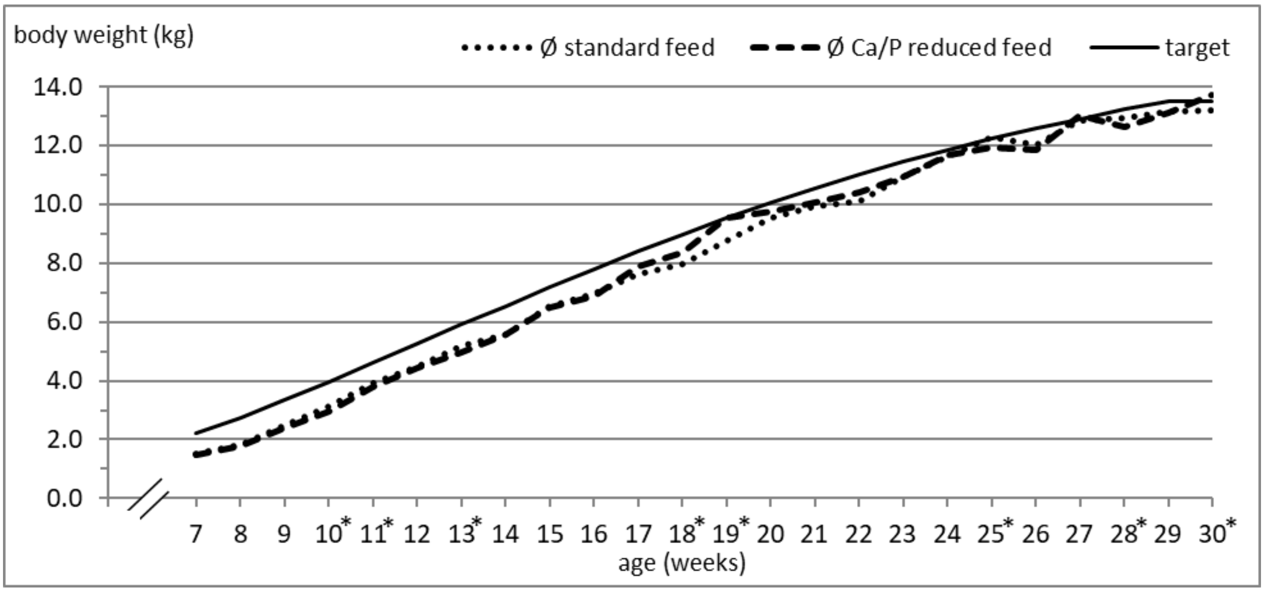

Figure 11: Body weight development of the hens in trial I separated per group; target ≙ target value from *Moorgut Kartzfehn Turkey Breeder GmbH Co. KG (*significant differences per group are marked with * next to the week number)

4.2.2 Feed intake

The feed intake of both groups exhibited a significant difference at three different weeks (weeks 17, 18, and 20). These weeks are marked with * on the x-axis (Figure 12). For all of them, a higher weekly mean was found in the group with Ca/P reduced feed.

Along the duration of the trial, the majority of weeks exhibited a tendency toward a higher weekly feed intake in the group with Ca/P reduced feed (except weeks 8–11). The mean difference of the daily feed intake per hen was 24.3 g, which equaled 6.8%. The feed intake showed an upwards trend up to week 18 and decreased afterwards with the lowest value in week 20 for both groups. Afterwards, the feed intake rose again until week 29. All weekly means and the significant differences between the groups can be found in the Appendix (Table A.- 11). As the SD equaled zero or 0.1 for all weekly means (N = 7 per barns, N = 14 by group), these values are not displayed.

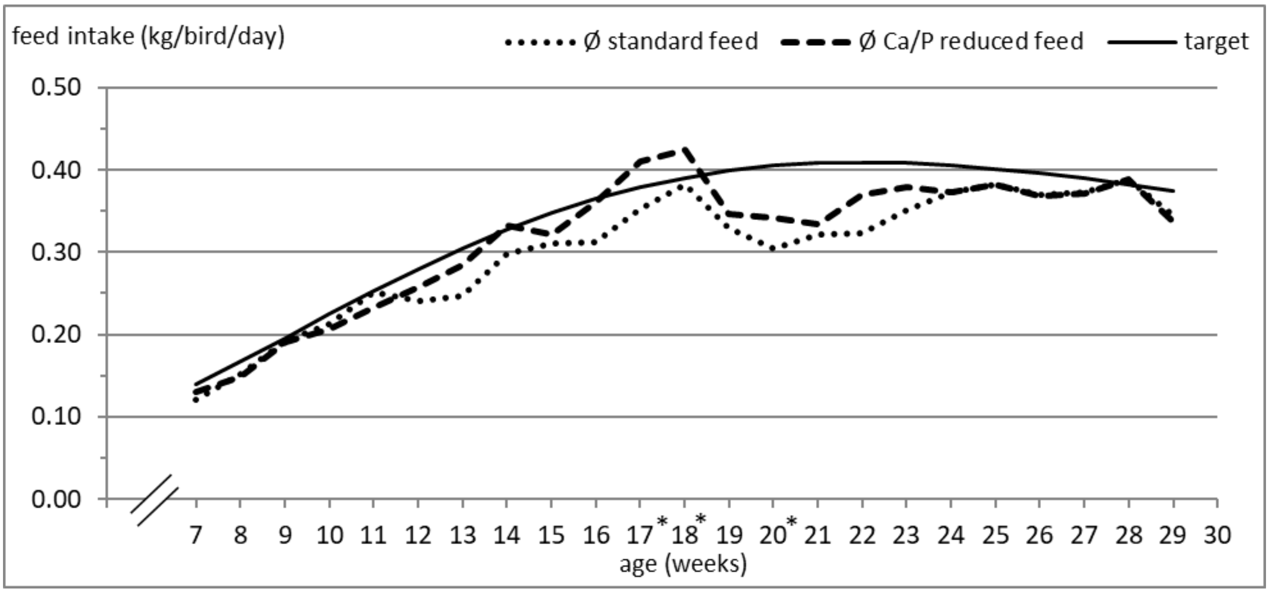

Figure 12: Development of feed intake of the hens in trial I separated by group (weekly averages); target ≙ target value from *Aviagen Turkey Ltd.* (n.d.)

4.2.3 Bone mineralization

In the group with standard feed, tibia bones from 26 birds were collected between week 19 and 29, whereas for the group with Ca/P reduced feed, they were collected from 29 birds between week 18 and 30. Among them, 10 bones from the group with standard feed and three from the group with Ca/P reduced feed could not be assigned to a precise week of age as the date of death was not unambiguous. However, as there would have been very few bones per group without them (especially in the standard feed group), these bones were also included in the evaluation. The median week of the analyzed bones related to a specific week was 23 and 21 for the standard and Ca/P reduced feed groups, respectively.

Furthermore, the percentage of bone ash from total bone weight (as dry matter) showed no significant difference between the treatments. It tended to be higher toward the end of the rearing period in both groups. The lowest value was observed in week 18 with 52.5% (Ca/P reduced feed group), whereas the highest value was found in week 28 with 64.0% (also Ca/P reduced feed group). The correlation between age and the percentage of bone ash (without splitting into the different treatments) was 0.66.

When comparing the groups according to Ca level, no significant difference was observed. The highest concentration of Ca in bone ash was found in week 29 with 36.6% (Ca/P reduced feed), whereas the lowest value was in week 20 with 32.6% (standard feed). The concentration of Ca in bone ash exhibited a correlation to bird age of 0.51.

Moreover, no significant difference was found between the treatments concerning the percentage of P in bone ash. The highest concentration of P was found in week 28 with 17.9% (Ca/P reduced feed), whereas the lowest value was found in week 25 with 16.2% (standard feed). For the percentage of P in bone ash, the correlation to bird age was 0.20.

All means are presented in Table 12, while boxplots for the percentages of P and Ca in bone ash are presented in Figure 13 and Figure 14. An evaluation of those results for each barn was not performed because only four tibias were taken from barn 2B and nine from barn 3A.

According to a visual inspection of all lost hens by a veterinarian, no culling was related to bone fractions or weak bones.

Table 12: Means ± SD of the analyzed values of bone ash, P in bone ash, and Ca in bone ash from tibias of lost animals between week 18 and 30 in trial I

Group	**Bone ash (% dry matter from total bone weight)**	**P in bone ash (%)**	**Ca in bone ash (%)**	**N**
Standard feed	58.5 ± 3.5	17.3 ± 0.3	34.9 ± 1.0	24
Ca/P reduced feed	59.4 ± 2.8	17.3 ± 0.2	35.4 ± 0.5	29
Significant difference	--	--	--	

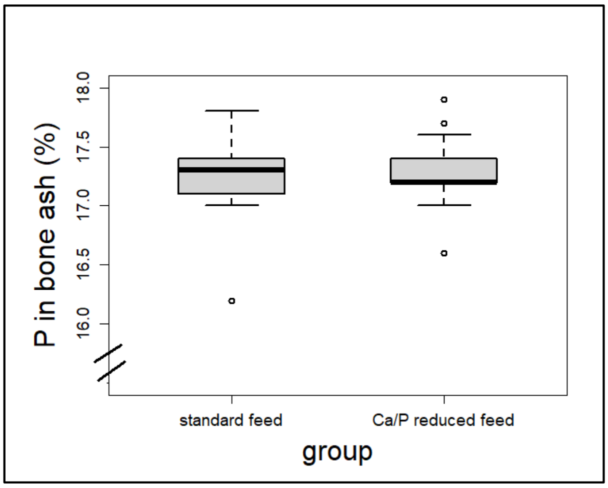

Figure 13: Boxplot for the analyzed proportion of P (%) in bone ash (by group)

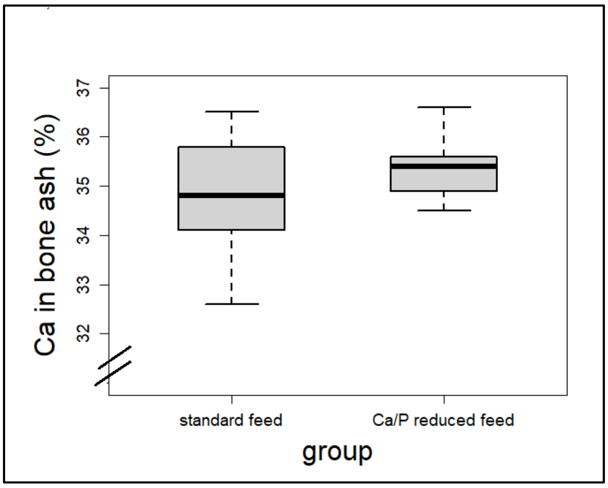

Figure 14: Boxplot for the analyzed proportion of Ca (%) in bone ash (by group)

4.2.4 Nutrients in manure

The highest mean proportion of dry matter in manure was found for barn 2A (69.1%), whereas the lowest was found for barn 2B (68.6%). Regarding the percentage of P, barns 2A, 3A, and 3B had the same mean concentration of 2.0%, whereas barn 2B had one of 1.9%. Regarding the percentage of Ca, the highest mean concentration was found for barns 2A and 2B (3.5%), whereas the lowest concentration was found for barns 3A and 3B (3.3%). The difference in mean values between the groups with standard feed (3.5%) and Ca/P reduced feed (3.3%) was significant (Table 13).

Figure 15 and Figure 16 present boxplots for the analyzed concentrations of P and Ca (related to dry matter) by group. For both factors, the visible variation in the values was higher for the group with Ca/P reduced feed than that with standard feed. This was also reflected by the variation coefficients of 17.9% (P related to dry matter) and 20.6% (Ca related to dry matter) for the group with standard feed and of 32.6% (P related to dry matter) and 36.2% (Ca related to dry matter) for the group with Ca/P reduced feed.

The Appendix contains all single values from all analyses (Table A.- 12) as well as boxplot elements for all displayed boxplots of trial I (Table A.- 13, Table A.- 14).

Table 13: Means ± SD of the analyzed concentrations (%) of dry matter, P, and Ca in manure samples (N = 9 per barn, trial I; significant differences per group are marked with * in the last column)

Analyzed concentration (%)	Barn 2A (standard feed)	Barn 2B (standard feed)	Barn 3A (Ca/P reduced feed)	Barn 3B (Ca/P reduced feed)	Ø Barn 2A + 2B (standard feed)	Ø Barn 3A + 3B (Ca/P reduced feed)	Significant difference per treatments
Dry matter	69.1 ± 3.4	68.6 ± 11.3	69.0 ± 6.8	69.1 ± 8.2	68.8 ± 1.2	69.0 ± 0.5	
P (related to dry matter)	2.0 ± 0.3	1.9 ± 0.4	2.0 ± 0.8	2.0 ± 0.4	2.0 ± 0.4	2.0 ± 0.7	
Ca (related to dry matter)	3.5 ± 0.7	3.5 ± 0.6	3.3 ± 1.2	3.3 ± 0.5	3.5 ± 0.7	3.3 ± 0.9	*

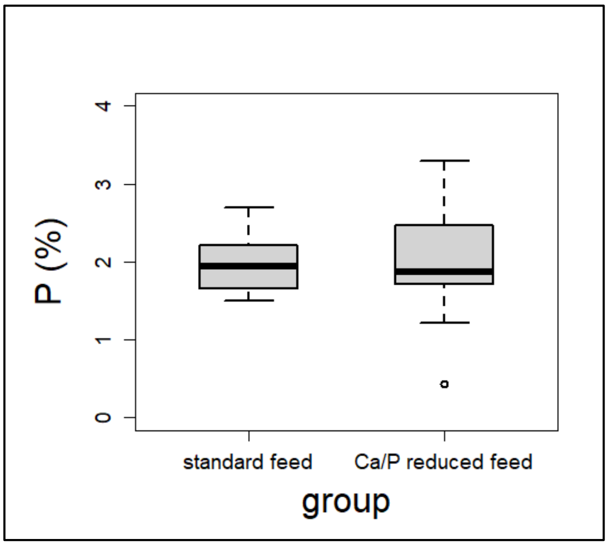

Figure 15: Boxplot for the analyzed levels of P (%) in manure in trial I (related to dry matter, N = 9)

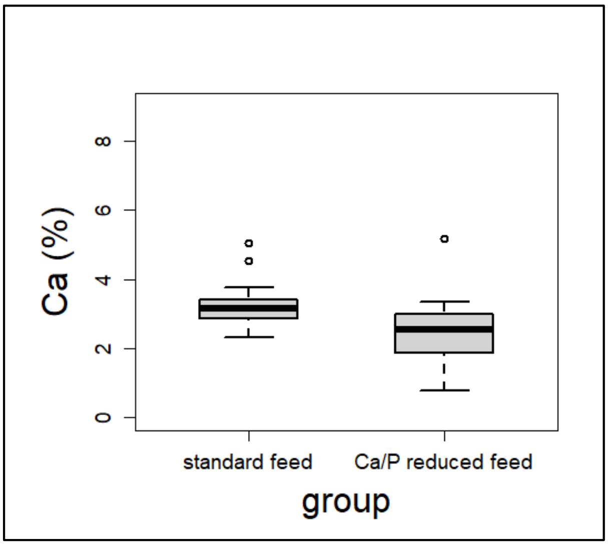

Figure 16: Boxplot for the analyzed levels of Ca (%) in manure in trial I (related to dry matter, N = 9)

4.2.5 Calculation of nutrient balance

The nutrient balance between the input of P and Ca in the feed and in the turkeys themselves as well as the output from the turkeys were calculated for each barn using the formula from Section 3.8. The results reflected the theoretical amounts of P and Ca in the litter.

Regarding the input of P by the turkeys, the values per barn ranged between 16 kg (barn 3B) and 17 kg (barn 2B). Considering the group averages, the input of P was the same for the groups with standard and Ca/P reduced feed. The input of P from feed ranged between 717 kg (barn 2B) and 793 kg (barn 3A). Concerning the means per group, the input of P by the feed was

48 kg higher for the Ca/P reduced feed group than for the standard feed group. Regarding the output of P through lost animals, the highest value was found for barn 2B (148 kg), while the lowest value was found for barn 2A (142 kg). Comparing the means of the groups, the input of P for the group with standard feed was 1 kg higher than for the group with Ca/P reduced feed (Table 14).

The amount of P in manure, calculated as the difference between input and output, differed between 586 kg (barn 2B) and 667 kg (barn 2A). Considering the averages of both groups, the calculated P in manure was 48 kg (7%) higher for the group with Ca/P reduced feed.

Furthermore, the input of Ca by the turkeys differed between 32 kg (barn 3B) and 34 kg (barn 2A). Comparing both treatments, the input of Ca for the group with standard feed was 2 kg higher than for the group with Ca/P reduced feed. A higher variation was observed for the input of Ca by the feed as the loads per barn differed between 906 kg (barn 3B) and 1307 kg (barn 2A). In general, the mean of the input from feed for the group with standard feed was 607 kg higher than that for the group with Ca/P reduced feed. The output of Ca from lost turkeys again differed less. The lowest value was found for barn 2A (278 kg), whereas the highest value was found for barn 2B (290 kg). Regarding the difference in output, the group with standard feed had an output 1 kg higher than the group with Ca/P reduced feed.

Comparing the calculated amounts of Ca in manure, the lowest value was found for barn 3B with 651 kg, whereas the highest was found for barn 2A with 1,062 kg. Considering the means of the groups, the calculated Ca in manure was 338 kg (33%) lower for the group with Ca/P reduced feed.

Table 14: Nutrient loads for the balance of P and Ca in trial I

Input/output in kg (source)	2A (standard feed)	2B (standard feed)	3A (Ca/P reduced feed)	3B (Ca/P reduced feed)	Ø Barn 2 (standard feed)	Ø Barn 3 (Ca/P reduced feed)
Input P (turkeys)	17	17	17	16	17	17
Input P (feed)	759	717	793	779	738	786
Output P (turkey)	142	148	142	146	145	144
Calculated P in manure	634	586	667	648	610	658
Input Ca (turkeys)	33	34	33	32	34	32
Input Ca (feed)	1,307	1,212	938	906	1,529	922
Output Ca (turkey)	278	290	279	287	284	283
Calculated Ca in manure	1,062	956	692	651	1,009	671

4.3 Results of trial II

4.3.1 Body weight development

Both groups exhibited their highest mean live weight in PW 1 with 13.2 kg (standard feed) and 13.1 kg (Ca/P reduced feed). Afterwards, the mean hen weights generally followed the same curve. From PW 1 to 10, the weight development exhibited a decreasing trend to 11.8 kg (both groups). From PW 11 onwards, the mean weights increased again up to 12.7 kg for both groups at the end of production (PW 24) (Figure 17).

Significant differences between the groups were found in PWs 4 and 18 (higher mean live weight in the group with Ca/P reduced feed) as well as PW 5 (higher mean live weight in the group with standard feed), which are marked with * on the x-axis. Significant differences between the barns was only observed in week 18 (Table A.- 16).

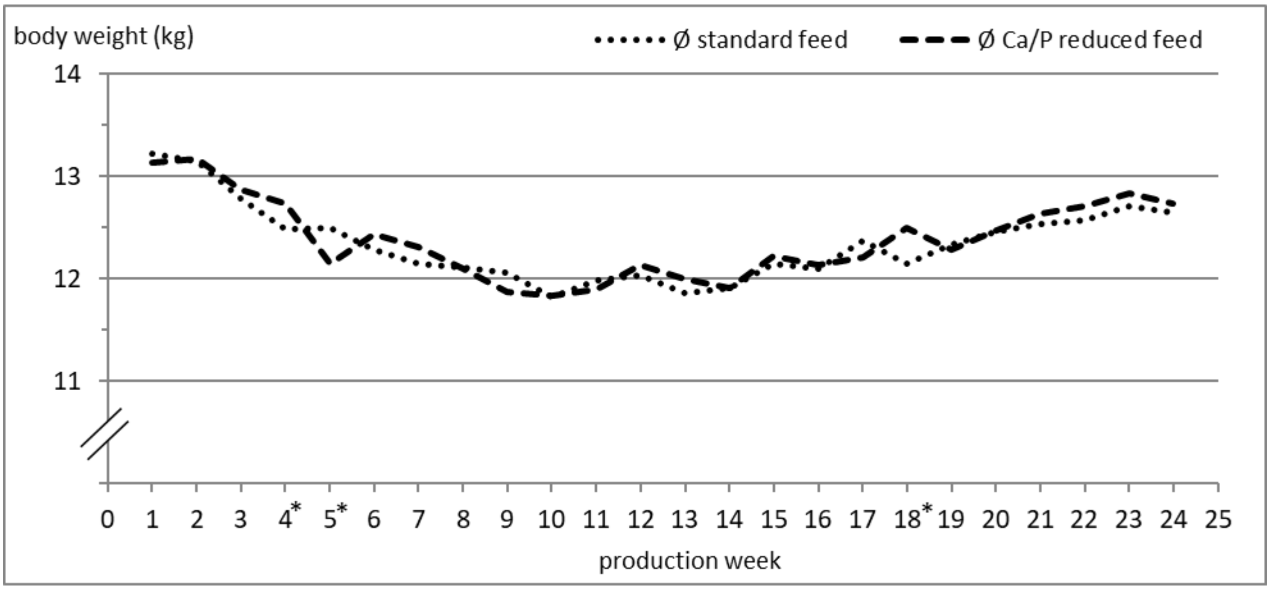

Figure 17: Body weight development of the hens in trial II separated by group (significant differences per group are marked with * next to the week number)

4.3.2 Feed intake

The feed intake only showed a significant difference between the treatments in PW 23, when the feed intake was higher in the group with standard feed. This week is marked with * on the x-axis (Figure 18).

For the majority of the other PWs, the weekly average feed intake in the group with standard feed tended to be above that in the group with Ca/P reduced feed, although exceptions were observed in PWs 2, 3, 8, 14, and 18. Overall, the differences between the groups varied between 0.9 g (0.4%) and 14.9 g (5.1%). The mean difference was 5.9 g (group with standard feed higher), which equaled 6.8% (Figure 18).

In general, the feed intake exhibited a decreasing trend between PW 1 and 4 from 0.25 kg per bird per day (standard feed) or 0.24 kg per bird per day (Ca/P reduced feed) down to 0.22 kg per bird per day for both groups. Afterwards, the average daily feed intake increased again, reaching its highest value in PW 21 with 0.32 kg per bird per day for the standard feed group and 0.31 kg per bird per day for the Ca/P reduced feed group. After PW 21, the feed intake of both groups fluctuated.

All weekly means and the significant differences between the groups can be found in the Appendix (Table A.- 17). As the SD equaled zero or 0.1 for all weekly means (N = 7 per barns, N = 14 by group), these values are not displayed.

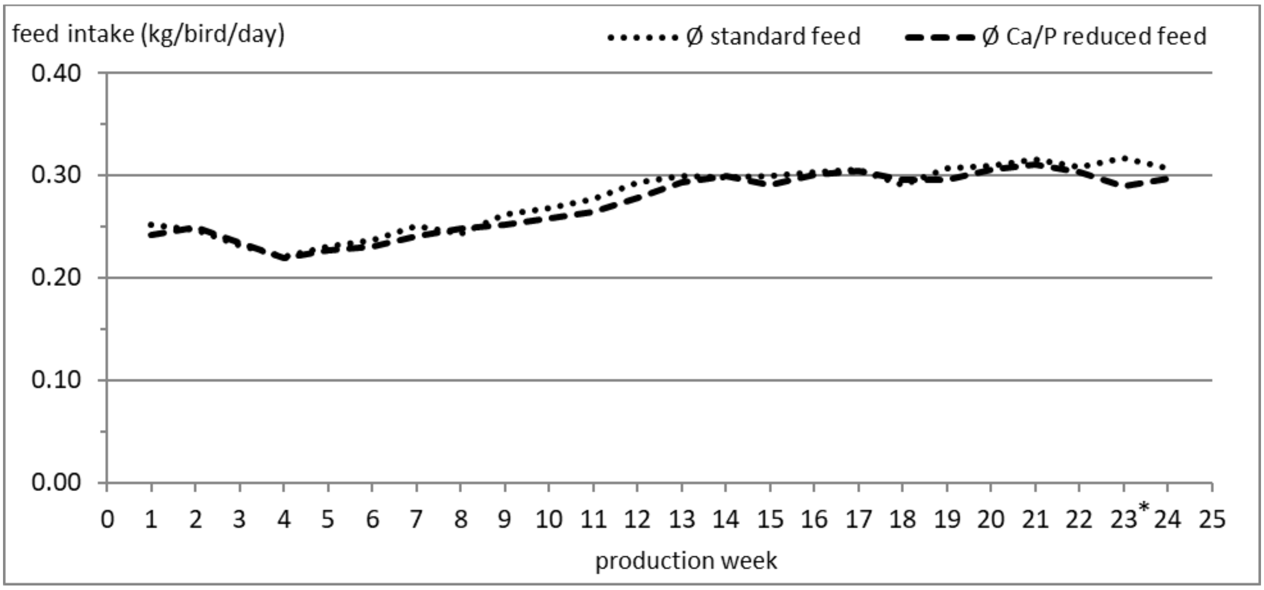

Figure 18: Development of feed intake of the hens in trial II separated by group (weekly averages)

4.3.3 Laying performance

Laying performance can be considered the total laying performance or the laying performance of settable eggs (net laying performance). The net laying performance excludes all laid eggs that were not used for the hatching process (see subsection 4.3.5.1). The presentation of results in this chapter focuses on the net laying performance; results for the total laying performance and detailed data on the net laying performance can be found in the Appendix (Table A.- 18, Table A.- 19).

The mean laying performance was 70.7% (± 3.2% SD) for the group with standard feed and 69.4% (± 3.8% SD) for the group with Ca/P reduced feed (excluding PW 1; see also Section 3.9). No significant difference was found between the values.

Comparing the groups each week, the laying performance tended to be higher for the group with standard feed for nearly all weeks; exceptions were PW 6, where both groups were at the same level, and PW 19, where a higher value for laying performance was found for the group with Ca/P reduced feed. A closer examination of the differences between the barns revealed that the laying performance of barn 2 tended to be above that of the other barns for the majority of weeks, while the laying performance of all other barns (1, 3, and 4) remained at a similar lower level. On average for all weeks, the laying performance of barn 2 was approximately 5% above the respective mean of barn 1, 3, and 4. The highest distinction was observed in PW 21 when the laying performance of barn 2 was 7% above the mean of the other three barns.

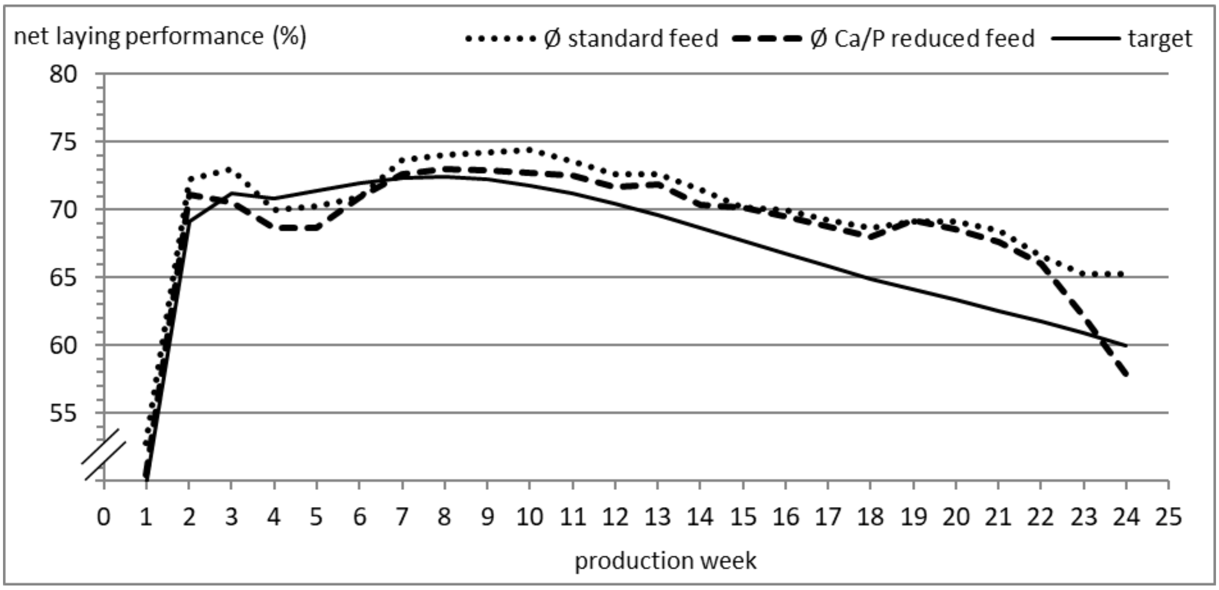

Figure 19: Development of the net laying performance (settable eggs) in trial II separated by group (weekly averages); target ≙ target value from *Moorgut Kartzfehn Turkey Breeder GmbH Co. KG*

4.3.4 Egg weights

The mean egg weights of both groups exhibited a positive trend from 83.7 g (standard feed) and 83.5 g (Ca/P reduced feed) in PW 1 up to 98.8 g (standard feed) and 97.5 g (Ca/P reduced feed) at the end of production (PW 24). A significantly higher mean egg weight in the standard feed group was observed in seven different weeks (PWs 6, 7, 11, 12, 14, 15, and 24); a significantly higher mean egg weight in the Ca/P reduced feed group was observed in PW 21. All weeks with significant differences are marked with * on the x-axis (Figure 20).

For the majority of PWs, the standard feed group tended toward a higher mean egg weight; exceptions were found in PW 9 (both groups on an identical level) and PWs 16, 19, 21, and 23 (higher mean egg weight in the Ca/P reduced feed group). The highest difference in mean egg weights between the groups was found in PW 14 (2.0 g), while the difference in mean egg weights between the groups was 0.5 g. Comparing the barns, the highest mean egg weight was found for barn 2 in 12 out of the 24 weeks and for barn 1 in seven weeks, while barns 3 and 4 had the highest mean weights only three times each. The highest difference between any two barns was observed in PW 14, when the mean egg weight of barn 2 was 2.9 g above that of barn 3.

Significant differences between the barns can be found in the Appendix (Table A.- 20). For PW 24, the ANOVA revealed a significant difference between the barns, but the post-hoc Tukey-HSD test was nonsignificant for the difference between any two barns.

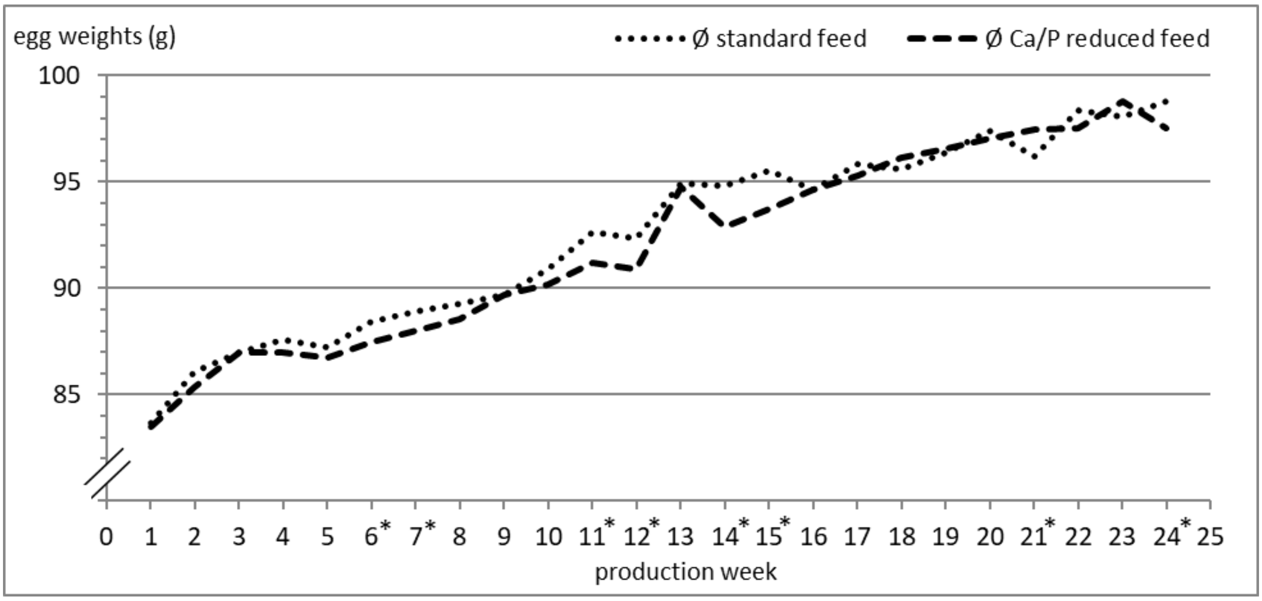

Figure 20: Egg weight development in trial II separated by group (significant differences per group are marked with * next to the week number)

4.3.5 Egg quality

In trial II, the quality of the brooding eggs was determined by two different factors. One factor was the number of eggs excluded from the hatching process immediately after collection and the sorting process on the farm. The other factor was the concentrations of Ca and P in brooding eggs from PW 21.

4.3.5.1 Culled eggs on the farm

Culled eggs on the farm were categorized into six different types (too small, dirty, cracked, double-yolked, misshapen, and thin eggs). All other eggs were categorized as settable eggs and used for the hatching process.

In total, 5.6% of all eggs were culled in the standard feed group (barn 1: 6.0%, barn 2: 5.2%), while 6.1% (barn 3: 6.0%, barn 4: 6.2%) of all eggs were culled in the Ca/P reduced feed group. In all barns, no differences were observed between the total percentages of cracked eggs (0.6% for all barns), thin eggs (0.4% for all barns), and misshapen eggs (0.2% for all barns); therefore, those results are not presented.

For the other three causes, the total percentages of culled eggs are displayed in Table 15. For the double-yolked eggs, the only difference occurred in barn 1; all other barns and both groups were identical for this type of egg. Higher numbers of eggs that were too small or dirty were observed in the Ca/P reduced feed group (barns 3 and 4).

Table 15: Proportion of culled eggs (trial II)

	Comparison by barn				**Comparison by group**	
Type of culled eggs	**Barn 1 (standard feed)**	**Barn 2 (standard feed)**	**Barn 3 (Ca/ reduced feed)**	**Barn 4 (Ca/P reduced feed)**	**Ø Barn 1+2 (standard feed)**	**Ø Barn 3+4 (Ca/P reduced feed)**
Dirty (%)	1.2	1.1	1.3	1.3	1.1	1.3
Double-yolked (%)	0.7	0.6	0.6	0.6	0.6	0.6
Too small (%)	1.4	1.3	1.4	1.5	1.3	1.5

4.3.5.2 Analyses of egg components

The egg components analyzed were albumen, egg yolk, and egg shell. The concentrations of albumen and egg yolk exhibited significant differences between the groups. The percentage of albumen was higher in the standard feed group, while the percentage of egg yolk was higher in the Ca/P reduced feed group (Table 16). Egg weights for both groups were nonsignificantly different (standard feed: 97.6 g, Ca/P reduced feed: 97.9 g).

Table 16: Means ± SD of the analyzed percentages of egg components for eggs from PW 21 in trial II (N = 20 per group)

Components	Correlation to egg weight	Ø Barn 1+2 (standard feed)	Ø Barn 3+4 (Ca/P reduced feed)	Significant difference
Egg weight (g)		97.64 ± 6.87	97.87 ± 6.53	
Albumen (%)	0.34	55.00 ± 2.53	53.19 ± 2.26	*
Egg yolk (%)	-0.33	33.04 ± 2.40	34.61 ± 1.72	*
Egg shell (%)	-0.17	11.95 ± 0.77	12.21 ± 0.81	
Ca in albumen (%)	-0.13	0.002 ± 0.005	0.001 ± 0.002	
P in albumen (%)	-0.10	0.007 ± 0.006	0.004 ± 0.005	
Ca in egg yolk (%)	0.06	0.15 ± 0.01	0.14 ± 0.01	
P in egg yolk (%)	-0.15	0.58 ± 0.02	0.57 ± 0.02	
Water in egg shell (%)	-0.27	23.45 ± 3.81	21.17 ± 3.42	
Ca in egg shell (%)	0.33	26.72 ± 1.60	27.67 ± 1.54	
P in egg shell (%)	0.10	0.11 ± 0.02	0.10 ± 0.02	
Total Ca in egg (%) (without shell)	-0.24	0.049 ± 0.006	0.049 ± 0.004	
Total P in egg (%) (without shell)	-0.41	0.19 ± 0.01	0.20 ± 0.01	
Total Ca in egg (%) (with shell)	0.08	3.24 ± 0.28	3.42 ± 0.25	*
Total P in egg (%) (with shell)	-0.40	0.21 ± 0.01	0.21 ± 0.01	

In all egg components, the respective proportions of Ca and P as well as water (for egg shell) did not exhibit any significant differences. When using the percentages of the egg components to calculate the total amounts of Ca and P within the eggs (with or without shell), the mean values did not exhibit any significant differences for total Ca without shell, total P without shell, and total P with shell. However, a significant difference was observed for total Ca in eggs including shell.

Analyses of significant differences between each barn were not conducted due to the small sample size (N = 10 per barn).

To determine the influence of egg weight on the concentrations of the different components, the correlations were calculated. The highest correlations were found between egg weight and total P concentration within whole egg without shell as well as total P concentration within whole egg with shell, with values of −0.41 and −0.40, respectively.

4.3.6 Brooding quality

The quality of all brooding eggs delivered to the hatchery can be evaluated using several parameters from the hatchery. Therefore, this study used fertility, hatchability, and number of dead embryos at screening. These factors were only separated between the groups with standard feed and Ca/P reduced feed, not between each barn. As the number of dead embryos at screening did not exhibit any clear difference between the groups, these results were left out. Details can be found in the Appendix (Table A.- 21).

4.3.6.1 Fertility

The mean fertility was 93.2% (±1.2% SD) in the standard feed group and 93.2% (±1.3% SD) in the Ca/P reduced feed group (excluding PW 1; see also Section 3.9). No significant difference was found between the values.

Comparing the weekly fertility between the groups, that of the Ca/P reduced feed group tended to be higher than that of the standard feed group for 13 of the 24 weeks. In nine different weeks, the fertility of the standard feed group was higher than that of the Ca/P reduced feed group. For two weeks, the fertility of both groups was identical (Figure 21, Table A.- 22).

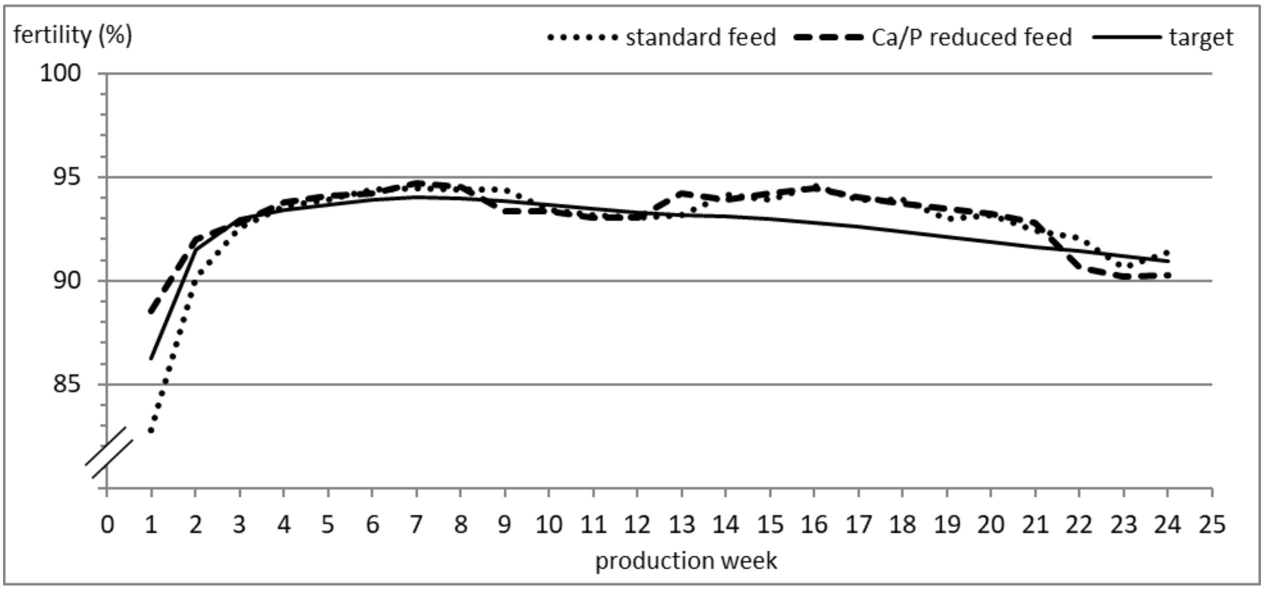

Figure 21: Development of fertility in trial II separated by group (weekly averages); target ≙ target value from *Moorgut Kartzfehn Turkey Breeder GmbH Co. KG*

4.3.6.2 Hatchability

The mean hatchability was 87.2% (±2.4% SD) in the standard feed group and 86.9% (±3.1% SD) in the Ca/P reduced feed group (excluding PW 1; see also Section 3.9). No significant difference existed between the values.

Comparing the hatchability between the groups, higher weekly values were observed for the standard feed group for 13 of the 24 weeks, whereas the Ca/P reduced feed group tended toward higher hatchability for 11 different weeks (Figure 22, Table A.- 23).

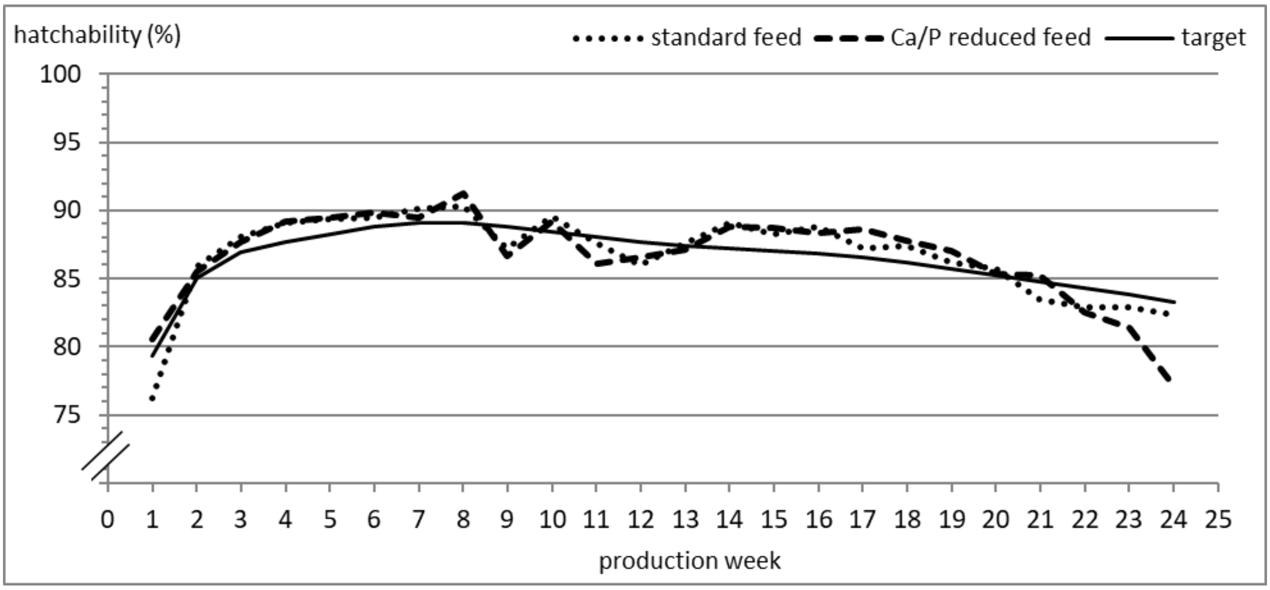

Figure 22: Development of hatchability in trial II separated by group (weekly averages); target ≙ target value from *Moorgut Kartzfehn Turkey Breeder GmbH Co. KG*

4.3.7 Body weights and fitness of the progeny

The mean progeny weight immediately after hatching in the groups started at 55.9 g (standard feed) and 55.5 g (Ca/P reduced feed) in PW 1 and rose to 65.7 g (standard feed) and 66.2 g (Ca/P reduced feed) in PW 24. In general, the curve exhibited a positive trend from the beginning to the end of production (Figure 23).

The mean weight of the hatchlings exhibited a significant difference between the treatments in PWs 17, 18, and 22 (marked with * on the x-axis). In those weeks, a higher mean body weight of the hatchlings was observed in the standard feed group. A tendency toward a higher mean weight for the standard feed group was also observed in PWs 1, 4–10, 13–16, 19, 21, and 23 (nonsignificant). A tendency toward a higher mean weight in the Ca/P reduced feed group was observed for PWs 2, 3, 12, 20, and 24 (nonsignificant). For PW 11, both groups exhibited the same mean weight of progeny. All mean weights can be found in the Appendix (Table A.- 24).

The fitness of the progeny was rated through subjective visible controls by skilled hatchery personnel. No difference was observed for this factor between groups (data not displayed).

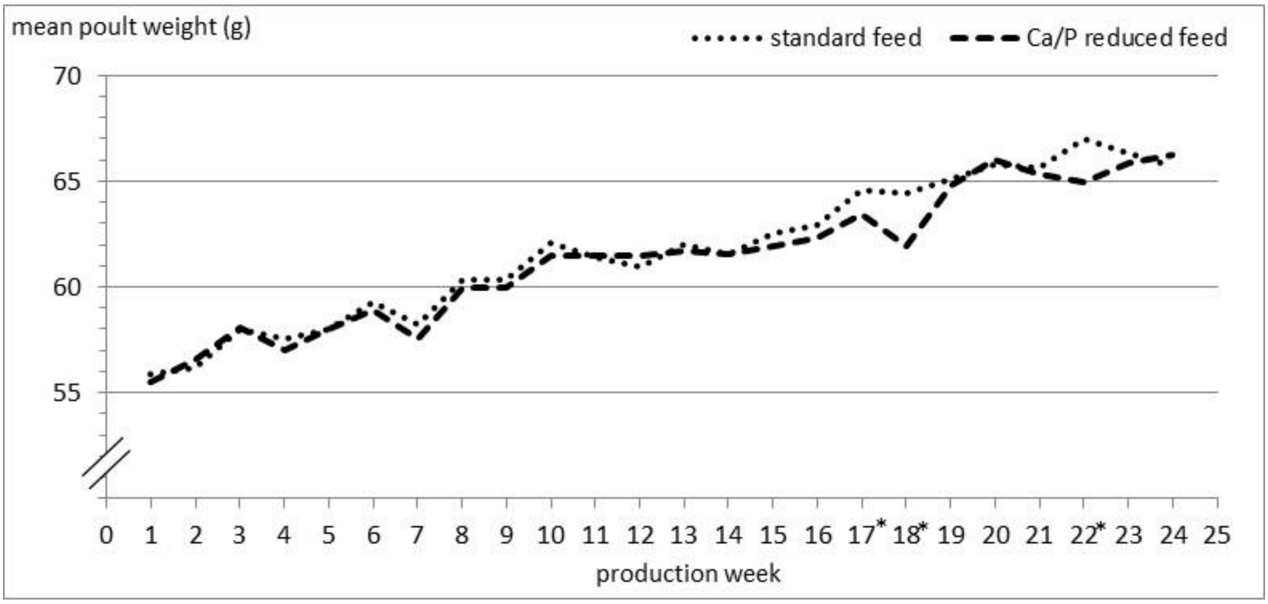

Figure 23: Development of mean body weight from the progeny per group (trial II)

4.3.8 Nutrients in manure

Regarding the proportion of dry matter and Ca in dry matter, the highest mean value was observed for barn 4. The highest mean percentage of P in dry matter was found for barn 1. The lowest mean values, however, were found for barn 2 for all analyzed factors. No difference for any factor between any two barns was found to be significant (Table 17). Comparing both treatments, the highest mean values were found for the Ca/P reduced feed group for all factors. However, none of the differences between treatments proved to be significant.

Boxplots for the analyzed percentages of P and Ca (in dry matter) are displayed in Figure 24 and Figure 25. Both figures suggest a higher variation for the standard feed group compared with the Ca/P reduced feed group. This was supported by the variation coefficients of 23.4% (P in dry matter) and 21.0% (Ca in dry matter) for the standard feed group and 19.4% (P in dry matter) and 19.5% (Ca in dry matter) in the Ca/P reduced feed group.

The Appendix contains the single values from all analyses (Table A.- 12) as well as the values of boxplot elements for all displayed boxplots (Table A.- 26, Table A.- 27).

Table 17: Means ± SD of the analyzed concentrations (%) of dry matter, P, and Ca in manure samples (N = 20 per barn, trial II; significant differences per group are marked with * in the last column)

Analyzed concentration (%)	Barn 1 (standard feed)	Barn 2 (standard feed)	Barn 3 (Ca/P reduced feed)	Barn 4 (Ca/P reduced feed)	Ø Barn 1+2 (standard feed)	Ø Barn 3 + 4 (Ca/P reduced feed)	Significant difference per treatment
Dry matter	51.7 ± 4.6	45.8 ± 7.2	49.7 ± 7.7	53.2 ± 6.5	48.8 ± 6.7	51.4 ± 7.2	
P (related to dry matter)	1.4 ± 0.3	1.4 ± 0.4	1.4 ± 0.3	1.4 ± 0.2	1.4 ± 0.3	1.4 ± 0.3	
Ca (related to dry matter)	5.1 ± 0.8	4.9 ± 1.3	5.0 ± 1.0	5.1 ± 0.9	5.0 ± 1.1	5.1 ± 1.0	

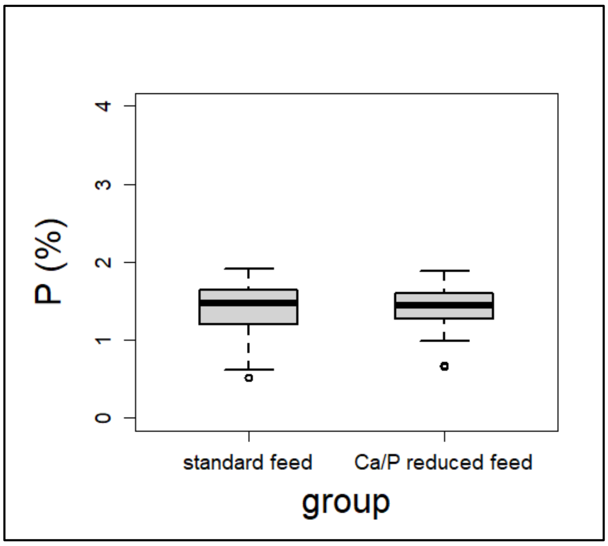

Figure 24: Boxplot of analyzed levels of P (%) in manure in trial II (related to dry matter)

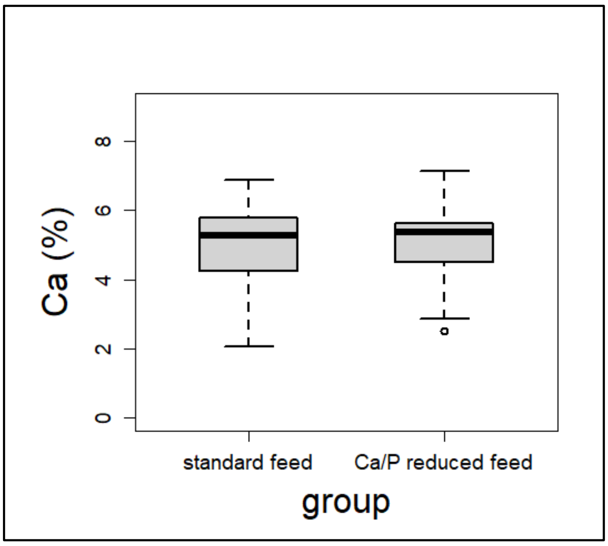

Figure 25: Boxplot of analyzed levels of Ca (%) in manure in trial II (related to dry matter)

4.3.9 Calculation of nutrient balance

The nutrient balance between the input of P and Ca in the feed and in the turkeys themselves as well as the output from the turkeys and eggs were calculated using the formula from Section 3.8 for each barn. The results reflected the theoretical amounts of P and Ca in the litter calculated for each barn (Table 18).

Regarding the input of P among the placed turkeys, the values varied between 135 kg (barn 1) and 141 kg (barn 3). In terms of the averages per group, the value of the Ca/P reduced feed group was 2 kg higher than that of the standard feed group. The input of P from feed had the lowest value of 500 kg (barn 4) and the highest value of 613 kg (barn 2). Comparing the averages between groups, that of the standard feed group was 90 kg higher than that of the Ca/P reduced feed group. The output of P from the animals again differed less. The highest value was found for barn 4 (135 kg), whereas the lowest value was found for barn 1 (129 kg). The output of P through eggs exhibited quite similar levels. The highest value was found for barn 2 (67 kg), whereas the lowest was found for barn 1 (63 kg).

Comparing the amount of calculated P in manure, the highest value was found for barn 2 (553 kg), while the lowest was found for barn 4 (438 kg). Regarding the averages of the groups, the calculated amount of P in manure was 92 kg lower (17%) for the Ca/P reduced feed group than for the standard feed group.

The input of Ca among the turkeys ranged between 265 kg (barn 1) and 277 kg (barn 3). The average value for the Ca/P reduced feed group was 4 kg higher than that for the standard feed group. The values of Ca input through feed varied between 2,528 kg (barn 4) and 2,737 kg (barn 2). Comparing the averages per group, the input of Ca through feed was 99 kg higher for the standard feed group than for the Ca/P reduced feed group. The output of Ca had the highest value for barn 4 (265 kg), whereas the lowest value was found for barn 1 (252 kg). Regarding the averages of the groups, the output of Ca was 5 kg higher for the Ca/P reduced feed group than for the standard feed group. The highest value was found for barn 3 (1,080 kg), while the lowest value was found for barn 1 (979 kg).

The comparison of the calculated Ca in manure revealed a range between 1,458 kg (barn 4) and 1,759 kg (barn 1). Considering the averages of the groups, the calculated Ca in manure was 235 kg (14%) lower for the Ca/P reduced feed group than for the standard feed group.

Table 18: Nutrient loads for the balance of P and Ca in trial II

Input/output in kg (source)	Barn 1 (standard feed)	Barn 2 (standard feed)	Barn 3 (Ca/P reduced feed)	Barn 4 (Ca/P reduced feed)	Ø Barn 1+2 (standard feed)	Ø Barn 3+4 (Ca/P reduced feed)
Input P (turkeys)	135	141	141	139	138	140
Input P (feed)	584	613	517	500	599	509
Output P (turkeys)	129	135	134	135	132	135
Output P (eggs)	63	67	66	66	65	66
Calculated P in manure	527	553	458	438	540	448
Input Ca (turkeys)	265	276	277	273	271	275
Input Ca (feed)	2,606	2,737	2,616	2,528	2,671	2,572
Output Ca (turkeys)	252	265	264	265	259	264
Output Ca (eggs)	979	1,030	1,080	1,078	1,004	1,079
Calculated Ca in manure	1,759	1,719	1,550	1,458	1,739	1,504

4.4 Results of trial III

4.4.1 Body weight development

Body weight development exhibited a positive trend from 5.3 kg (standard feed) and 5.5 kg (Ca/P reduced feed) at the beginning of the trial (week of live [LW] 12) to 13.2 kg (standard feed) and 13.0 kg (Ca/P reduced feed) at the end of the trial (LW 29).

Comparing the groups, the Ca/P reduced feed group exhibited a significantly higher body weight at four different week (LW 12, 21, 24, and 26). Those weeks are marked with * on the x-axis (Figure 26).

Moreover, the majority of weeks exhibited a tendency toward a higher mean body weight for the Ca/P reduced feed group. Exceptions (higher mean weight for standard feed) were found only in weeks 16, 28, and 29. All single values can be found in the Appendix (Table A.- 28).

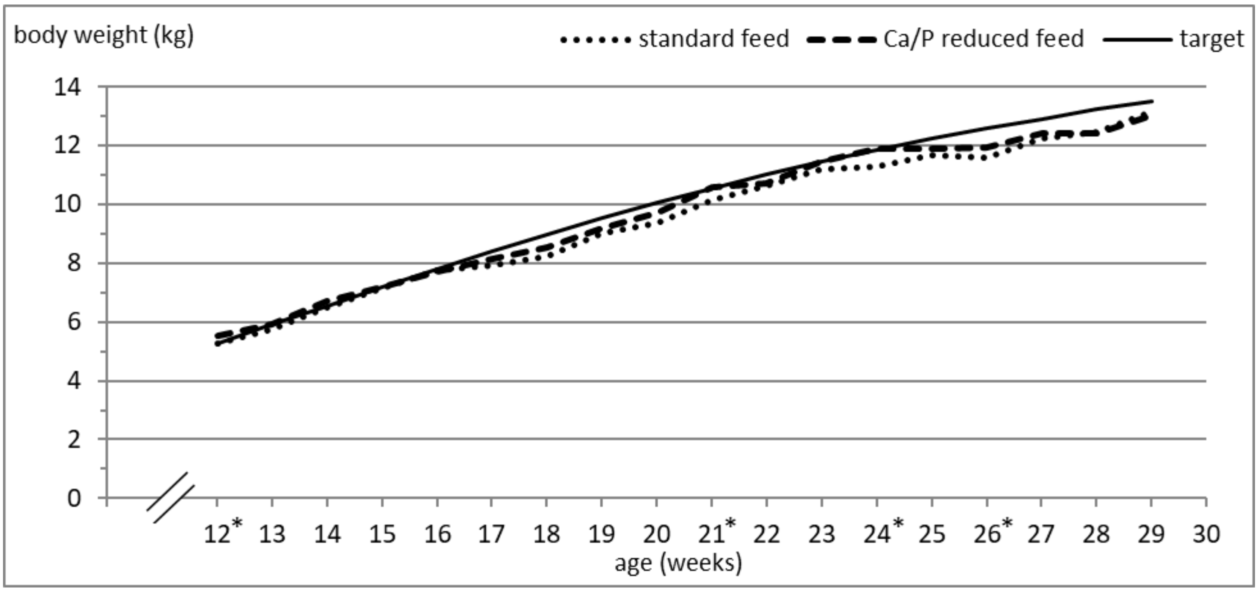

Figure 26: Body weight development of hens in trial III separated by group; target ≙ target value from *Moorgut Kartzfehn Turkey Breeder GmbH Co. KG* (significant differences per group are marked with * next to the week number)

4.4.2 Feed intake

The feed intake in trial III was calculated based on the amount of delivered feed. Therefore, the total amount of delivered feed per feeding phase was divided by the number of days for this period and the mean number of animals per group.

For all four feeding phases (which equaled weeks 11 to 27), the mean feed intake was higher (or equal) for the Ca/P reduced feed group than for the standard feed group. The mean difference was 0.02 kg (5%), varying between the lowest value of 0 kg for the feeding phase "JP 1" and the highest value of 0.03 kg (9%) in the feeding phase "PA 5 Plus."

Table 19: Calculated feed intake for the group with standard feed and the group with Ca/P reduced feed in trial III (kg/bird/day)

Feeding phase (weeks)	Barn 2 (standard feed)	Barn 3 (Ca/P reduced feed)
PA 5 (11–13)	0.30	0.32
JP 1 (14–17)	0.35	0.35
PA 5 Plus (18–19)	0.35	0.39
JP 2 (20–27)	0.37	0.38

4.4.3 Nutrients in manure

The proportion of dry matter in the manure was similar for both groups, with 68.6% in the standard feed group and 68.7% in the Ca/P reduced feed group. The percentages of P were also very close, with 1.4% in the standard feed group and 1.5% in the Ca/P reduced feed group. A larger difference was found for the percentage of Ca in manure, with 3.7% in the standard feed group and 2.3% in the Ca/P reduced feed group. This difference was significant (Table 20). The analyzed concentration of all samples can be found in the Appendix (Table A.- 39).

Boxplots depicting the proportions of the elements (related to dry matter) are displayed in Figure 27 and Figure 28. For the percentage of Ca, the figures suggest a higher variation of the analyzed values for the group with standard feed. This was confirmed by a variation coefficient of 27.7% for the level of Ca (related to dry matter) within the group with standard feed and 27.2% within the group with Ca/P reduced feed. For the percentage of P (related to dry matter), the variation coefficient was 18.3% for the group with Ca/P reduced feed and 13.3% for the group with standard feed.

Tables containing the values of the elements in the displayed boxplots can be found in the Appendix (Table A.- 30, Table A.- 31).

Table 20: Means ± SD of the analyzed concentrations (%) of dry matter, P, and Ca in manure samples (N = 10 per barn, trial III; significant differences per group are marked with * in the last column)

Analyzed concentration (%)	Barn 2 (standard feed)	Barn 3 (Ca/P reduced feed)	Significant difference between groups
Dry matter	68.6 ± 11.3	68.7 ± 11.3	
P (related to dry matter)	1.4 ± 0.2	1.5 ± 0.3	
Ca (related to dry matter)	3.7 ± 1.0	2.3 ± 0.6	*

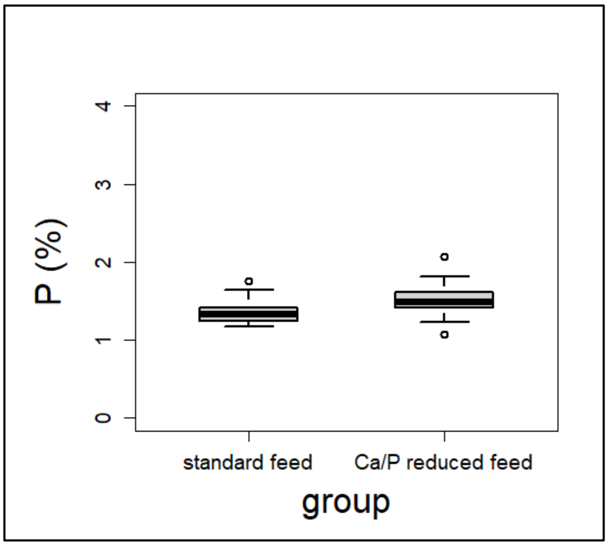

Figure 27: Boxplot of analyzed proportion of P (%) in manure in trial III (related to dry matter; by group)

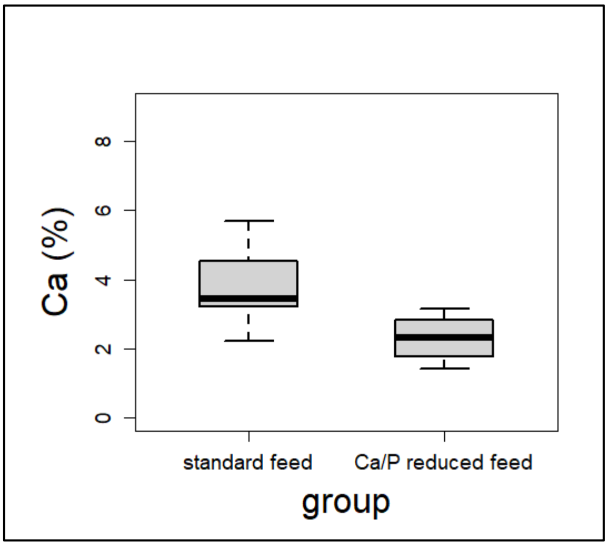

Figure 28: Boxplot of analyzed proportion of Ca (%) in manure in trial III (related to dry matter; by group)

4.4.4 Calculation of nutrient balance

The nutrient balance between the input of P and Ca in the feed and in the turkeys themselves as well as the output from the turkeys were calculated for each barn. Using the formula from Section 3.8, the theoretical amounts of P and Ca in the litter were also calculated for each barn (Table 21).

The input of P among the turkeys at the beginning of the trial was 127 kg in both groups. The P input from feed exhibited a deviation, with 1,100 kg for the standard feed group and 958 kg for the Ca/P reduced feed group. The output of P was 80 kg and 76 kg for the groups with standard feed and Ca/P reduced feed, respectively.

Comparing the amounts of calculated P in manure, that of the standard feed group was 1,147 kg while that of the Ca/P reduced feed group was 1,010 kg. This represented a reduction of 137 kg (12%).

The input of Ca in turkeys was 248 kg for the standard feed group and 250 kg for the Ca/P reduced feed group. Moreover, the input of Ca through feed was 1,820 kg for the standard feed group and 1,134 kg for the Ca/P reduced feed group. The output of Ca from turkeys was 157 kg and 150 kg for the groups with standard and Ca/P reduced feed, respectively.

Lastly, the amount of calculated Ca in manure was 1,911 kg for the standard feed group and 1,234 kg for the Ca/P reduced feed group, representing a reduction of 677 kg (35%).

Table 21: Nutrient loads for the balance of P and Ca in trial III

Input/output in kg (source)	Barn 2 (standard feed)	Barn 3 (Ca/P reduced feed)
Input P (turkeys)	127	127
Input P (feed)	1,100	958
Output P (turkeys)	80	76
Calculated P in manure	1,147	1,010
Input Ca (turkeys)	248	250
Input Ca (feed)	1,820	1,134
Output Ca (turkeys)	157	150
Calculated Ca in manure	1,911	1,234

4.5 Results of trial IV

4.5.1 Body weight development

The body weight development in both groups was initially equal, with 12.3 kg in PW 12 for both groups. Afterwards, the highest mean weight for both groups was found in PW 20, with 12.8 kg (Ca/P reduced feed) and 12.7 kg (standard feed), whereas the lowest weight for both groups was observed in PW 12 (Figure 29).

The mean hen body weight was significantly higher for the group with Ca/P reduced feed in PWs 14 and 18. Those weeks are marked with * on the x-axis.

Between PW 14 and 20, the group with Ca/P reduced feed also exhibited a tendency toward a higher mean body weight. From PW 20 to 26, the differences between the means of the groups became smaller. In PW 20 and 24, the group with Ca/P reduced feed exhibited a tendency toward a higher mean weight, whereas in PW 22 and 26 the same tendency was found in the group with standard feed (Table A.- 32).

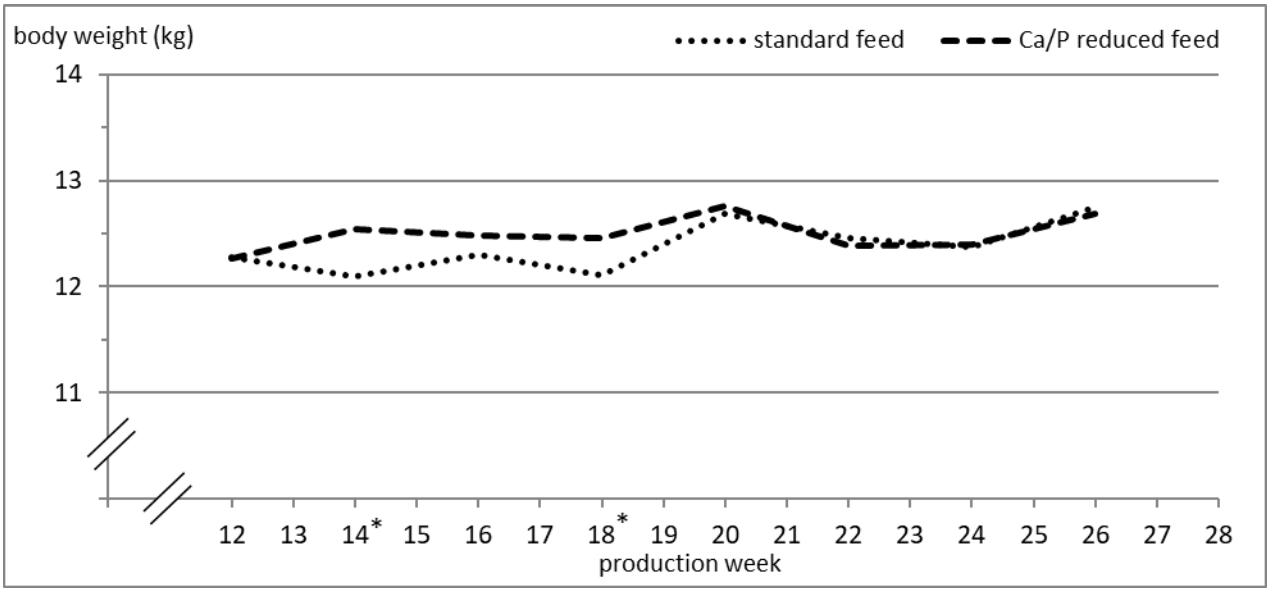

Figure 29: Body weight development of hens in trial IV separated by group (significant differences per group are marked with * next to the week number)

4.5.2 Feed intake

The feed intake in trial IV was calculated using the amount of delivered feed. Therefore, the total amount of delivered feed within the trial period was divided by the number of days in the period and the mean number of animals per group. For this calculation, it must be noted that the deliveries also included feed for barn 2 (Ca/P reduced feed) and barn 3 (standard feed), although none were part of the trial due to different sizes and numbers of animals compared with barns 1 and 4. The factors for this rough calculation and its results are given in Table 22.

The estimated mean feed intake was approximately 0.35 kg/day per hen and 0.34 kg/day per hen for the groups with standard and Ca/P reduced feed, respectively.

Table 22: Calculated feed intake for the groups with standard feed and Ca/P reduced feed in trial IV within the trial period (PW 12–26)

Calculation factor	Barn 1+2 (Ca/P reduced feed)	Barn 3+4 (standard feed)
Total amount of delivered feed (kg)	105,370	75,180
Mean number of animals	3,348	2,425
Estimated mean feed intake in the trial period (kg/bird/day)	0.35	0.34

4.5.3 Laying performance

This section presents results on the laying performance of settable eggs (see also Section 4.3.3); results for the total laying performance and detailed data on the net laying performance can be found in the Appendix (Table A.- 33, Table A.- 34).

The mean laying performance was 68.2% (±3.7% SD) for the group with standard feed and 68.3% (±4.0% SD) for the group with Ca/P reduced feed. No significant difference was found between the values.

Comparing the treatments, the laying performance within the trial period exhibited a tendency toward higher values in the group with Ca/P reduced feed in PWs 13 and 15–21. A tendency toward higher laying performance for the group with standard feed was observed for PWs 12, 14, and 22–26. The differences per week varied between 0.1 and 1.4 percentage points.

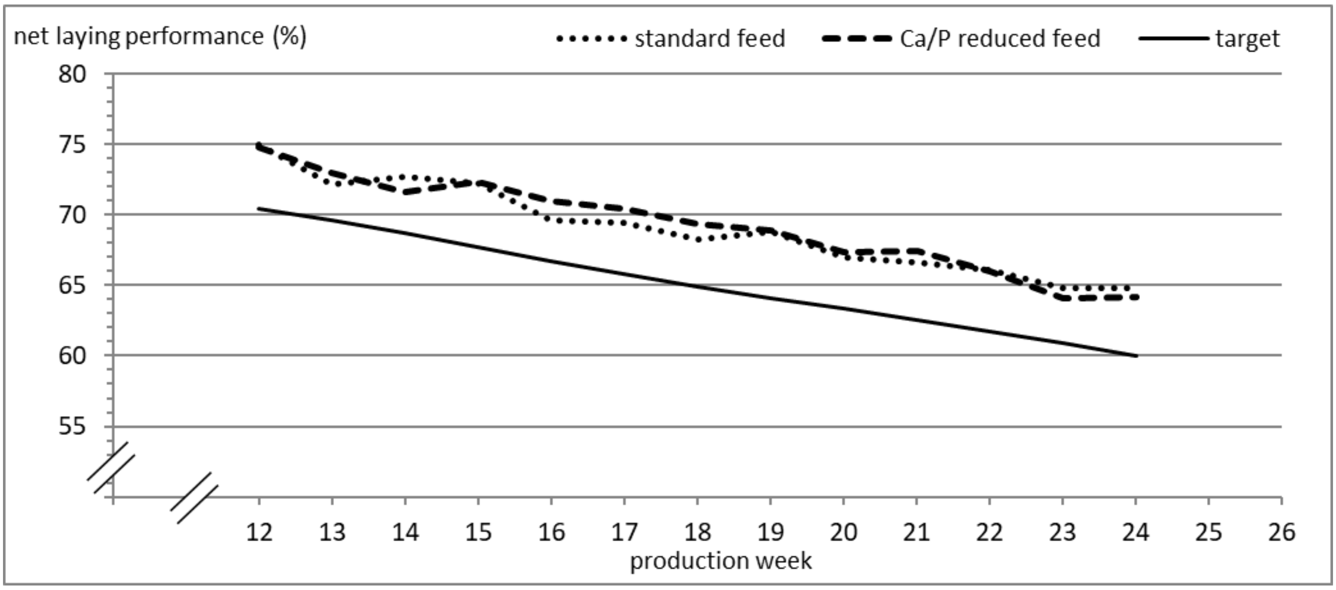

Figure 30: Development of net laying performance (settable eggs) in trial IV separated by group (weekly averages); target ≙ target value from *Moorgut Kartzfehn Turkey Breeder GmbH Co. KG*

4.5.4 Egg weights

The mean egg weights of both groups exhibited a nearly positive trend from 91.5 g (standard feed) and 92.6 g (Ca/P reduced feed) in PW 14 up to 98.6 g (standard feed) and 96.6 g (Ca/P reduced feed) at the end of the trial (PW 26). A dip in the positive trend was found in PW 16, where the mean egg weight of both groups was lower than the respective value in PW 14. For PW 14, the only significant difference observed was a higher mean egg weight for the group with Ca/P reduced feed. This week is marked with * on the x-axis (Figure 31, Table A.- 35).

For PWs 14, 16, and 22, a tendency toward a higher mean egg weight was observed for the group with Ca/P reduced feed. For the other PWs (18, 20, 24, and 26), a tendency toward a higher mean egg weight was observed for the group with standard feed. The differences varied between 2.2 g higher for the group with Ca/P reduced feed and 1.4 g higher for the group with standard feed. The mean of all differences was zero.

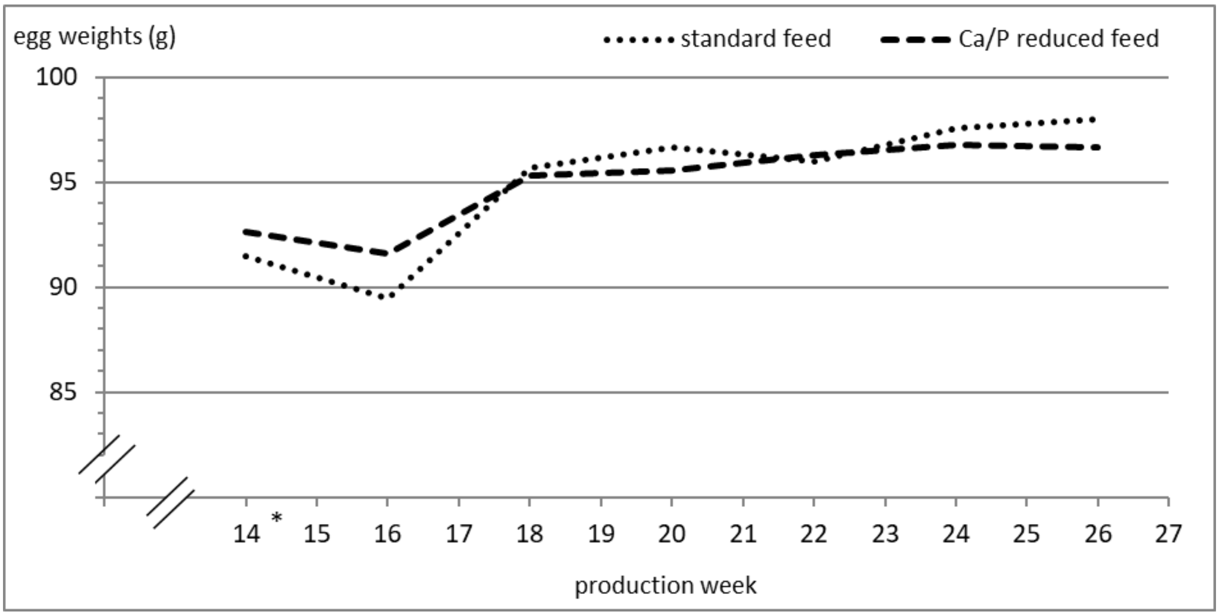

Figure 31: Egg weight development in trial IV separated by group (significant differences per group are marked with * next to the week number)

4.5.5 Culled eggs on the farm

In trial IV (conducted simultaneously to trial II), the quality of the brooding eggs was determined by the number of culled eggs. This refers to the number of eggs excluded from the hatching process immediately after collection and the sorting process on the farm.

The highest percentage of culled eggs were in the category of thin eggs, which accounted for 0.8% of all collected eggs for the group with Ca/P reduced feed and 0.9% for the group with standard feed (Table 23). In total, 3,828 out of 130,264 collected eggs were culled in the group with Ca/P reduced feed, which equaled 2.9%, whereas a total of 4,174 out of 135,669 collected eggs were culled in the group with standard feed, which equaled 3.1%.

Both groups exhibited different percentages of culled eggs for each category. In the Ca/P reduced feed group, higher percentages of culled eggs was found in the dirty, double-yolked, and misshapen categories. In the standard feed group, higher percentages of culled eggs were found in the cracked, thin, and too small categories. As the proportion of cracked eggs was identical for both groups (0.2%), this result is not displayed.

Table 23: Proportion of culled eggs (trial IV)

Type of culled eggs	Barn 1 (Ca/P reduced feed)	Barn 4 (standard feed)
Dirty (%)	0.6	0.5
Double-yolked (%)	0.7	0.6
Misshapen (%)	0.3	0.2
Thin (%)	0.8	0.9
Too small (%)	0.5	0.7

4.5.6 Brooding quality

In trial IV, to determine the quality of brooding eggs delivered to the hatchery, the parameters of fertility, hatchability, and number of dead embryos at screening were used. They were only separated between the standard and Ca/P reduced feed groups, not between the barns.

As the number of dead embryos on the screening date did not exhibit any clear difference between groups, the results were left out. Details can be found in the Appendix (Table A.- 36).

4.5.6.1 Fertility

For fertility in trial IV, only six weeks of the laying period were considered. This is because in some weeks the eggs were removed from production to be sold. Therefore, for many PWs, the overall fertility was calculated from one group only. Those weeks are displayed as "uncertain" in Figure 32 below. For the evaluation, only weeks with a clear distinction between the groups were considered.

The fertility of these weeks was 91.2% (±1.9% SD) in the standard feed group and 91.7% (±1.1% SD) in the Ca/P reduced feed group. No significant difference existed between the values.

Comparing the fertility between the groups, the higher level was found for the group with standard feed in four out of six weeks (PWs 19, 21, 25, and 26), whereas for the other weeks the higher fertility was found for the group with Ca/P reduced feed (PWs 12 and 20). The absolute difference of the groups varied between 0.7% (PW 12) and 2.4% (PW 26). The mean difference was 0.5%, with a higher level for the group with standard feed. The particular levels of fertility for all weeks can be found in the Appendix (Table A.- 37, Figure 32).

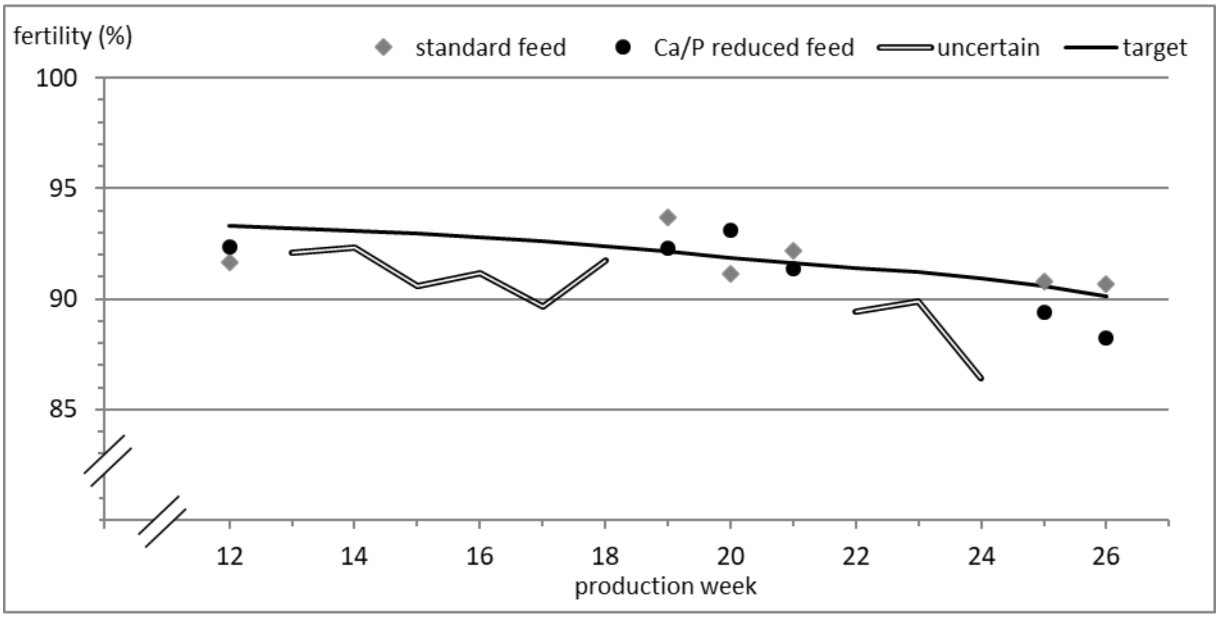

Figure 32: Development of fertility in trial IV separated by group or, if not possible, declared "uncertain" (weekly averages); target ≙ target value from *Moorgut Kartzfehn Turkey Breeder GmbH Co. KG*

4.5.6.2 Hatchability

For hatchability in trial IV (analogous to fertility), only six weeks of the laying period were considered (parallel to fertility) for the reason stated above. Therefore, for many PWs, the overall hatchability was calculated from one group only. Those weeks are displayed as "uncertain" in Figure 33 below. For the evaluation, only weeks with a clear distinction between the groups were considered.

The hatchability for these weeks was 82.9% (±4.1% SD) in the group with standard feed and 87.7% (±6.4% SD) in the group with Ca/P reduced feed. No significant difference existed between the values.

When comparing the groups, the hatchability of the standard feed group was observed to be higher than that of the Ca/P reduced feed group for the majority of weeks (PWs 12, 19, 20, 21, and 26). Omitting the extreme values from PW 20 (see also Section 5.4.5), the mean difference between the groups was 1.1% (higher for standard feed). The differences per week varied between 0.4% (PW 12) and 3.3% (PW 19; Figure 33). All hatchability values can be found in the Appendix (Table A.- 38).

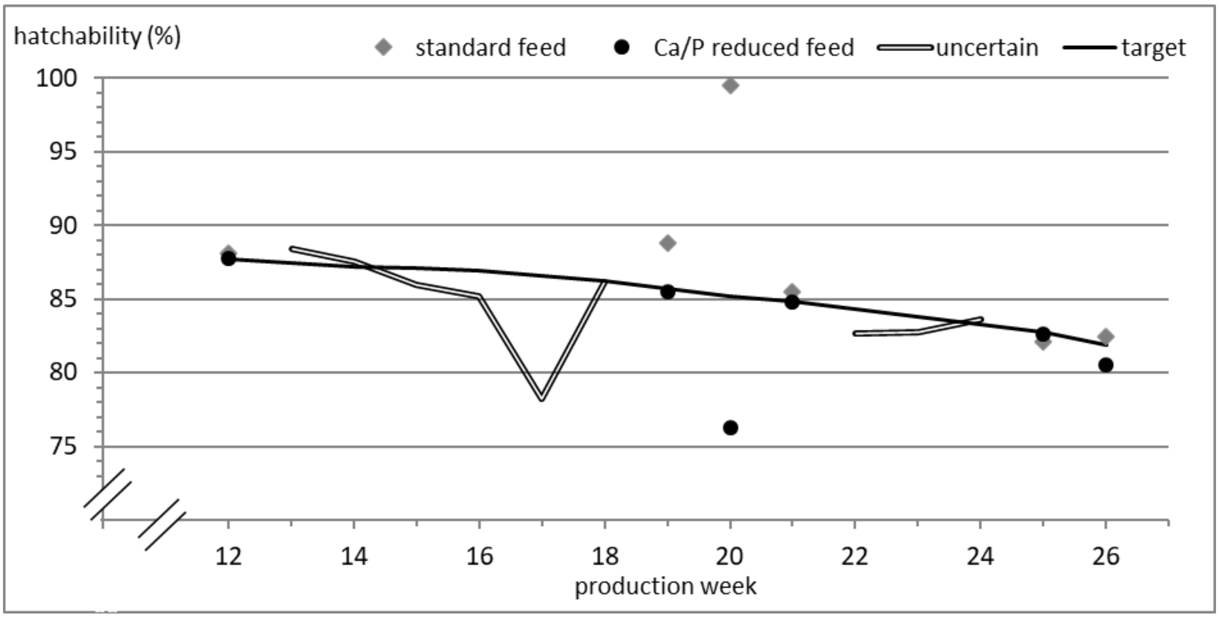

Figure 33: Development of hatchability in trial IV separated by group or, if not possible, declared "uncertain" (weekly averages); target ≙ target value from *Moorgut Kartzfehn Turkey Breeder GmbH Co. KG*

4.5.7 Body weights and fitness of the progeny

Similar to fertility and hatchability, the body weights of the progeny were only considered for PWs with a clear distinction between the groups. No differences between the treatments were significant. For PWs 19 and 22, a tendency toward a higher mean poult weight was found in the group with Ca/P reduced feed. For PWs 25 and 26, however, a tendency toward a higher mean weight was observed for the group with standard feed (Table 24).

The fitness of the progeny was rated through subjective visible controls by skilled hatchery personnel. No difference was observed for the factor when the groups were compared.

Table 24: Development of mean weight ± SD of N = 100 poults per group per week (trial IV; significant differences are marked with * in the last column)

PW	Ca/P reduced feed (barn 1) (g)	Standard feed (barn 4) (g)	Significant difference
19	64.8 ± 4.8	64.2 ± 5.2	
22	65.0 ± 4.9	64.4 ± 4.7	
25	66.2 ± 5.2	67.5 ± 5.3	
26	66.3 ± 5.0	66.7 ± 5.1	

4.5.8 Nutrients in manure

Higher mean concentrations of dry matter and Ca (in dry matter) were observed in the manure from barn 4 (standard feed). A higher mean concentration of P (in dry matter) was observed in the manure from barn 1 (Ca/P reduced feed). No difference in any factor was significant (Table 25). The analyzed levels of all samples can be found in the Appendix (Table A.- 39).

Boxplots depicting the proportion of each element (in dry matter) are displayed in Figure 34 and Figure 35. In both, the figures suggest a higher variation of the analyzed levels for the group with standard feed. This was supported by a variation coefficient of 37.3% for the proportion of P (in dry matter) in the Ca/P reduced feed group and one of 38.1% in the standard feed group. Regarding the proportion of Ca (in dry matter), the variation coefficient was 31.9% for the Ca/P reduced feed group and 33.8% for the standard feed group.

Tables containing the values of the elements in the displayed boxplots can be found in the Appendix (Table A.- 40, Table A.- 41).

Table 25: Means ± SD of the analyzed concentrations (%) of dry matter, P, and Ca in manure samples (N = 10 per barn, trial IV; significant differences per group are marked with * in the last column)

Analyzed concentration (%)	Barn 1 (Ca/P reduced feed)	Barn 4 (standard feed)	Significant difference per group
Dry matter	39.0 ± 3.3	41.7 ± 6.6	
P (related to dry matter)	1.2 ± 0.5	1.2 ± 0.4	
Ca (related to dry matter)	4.3 ± 1.4	4.8 ± 1.6	

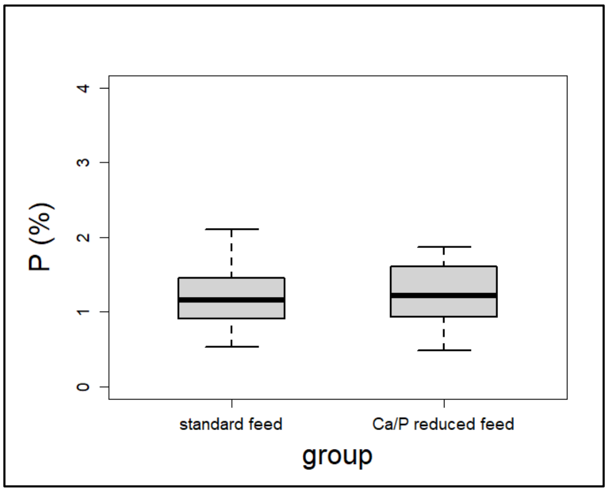

Figure 34: Boxplot of analyzed levels of P (%) in manure in trial IV (related to dry matter)

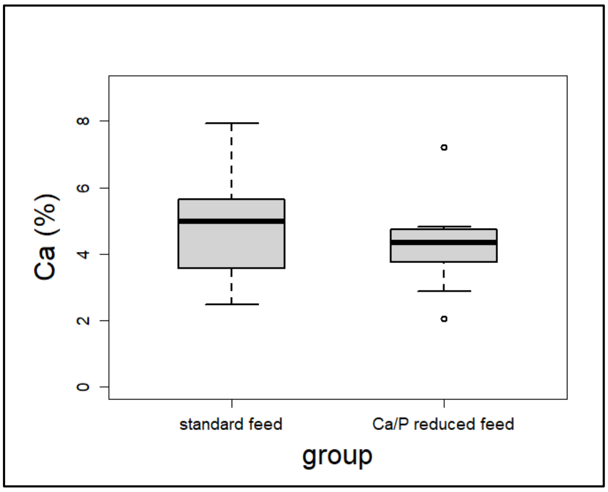

Figure 35: Boxplot of analyzed levels of Ca (%) in manure in trial IV (related to dry matter)

4.5.9 Calculation of nutrient balance

The nutrient balance between the input of P and Ca in feed and in the turkeys themselves as well as the output from the turkeys and eggs were calculated for each barn using the formula from Section 3.8. The results were the theoretical amounts of P and Ca in the litter (Table 26).

Regarding the input of P among the turkeys, the calculated amounts were 108 kg and 103 kg for the standard feed and Ca/P reduced feed groups, respectively, which equated to a difference of 5 kg. Regarding the input of P from feed, the calculated amounts were 398 kg and 298 kg for the standard feed and Ca/P reduced feed groups, which equated to a difference of 100 kg. A higher output of P was found in the group with standard feed (111 kg), whereas the output in the group with Ca/P reduced feed (107 kg) was 4 kg less. The output of P in eggs was 36 kg for the group with standard feed and 43 kg for the group with Ca/P reduced feed.

Overall, the calculated amount of P in the manure of the Ca/P reduced feed group was 141 kg, which was 218 kg (61%) less than that of the standard feed group (359 kg).

The input of Ca in the turkeys in barn 1 (Ca/P reduced feed) was 203 kg, whereas that in barn 4 was 9 kg higher (212 kg). For the input of Ca in feed, the higher value was found in the standard feed group (1,777 kg); the corresponding value in the Ca/P reduced feed group was 108 kg lower (1,669 kg). In terms of Ca output, the levels differed by 9 kg, with 209 kg of Ca in the Ca/P reduced feed group and 218 kg in the standard feed group. The output of Ca in eggs was 550 kg for the standard feed group and 558 kg for the Ca/P reduced feed group.

Lastly, the calculated Ca levels in manure exhibited a difference of 116 kg. The value in the group with Ca/P reduced feed was 1,104 kg, which was 10% lower than that in the group with standard feed (1,220 kg).

Table 26: Nutrient loads for the balance of P and Ca in trial IV

Input/output in kg (source)	Barn 1 (Ca/P reduced feed)	Barn 4 (standard feed)
Input P (turkeys)	103	108
Input P (feed)	298	398
Output P (turkeys)	107	111
Output P (eggs)	34	36
Calculated P in manure	141	359
Input Ca (turkeys)	203	212
Input Ca (feed)	1,669	1,777
Output Ca (turkeys)	209	218
Output Ca (eggs)	558	550
Calculated Ca in manure	1,104	1,220

5 Discussion

5.1 Evaluation of trial conditions

5.1.1 Evaluation of methods and mistakes

All four parts of this study were conducted within the normal production process of brooding eggs and poults at *Moorgut Katzfehn Turkey Breeder GmbH*. This experimental setup led to disadvantages as well as some advantages concerning the implementation itself and the informative value of the results.

One advantage of this kind of trial was reduced costs. No barn, animal, technique, or infrastructure needed to be established or purchased to conduct it. Due to long production phases up to approximately one year or even longer, a trial with turkey breeders would result in extremely high costs if the entire infrastructure had to be created at a research institute. The mode in the present study, however, led to a much lower barrier to planning and conducting a trial with turkey breeders, enabling findings for this species to be obtained. Another advantage of this kind of trial involved the infrastructure itself. As the buildings, technique, and staff were used to work in the production of turkey brooding eggs, trials within the normal production process ensured the same standard as usual in this business, which certainly would not have been achieved with inexperienced infrastructure.

However, the disadvantages of this kind of trial must be considered. Some parameters such as feed intake, laying performance, and parameters concerning brooding quality and manure collection were only available as group averages without values for single individuals or more than two groups of animals. This led to a lower statistical power and the exclusion of many statistical tests as there were only one or two replicates per group. To improve the statistical validity, more replicates per factor would have been very useful.

Another factor causing reduced statistical validity was the small sample size for some factors. For example, for the egg components and the analyses of manure, more replicates per group would have improved the statistical validity. By contrast, the high external costs for laboratory analyses required lower numbers to minimize costs. For this point, both the statistical power and laboratory costs had to be considered to determine a suitable number of replicates.

Another disadvantage of this kind of trial within an operating production cycle is the occurrence of external effects that cannot be excluded but may lead to bias. Those effects refer to the farm, the barn and its location, and the season in which the different parts of the trial were conducted. The period of time observed in trial I started in December 2019 and ended in May 2020, trial II lasted from May 2020 to November 2020, and trials III and IV started in February 2021 and ended in July and June 2021, respectively. Therefore, a seasonal effect between the trials on different performance data, such as weight, feed intake, and laying performance, could not be excluded.

Trials III and IV were conducted in parallel. The advantage of this design was that results were obtained more rapidly. On the other hand, it would have also been interesting to observe a total period from the first day of life up to end of production to determine whether there might have been any later influences of reduced dietary levels of Ca and P, such as was done in trials I and II. As a trial with two different feeding groups cannot be realized on every farm (groups should preferably have equal barns concerning space, number of pieces of feed and water equipment, and similar climate and management conditions), the number of possible farms was limited. Therefore, a parallel rearing and laying period seemed to be a good compromise.

Furthermore, at the end of trial II (PW 23/24), the animals in three barns fell ill with *Ornithobacterium rhinotracheale* (ORT). As this had an influence on the performance of the animals, this topic is discussed further in Section 5.4.

The evaluation of the ingredients and nutrient concentrations in trials I and II revealed slight differences (Table A.- 1), although the feed mill was taught to optimize the trial feed based on the respective standard feed. For all used feed types, the trial feed had lower concentrations of Ca carbonate and monocalcium phosphate and a higher concentration of wheat bran. Even if the differences seem tiny, this may have had an influence on the animals. This error was corrected in trials III and IV.

A serious issue was found in the optimization of dietary levels of Ca and P in trials I and II. In all calculations of the required levels of Ca and P for turkeys, the optimization was performed only based on the level of av. P without considering the level of total P. Additionally, the Ca/P reduced feed contained no phytase while the standard feed did. Afterwards, it transpired that the trial feed indeed had a lower level of av. P but that of total P was higher for some phases in the rearing period that were fed between weeks 14 and 19 (Table A.- 1). This certainly influenced the amount of P that could be found in the manure at the end of the trial and weakened the information value of this factor concerning the levels of P significantly. In trials III and IV, this error was resolved (Table A.- 2) using a different calculation system for dietary levels of Ca and P based on av. P as well as total P (see also Section 3.2.2).

5.1.2 Evaluation of quality from the factorial approach for requirements with analyzed values throughout the trial

To obtain an idea of the requirements of Ca and P for turkey breeder hens, a factorial approach was used to calculate them in preparation for the four trials. Therefore, many assumptions were made and some data were taken over from other species (see also Section 3.2.2). Some of them could be proven in this study.

The feed intake was estimated from monitored feed intake data from turkey breeders from *Moorgut Kartzfehn Turkey Breeder GmbH & Co. KG* between 2016 and 2019. In trial I, it was found that this particular flock had a feed intake approximately 15% below the assumed value. Therefore, the feed intake in trial II was set at 85% of the estimated level, which led to higher dietary levels of Ca and P. Evaluating the feed intake in trial II subsequently, it was found that the real feed intake was again higher than the lowered estimation and nearly at the initially estimated level. Hence, the dietary levels of Ca and P in trial II seemed to have been above the real requirement level of the animals.

In trials III and IV, the feed intake could not be measured directly on the farm, but the deliveries of feed were recorded. This led to inaccurate estimates of feed intake data. In trial III, it was found that the estimated feed intake was between 95% and 109% of the estimated value (per feeding phase). In trial IV, the feed intake was set to 85% of the initially estimated level (simultaneously to trial II) because this particular flock also exhibited a lower feed intake level at the beginning of production. In the end, a rough estimation of the mean feed intake was found to be approximately 20% higher than the mean of the expected values. Hence, the dietary levels of Ca and P in trial IV seemed to have been above the requirement level of the hens (similar to trial II).

For the calculation of the requirements of Ca and P for rearing hens (trials I and III), also body weight was a factor. In trial I, the body weight of both groups was found to be between 70% and 82% of the estimated values for weeks 7–10 and later rose to between 86% and 102%. In trial III, the body weight data resulted in weights between 92% and 110% of the estimated value. Overall, it can be inferred that the assumed body weight data were quite suitable.

In trials II and IV, the egg weight was used as a factor for calculating the requirements. The determined egg weight in trial II was between 98% and 104% of the assumed egg weight, whereas that in trial IV was between 96% and 103% of the assumed egg weight. These values being around 100% implied high suitability of the assumed egg weight data.

The requirements for egg production in trial II were calculated using estimates of egg components. The proportion of Ca in a turkey egg was estimated to be 3.7%, while that of P was estimated to be 0.17%. The results of trial II also included analyses of egg components (see subsection 4.3.5.2). Therefore, those values could be used to improve the estimates in trial IV. The new estimate of the proportion of Ca was set to 3.3%, while that of P was set to 0.21%. The high match of the assumed values in trials II and IV indicated that those estimations were close to the actual proportions.

To obtain an idea of the influence of body weight, egg weight, and feed intake on the calculated concentrations of P and Ca in the Ca/P reduced feed, the factorial approach (see also Section 3.2.2) was recalculated with levels of 20% above and 20% below the assumed value and their combinations. The minimum and maximum in these scenarios can be found in Table 27, while all values of the calculated scenarios are provided in the Appendix (Table A.- 42-45).

To determine the minimum and maximum of the calculated concentrations, it was assumed that concentrations would have been rounded up to one decimal place if they had been used in a feed formula. For the trial periods of all trials, the minimal calculated concentration of P was 0.3% or 0.4% and the maximal concentration was 0.6% or 0.7%. This equals a range between −40% and +150% when compared with the concentrations used throughout the trials.

For Ca, the minimal calculated concentration of the scenarios in the rearing trials (trials I and trial III) was between 0.2% and 0.5%, whereas the maximal calculated concentration was between 0.5% and 1.2%. This equals a range between −64% and +50% when compared with the concentrations used throughout the trials. For the trials in the laying period (trials II and IV), the minimal calculated concentration out of all scenarios was 1.3% or 1.5% and the maximal value was 2.9% or 3.2%. If these values are compared with the concentrations used throughout the trials, this equals a range between −54% and +23%.

Examining the absolute values, the calculated concentrations out of the scenarios did not differ widely from the concentrations used in the present study. However, the percentage difference indicated that tiny changes could result in completely different concentrations that possibly would not meet the requirements of the animals.

Table 27: Minimum and maximum concentrations of av. P/total P and Ca for different scenarios compared with the used concentrations (trials I–IV)

Trial	Week of live (feeding phase)	Concentration of av. P (trials I and II) or total P (trials III and IV) (%)				Concentration of Ca (%)			
		Used concentration	**Calculated concentration**	Minimum from all scenarios *	Maximum from all scenarios *	**Used concentration**	**Calculated concentration**	Minimum from all scenarios *	Maximum from all scenarios *
Trial I	**LW 7–10 (PA 4 P-)**	0.50	0.45	0.4	0.7	0.80	0.74	0.5	1.2
	LW 11–13 (PA 5 P-)	0.42	0.42	0.3	0.7	0.67	0.61	0.5	1.0
	LW 14–15 (JP 1 P-)	0.41	0.41	0.3	0.7	0.65	0.55	0.4	0.9
	LW 16–19 (PA 5 Plus P-)	0.40	0.39	0.3	0.6	0.63	0.46	0.4	0.7
	LW 20–27 (JP 2 P-)	0.35	0.35	0.3	0.6	0.56	0.27	0.2	0.5
Trial II	**LW 30–47 (Layer P-)**	0.30	0.24	0.3	0.7	2.80	2.66	1.5	3.2
	LW 48–56 (Layer P- II)	0.24	0.24	0.3	0.6	2.60	2.63	1.5	3.2
Trial III	**LW 11–13 (PA 5 P-)**	0.60	0.42	0.4	0.7	0.67	0.61	0.5	1.0
	LW 14–17 (JP 1 P-)	0.50	0.40	0.4	0.6	0.65	0.51	0.4	0.8
	LW 18–19 (PA 5 Plus P-)	0.50	0.40	0.4	0.6	0.63	0.43	0.3	0.7
	LW 20–27 (JP 2 P-)	0.50	0.35	0.3	0.6	0.56	0.27	0.2	0.5
Trial IV	**LW 43–57 (Layer P-)**	0.50	0.22	0.3	0.7	2.80	1.88	1.3	2.9

* rounded up to one decimal place

Considering all calculations, the verifiable estimated factors (body weight, egg weight, and feed intake) accorded well with the assumed values and results in the present study, which suggested that the estimations may be used again for comparable calculations and also that the calculated dietary levels should be close to the actual requirements. Issues still remain for the estimated factors that could not be verified throughout the trials. For those factors, further studies must be conducted to prove them right or wrong. As body weight, feed intake, and egg weight may have a huge impact on the factorial approach to the requirements of Ca and P, they should be handled with care and input values should be checked for credibility, especially in differing conditions (e.g., summer/winter).

5.1.3 Potential impact of actual nutrient concentrations on the results

In general, the deviation of the analyzed Ca level from the target level was higher than that for total P levels. For the rearing periods, the largest proportion of those differences could be classified as the analyzed Ca level being above the calculated level. For the group with Ca/P reduced feed, these deviations tended to be higher than for the group with standard feed. As the P levels fit quite well, this might have had an effect on the ratio of Ca and P and thus also on the interaction and utilization of those minerals. The ratio of Ca:total P had values between 1.1 and 3.6 (mean: 1.5) for standard feed and between 0.9 and 2.3 (mean: 1.3) for Ca/P reduced feed in trial I. In trial III, the ratios differed between 1.8 and 3.2 (mean: 2.2) for standard feed and between 1.0 and 2.3 (mean: 1.8) for Ca/P reduced feed. Driver et al. (2005) conducted a trial with broiler chickens using different ratios of Ca:total P and did not observe any negative effect on the animals. Furthermore, the standard protocol from the *WPSA* for determining the pcdP allows a ratio between 1.3:1 and 1.4:1 (Ca:total P) and does not prescribe a fixed ratio, indicating that the particular ratio is no target value but is flexible and a result of the optimization of the feed for both minerals (WPSA Working Group No. 2, 2013).

Assuming that the overall Ca level in the group with Ca/P reduced feed might have been slightly above the calculated levels, this certainly would reduce the absolute reduction of Ca. Possible effects on physical development and the performance of the animals might have been visible with a greater reduction of Ca in the rearing periods only. For the laying period, however, the overall Ca level tended to be slightly below the calculated levels for both the standard feed and Ca/P reduced feed groups; therefore, it probably had no effect on the comparison of the groups. In accordance with that, Bradbury et al. (2014) conducted a trial with broiler chickens and found that the concentration of P (especially NPP) had a greater influence on the performance of the birds than the concentration of Ca.

5.2 Effects on growth and feed intake of turkey breeder hens

For both rearing periods (trials I and III), the weight data did not exhibit any clear effects concerning a higher mean weight of the hens in either group. In trial I, all throughout the trial period, the group with standard feed exhibited a higher mean body weight for five different weeks, while the group with Ca/P reduced feed exhibited a higher mean body weight in three weeks. In trial III, the group with Ca/P reduced feed exhibited a higher mean body weight for four different weeks, while the group with standard feed did not exhibit a higher mean body weight for any week. When comparing the weight data of these two trials, it must be kept in mind that trial III started later than trial I (week 11 compared with week 7). An influence of these four weeks on the total weight development may be conceivable, but it seems to be improbable because of the small number (four weeks out of 30). The more likely explanation for the observed differences in body weight is that Ca/P reduced feed in the rearing period did not have an effect on the weight development of the hens and that the observed differences (either in trial I or III and also within the two) were random effects.

Viveros et al. (2002) conducted a trial with broiler chicks (0–6 weeks of age) and found that a decreased concentration of NPP (0.22% compared with 0.35% in weeks 0–3, 0.14% compared with 0.27% in weeks 3–6) led to a decrease in weight gain. These findings were supported by a trial with broilers by Bradbury et al. (2014). The authors found that groups with a higher amount of NPP (concentrations between 0.24% and 0.7%) exhibited higher body weight and feed intake. An influence of the also varied Ca concentration (between 0.64% and 1.2%) could also have had an influence on the results, but this influence was analyzed to be lower than that of the concentration of NPP. In a trial by Walk et al. (2014), however, a reduction of the dietary levels of Ca (0.82% compared with 0.98%) and P (0.6% total P/0.3% av. P compared with 0.76% total P/0.45% av. P) exhibited no influence on body weight and feed intake. This phenomenon was also observed in a trial by Rousseau et al. (2012) with finishing broilers, where different dietary levels of Ca (0.37%, 0.57%, and 0.77%) and NPP (0.18% and 0.32%) had no effect on growth performance.

Regarding mean hen weights in the laying periods (trials II and IV), the data were observed to fluctuate as much as they did in the rearing periods. In trial II, higher body weight in the group with standard feed was observed for two weeks throughout the trial, while for one week a higher mean body weight was observed in the group with standard feed. In trial IV, the mean weight between two different weeks was higher for the group with reduced dietary levels of Ca and P.

In all other weeks, no difference in body weights was found between treatments. No effect on the body weight of turkey breeder hens was also observed in a trial by Slaugh et al. (1989) concerning different dietary levels of av. P (0.70%, 0.50%, 0.30%, and 0.15%). Another trial with laying hens by Jing et al. (2018) found no effect on the body weight or feed intake of the animals related to av. P levels between 0.15% and 0.45% (increments of 0.05%).

Of course, body weight and feed intake might be interlinked. Comparing the results concerning feed intake revealed that feed intake exhibited a tendency towards higher values for the group with Ca/P reduced feed in all four trials. In trial I, this difference was even significant for three out of 30 weeks. In trial II, however, the difference between the treatments was significant for one week only with a higher value in the group with standard feed. The overall mean feed intake of the group with Ca/P reduced feed was above that of the group with standard feed in all four trials (Figure 36). Furthermore, the course of the feed intake in both rearing trials (trials I and III) exhibited a tendency toward higher values in the trial group (reduced dietary levels of Ca and P) with only a few outliers. The same tendency was found in trial IV where the overall mean feed intake turned out to be higher in the group with lower dietary levels of Ca and P. Regarding this result of trial IV as well as trial III, it must be kept in mind that they refer to rough calculation and are not based on daily or weekly measurements. Nevertheless, the results of these periods supported the findings from trial I.

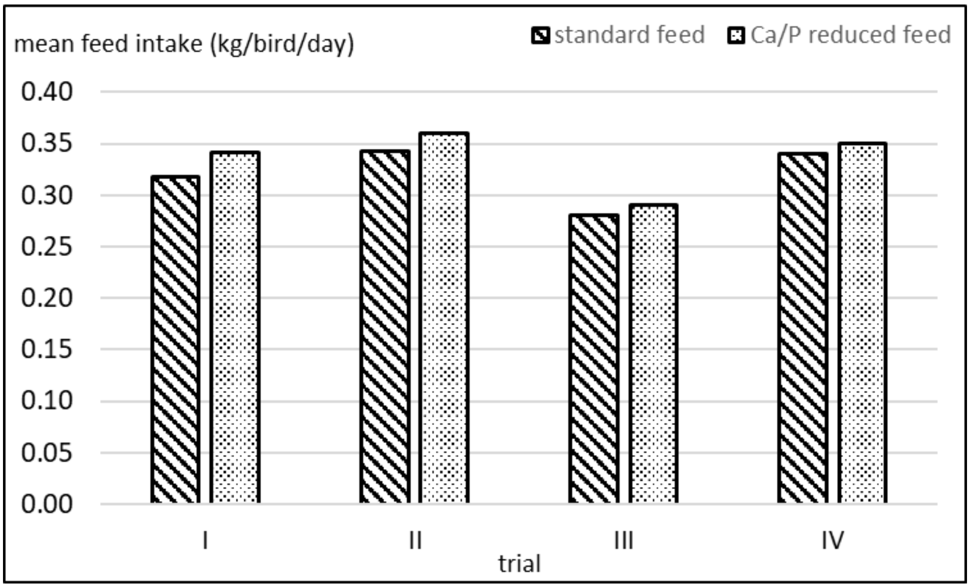

Figure 36: Mean feed intake of the group with standard feed compared with the group with Ca/P reduced feed (trials I–IV)

Similar effects concerning feed intake were observed in an older study with different Ca levels in broiler chicken feed. The authors found that the feed intake was significantly lower with a high level of Ca in the feed using the same level of P (Sebastian et al., 1996). Rama Rao et al. (2006) found that feed intake as well as weight gain decreased when Ca levels were increased while using the same level of P. This effect on the feed intake was also found to be a trend in the present study (trials I, III, and IV). A tendency toward higher body weights and higher body weight gains in the group with less Ca in the feed, however, was only observed in trial III and a part of trial IV and could not be confirmed with these trials.

The results of trial II differ from the results of the other three trials in terms of feed intake data. In this trial, the group with standard feed with higher dietary levels of Ca and P exhibited a tendency towards a higher feed intake for the majority of the time (although the mean feed intake of the group with standard feed was lower compared with the group with Ca/P reduced feed). The evaluation of the diagram for the data on feed intake in trial II shows that that the differences in this trial were very small. Actually, the largest difference between the groups was 8.5% (27.0 g) in this trial, whereas that in trial I, for example, was 19% (69.8 g). This relation demonstrates that the differences in trial II were indeed smaller compared with those in other trials but only when compared with trial I (rearing period). Unfortunately, a comparison with trials III (rearing period) and IV (laying period) appears inappropriate because of the rough calculation of these values. It might be possible that the differences in general were higher in the trials involving rearing periods. This hypothesis fits the higher percentages of reduction in the dietary level for the trials in the rearing period. In particular, the reduction of the dietary levels of Ca was noticeably higher in the trials involving rearing periods, which leads to the assumption that a higher percentage of reduction in Ca causes a larger difference in feed intake.

Although the mean feed intake data (Figure 36) exhibited quite a clear trend towards a higher feed intake for the group with Ca/P reduced feed, this tendency was never observed for body weight data. As the levels of energy and protein were identical for the same time in one trial, the group with a higher feed intake (the group with reduced dietary levels of Ca and P) overall ingested greater amounts of energy and protein. As this did not result in higher body weight at this point of the evaluation, it might be possible that the higher feed intake resulted in another output, such as greater performance (see Section 5.4), higher robustness against diseases, or rather more feces.

In general, the mean body weight of both rearing periods was below the target curve for the majority of weeks, and it seems that the turkeys caught up in terms of weight by the end of the rearing. Especially between weeks 19 and 27 in both rearing trials, a visible dip occurred in body weight in both groups. The temporal difference between the target curve and the body weight curve suggests rethinking either the target curve or the feeding regime to bring the curves closer together. It is possible that this dip was caused by an inappropriate nutrient concentration of the feed during this period. If this effect was also visible in a higher number of flocks, the feeding regime and its nutrient concentration should be edited.

For the laying periods, however, there was no target curve to synchronize with, but it is striking that the body weight curve in trial II exhibited a long dip downwards after the start of laying, where the mean weight also fell below 12 kg. This decreasing trend did not manifest before the middle of the laying period. Moreover, in trial IV, the body weight curve exhibited a rising trend after the trial started in PW 12. This phenomenon was described by Cherms et al. (1976). In their trial, feed intake and body weight declined when the laying performance increased. To buffer this effect, a higher concentration of nutrients might be useful at the beginning of the laying period. In fact, the standard feeding program used only one feed type for the whole laying period, which should be reconsidered because the influential factors, such as body weight, feed intake, egg weight, and laying performance, are not the constant throughout the laying period of turkey breeder hens.

5.3 Effects on bone mineralization

Rath et al. (2000) defined the amount of bone ash as well as the concentration of minerals as indicators of good stability in poultry bones. In trial I in the present study, the amount of bone ash and the concentrations of Ca and P exhibited no significant difference between the treatments. A tendency toward higher values was observed for the concentrations of bone ash and Ca, where the group with Ca/P reduced feed exhibited slightly higher values. This leads to the conclusion that the stability of the bones tended to be better in the group with Ca/P reduced feed. In a trial with broiler chickens, Rousseau et al. (2012) found that the concentration of bone ash decreased with a lower concentration of Ca and an identical level of NPP (Ca concentrations: 2.8%, 3.2%, 3.6%, 4.0%, and 4.4%). The authors thus suggested that "Ca is the limiting factor for bone mineralization". Similar effects were also found in trials with laying hens (increasing bone stability with increasing Ca concentration between 2.5% and 5.0%; Roland et al., 1996) and duck breeders (increasing ash content with increasing concentrations of Ca between 2.8% and 4.4%). This effect could not be observed in the present study, which might refer to the simultaneous reduction of Ca and P concentrations. Toward this hypothesis, Walk et al. (2014) also found lower concentrations of tibia ash in a trial with broiler chickens fed a diet with reduced dietary levels of both Ca (0.82% compared with 0.98%) and P (0.6% total P/0.3% av. P compared with 0.76% total P/0.45% av. P). Another trial with broiler chickens focusing on different ill effects on the legs of the animals referred to different Ca levels in the feed. These effects were alleviated by diets with reduced Ca levels (Rama Rao et al., 2006). This result also supports the findings of the present study as the group with reduced levels of Ca and av. P in the feed exhibited a tendency toward better bone mineralization results. In this trial, however, no ill effects for either group could be observed by veterinarian inspection of the lost animals. Therefore, a comparison of this factor was not made.

Focusing on that tendency concerning the stability of bones, it must be highlighted that the observed difference turned out to be nonsignificant, which means that the difference could not be proven statistically. Additionally, all of the differences between the groups were below 1%. These two issues lowered the resilience of those differences.

Furthermore, the bones of the animals, especially the tibia, reflected the marginal mineral supply of the animals quite directly (Pongmanee et al., 2020; Roberson, 2004). No difference could be observed between the treatments, which led to the assumption that no Ca or P concentration in trial I was below the requirements of the turkeys.

In the present study, the correlation between age and the concentration of bone ash (without splitting into groups with standard feed and Ca/P reduced feed) was calculated to be 0.66 (see also Section 4.2.3). Following the interpretation suggestions by Cohen (1988), a correlation above 0.50 may be classified as a high relation. This leads to the assumption that a strong relation exists between the age of turkeys and the percentage of bone ash, with older turkeys exhibiting a higher concentration of bone ash. Following Rath et al. (2000), this leads to the assumption that older turkeys exhibit higher bone stability. This assumption was supported by a trial by Rath et al. (2000), where 75-week-old laying hens exhibited stronger bones compared with the laying hens at 25 weeks of age.

The observed correlation of 0.51 between the concentration of Ca in the bone ash (without splitting into groups with standard feed and Ca/P reduced feed) and the age of turkeys suggests a strong relation as birds of older age seemed to have a higher concentration of Ca in the bone ash. However, the correlation between the concentration of P in bone ash (without splitting into groups) and age in this trial was 0.20. According to Cohen (1988), this suggests a low negative relation, meaning that older age results in a lower proportion of P in the bone ash. In general, the percentage of P in bone ash exhibited a low range between 16.2% and 17.9% in this trial, which seems to reduce the validity of that statement.

In the results of the present study, the effect of age on the factors may be neglected because of a similar age distribution of the analyzed bones. This was reflected in the median being 23 and 21 for the groups with standard and Ca/P reduced feed, respectively.

Lastly, the general proportion of bone ash (58.5%/59.4%) in the present study seemed to be high compared with other studies. Shastak et al. (2012a), for example, conducted a trial with broiler chickens and found levels between 41.5% and 51.2%. For fattening turkeys at the age of 16 weeks, Kozłowski et al. (2010) found bone ash contents between 51.27% and 55.39%. The higher concentration of bone ash in the present study may be related to the older age of the turkeys. No comparative value for this factor could be found for turkey breeder hens in rearing.

5.4 Effects on the performance of turkey breeder hens

When evaluating the performance of turkey breeder hens through this thesis, only the results from both laying periods (trials II and IV) were considered. Within these trials, the overall performance was described by the laying performance, egg weight, and egg quality (culled eggs and egg components), brooding quality (fertility and hatchability) and the weights and fitness of the progeny.

5.4.1 Laying performance

The laying performance of both groups in trials II and IV was above the target from *Moorgut Katzfehn Turkey Breeder GmbH & Co. KG* nearly all of the time. This is an indicator that both flocks that were monitored in the present study may be classified as quite “good” flocks without greater issues concerning diseases or management problems. An influence of a short disease, however, was observed at the end of trial II. In PW 23, an *Ornithobacterium rhinotracheale (ORT)* infection was diagnosed in the animals in barn 3 and also in barns 1 and 4 in PW 24. This infection caused a severe reduction in laying performance, especially for the group with Ca/P reduced feed (barns 3 and 4). Therefore, these data could be evaluated up until PW 22 only.

In trial II, the laying performance of the group with standard feed tended to be higher than that of the group with Ca/P reduced feed the majority of time. This result was influenced by a visibly higher laying performance in barn 2 compared with the other three barns. Indeed, the laying performance of barns 1, 3, and 4 were of a similar level. No reason could be found for the higher laying performance of barn 2, so it can be assumed that it is either due to management differences, location effects, or random effects. The overall dip in the laying performance at the beginning of the trial could be traced back to management issues on the farm. In that period of time, many changes in staff caused nervousness for the turkeys.

Leaving out barn 2, the laying performance in trial II was nearly identical for the groups with standard feed and reduced dietary levels of Ca and P all of the time. The same result was found in trial IV, where only tiny differences were visible between groups. Overall, the laying performance seemed not to be influenced by a reduction of the dietary levels of Ca from 2.90% to 2.80% or 2.60% as well as a reduction of the dietary level of P from 0.36% to 0.30% or 0.24% (av. P) or from 0.65% to 0.50% (total P).

This result is supported by the findings of Austic and Keshavarz (1988) as well as Clunies et al. (1992). Neither trial with laying hens detected negative effects on the laying performance when the dietary Ca level was reduced to 2.5% or even 2.0%. Potter et al. (1974) also postulated that dietary Ca levels below 2.0% could reduce the laying performance of turkey breeder hens. As the lowest dietary Ca level in these trials was 2.60%, the reduction in this mineral could have been even greater with probably also no negative effect on the laying performance.

For the dietary level of P, the lowest used level of total P (0.50%) was even below the dietary level in the trial by Potter et al. (1974) at 0.64%. No negative effect on the laying performance could be determined in the trial. A more recent trial by Godwin et al. (2005) with turkey breeder hens also published no effect on the laying performance when the level of av. P was reduced to 0.17% (without the addition of phytase) between weeks 31 and 62 of age. For laying hens, Pongmanee et al. (2020) reported no negative effects on the laying performance with a dietary level of 0.19% av. P compared with 0.38% av. P. Moreover, a meta-analysis by Ahmadi and Rodehutscord (2012) reported an optimum for the laying performance of laying hens at the age of 36–76 weeks, with a minimal higher level of 0.22% NPP.

In addition, Potter et al. (1974) found laying performance to be highly correlated to feed consumption. When evaluating the results of the present study, such relation could not be supported. Although different feed intakes were observed for the groups with standard feed and Ca/P reduced feed in trials II and IV (laying periods) as well as for the rearing periods, no difference in the laying period could be determined in any trial. It might be possible that the differences in feed consumption in the present study were too small to observe any effect.

5.4.2 Egg weight

The egg weight data from trial II revealed a slightly higher mean egg weight for the group with the standard diet for the majority of weeks. For seven weeks, the difference from the mean egg weight of the Ca/P reduced group even turned out to be significant. This suggests a generally higher egg weight for the group with standard dietary levels of Ca and P. On the other hand, the Ca/P reduced feed group also exhibited a higher mean egg weight for some PW (significant at one week), which weakens this statement. Overall, a higher number of significantly higher egg weights was found for the group with standard feed.

Barn 2, which had a noticeably higher overall laying performance, did not exhibit a higher or lower mean egg weight, which led to the assumption that a high (or low) laying performance is not correlated with a high (or low) egg weight. This may also be supported by the fact that the overall egg weight in trials II and IV increased from the beginning of the trials until the end while the laying performance in both decreased toward the end of the trials.

Overall, the average of all differences between the mean egg weights in the groups with standard feed and Ca/P reduced feed was 0.5 g. This result also weakens the statement of a generally higher mean egg weight in the group with standard feed.

In trial IV, the egg weight data did not exhibit any clear differences. Only for one PW was the mean egg weight of the group with standard feed significantly higher than that of the Ca/P reduced feed group. The overall average of all single differences between the mean egg weights of the two groups was zero.

Overall, a reduction of the dietary levels of Ca from 2.90% to 2.80% or 2.60% and a reduction of the dietary level of P from 0.36% to 0.30% or 0.24% (av. P) or from 0.65% to 0.50% (total P) exhibited no negative effect on the egg weight of turkey breeder hens in this trial.

Similar results were published by Austic and Keshavarz (1988) and Roland et al. (1996). In these trials, different dietary Ca levels between 2.0% and 5.0% did not exhibit any effects on the egg weights of laying hens. Even in a trial with turkey breeders back in 1974 with dietary Ca levels between 0.99% and 3.33% and total P levels between 0.64% and 0.82%, egg weight did now exhibit any variation concerning the different dietary levels. A more recent trial with turkey breeders by Godwin et al. (2005) focusing on different dietary levels of av. P (0.55%, 0.35%, and 0.17%, each with and without the addition of phytase) also revealed no effects on egg weight related to the level of P.

Another trial with broiler breeder chickens from 2012, however, detected a lower egg weight in the group with a dietary level of 0.15% NPP compared to the group with a dietary NPP level of 0.40% (Ekmay et al., 2012). This tendency was also visible in trial II in the present study but the significance could not be proven. The slightly higher egg weight for the group with standard feed might also have been influenced by the higher feed intake in the group with standard feed (see also Section 5.2).

In trial IV, the mean body weight of the group with reduced dietary levels of Ca and P exhibited a tendency toward a higher mean weight between PWs 12 and 18. However, for PWs 14 and 16, a higher mean egg weight was found in the group with standard feed. This indicates that a high body weight might have a negative effect on egg weight. This hypothesis is not in line with the findings of Bish et al. (1985). In their trial with three groups of laying hens (of high, medium, and light body weight), the hens with a high body weight produced eggs of a higher weight than the other two groups. Similar results to this trial were published by Leeson and Summers (1983) for broiler breeder hens. It is possible that the findings of the present work differ from those trials because the differences in body weight and egg weight were too small to classify a difference and the observed difference was due to random effects.

5.4.3 Culled eggs on the farm

The proportion of culled eggs classified in different categories exhibited only very small differences in trials II and IV. In trial II, minimal differences were observed for the total percentage of culled eggs, which was 0.5% higher for the trial group (with reduced dietary levels of Ca and P) than for the group with standard feed (standard: 5.6%, trial: 6.1%). Similar to the results for laying performance, it was striking that the results from barn 2 (5.2%) differed from those of barn 1 (6.0%), barn 3 (6.0%), and barn 4 (6.2%). The differences could probably be explained by either management differences, location effects, or random effects.

Nearly all observed differences in the percentage of culled eggs within one classification between the groups were 0.1% or 0.0%. As the differences were that small, probably all of them were due to random effects. In both trials, the only difference above 0.1% was observed for the percentage of small eggs, which was 0.2% for both groups. However, for this classification, the number of culled eggs was higher for the group with reduced dietary levels of Ca and P in trial II and vice versa in trial IV. As no reason could be found for this result and as 0.2% is a tiny difference, these variations were probably due to random effects.

The second difference above 0.1% was obvious for the proportion of dirty eggs in trial II. In this classification, the percentage of culled eggs for the group with Ca/P reduced feed was 0.2% above the number of dirty eggs in the group with standard feed. Examining trial IV, the difference in the classification of dirty eggs exhibited the same direction but only a difference of 0.1%. As no reason could be found for this variation (which was certainly still tiny), it was probably due to random effects.

In general, the proportion of culled eggs seemed to be higher in trial II than in trial IV. However, it must be kept in mind that the trial period of trial IV started in PW 12 and trial II had already started in PW 1. It is likely that more eggs were culled at the beginning of a laying period when the hens started laying.

Overall, the reduction of the dietary Ca level from 2.90% to 2.80 or 2.60% and the reduction of the dietary level of P from 0.36% to 0.30% or 0.24% (av. P) or from 0.65% to 0.50% (total P) exhibited no measurable effects on the percentages of eggs that were cracked, dirty, double-yolked, misshapen, thin, and too small. Therefore, it seems that egg quality as well as shell quality were not negatively influenced by this reduction (see also Section 5.4.4).

A comparison of the other trials and studies for this part of the results relating to the number of culled eggs was omitted because no trials with similar methods could be found.

5.4.4 Egg components

The data concerning egg components were from 20 eggs per group (10 per barn) in trial II. Due to this limitation to only one trial, no comparison between the trials could be performed for this factor. The small number of replications also reduced the information value of this factor and led to uncertainties regarding the hypothesis of this chapter.

Nevertheless, the egg component data led to some tendencies and assumptions that could be evaluated. For example, the group with reduced dietary levels of Ca and P exhibited significantly more egg yolk compared with the group with standard feed. The group with standard feed, however, exhibited significantly more albumen (see subsection 4.3.5.2). For this variation, no reason could be found.

Another significant difference was found concerning the total amount of Ca in eggs (shell included) being higher in the group with Ca/P reduced feed. Moreover, the proportion of Ca within the egg shell tended to be higher for the group with Ca/P reduced feed, but this difference was nonsignificant. These results suggested higher egg stability for the group with a reduced dietary level of Ca and P. This assumption was supported by a higher proportion of water within the shell of the group with standard feed. The group with reduced dietary levels of Ca and P also had slightly higher concentrations of egg shells compared with the group with standard feed (nonsignificant).

The mean proportions of Ca and P were higher in egg yolk and albumen for the group with standard feed; however, the mean proportions of Ca and P in the shell were higher for the group with Ca/P reduced feed. Both differences were nonsignificant but indicated that differences between the groups might be found if the analyses were repeated with a higher number of analyzed eggs.

Overall, the results concerning egg components exhibited a possible tendency toward better egg shell quality for the group with reduced dietary levels of Ca and P. Due to uncertainties, this tendency should not be taken for granted. Nevertheless, at least no negative effect could be observed due to a reduction of the dietary Ca level from 2.90% to 2.80 or 2.60% and a reduction of the dietary level of P from 0.36% to 0.30% or 0.24% (av. P) or from 0.65% to 0.50% (total P).

In a trial with turkey breeder hens, Potter et al. (1974) found that a dietary level of 0.99% Ca had a negative effect on egg shell thickness and the percentage of egg shell from the total egg weight. The dietary Ca levels of 1.77%, 2.55%, and 3.33% had no significant influence on these two factors. A trial with laying hens by Ruhnke et al. (2021) also reported a higher shell weight, shell thickness, and shell breaking strength with a higher dietary level of 4.0% compared with 2.0%. An allegedly positive effect of the reduction of dietary Ca level on egg shell quality, as reported in this trial, however, was not observed in the trial by Potter et al. (1974). Different dietary levels of total P (0.64% to 0.82%) in the trial by Potter et al. (1974) exhibited no influence on egg shell quality, which could not be related to the present study because of unsuitable dietary levels of P.

As Comar and Driggers (1949) reported that 60% to 75% of Ca in the shell is directly ingested from the feed, the simultaneous reduction of Ca and P might lead to a reduced absorption of Ca and therefore to a lower proportion of Ca in the shell. This effect could not be observed in this work. Jing et al. (2018) conducted a trial with laying hens and found that a lower value of P (0.15% av. P compared with 0.45% av. P) tended to increase the absorption and retention of Ca. This leads to increased eggshell thickness as it was also observed in this work. The same hypothesis was published by Ekmay et al. (2012), who found that hens fed a lower dietary level of NPP (0.15% compared with 0.40%) had lower egg weights but a higher egg shell quality. Pongmanee et al. (2020) reported no effect on the egg shell thickness of laying hens using a dietary level of 0.19% av. P compared with 0.38% av. P.

Jing et al. (2018) also determined the proportions of Ca and P within egg yolk and albumen. The proportions of egg yolk and albumen were not affected by different levels of dietary P (0.15% av. P compared with 0.45% av. P). The same result was published by Godwin et al. (2005) for dietary levels of total P (0.55%, 0.35%, and 0.17%). This increases the probability that the observed differences concerning this factor in the present study were due to random effects.

The calculation of the correlation between egg weight and egg components exhibited no correlation of 0.5 or higher, which could be considered a high correlation (Cohen, 1988). A moderate correlation of between 0.3 and 0.5 was found five times (out of a total of 14 calculated concentrations). The correlation of egg weight to albumen was 0.34, while that of egg weight to egg yolk was −0.33. Hence, in the present study, heavier eggs tended to have more albumen but less egg yolk compared with lighter eggs. Another positive moderate correlation of 0.33 was found for egg weight and the concentration of Ca in shell. This means that in this study, the heavier eggs tended to have a higher concentration of Ca in the egg shell. According to the value, the highest correlation was calculated between the egg weight and the concentration of total P in the whole egg (with and without shell). The values were −0.41 and −0.40, indicating that the heavier eggs tended to have a lower concentration of P in the whole egg in the present study. The other nine calculated correlations could be classified as "small" according to Cohen (1988). For these ingredients, it was concluded that the egg weight had a small influence on their concentration only.

5.4.5 Fertility and hatchability

The fertility of both groups in trial II was similar all throughout the laying period. A dip for both groups was observed between PW 11 and PW 13, which might be due to many changes in staff causing nervousness for the turkeys. Another dip from PW 22 (group with Ca/P reduced feed) or PW 23 (group with standard feed) until the end of production probably resulted from the infection with ORT (see also Section 5.4.1).

The hatchability curve in trial II generally followed the fertility curve. For example, the same dip at the end of production was visible here although it started already in PW 21 for the group with standard feed (but more moderate). Between PW 8 and 13, the curve for both groups exhibited higher and lower values around the target curve. This could be traced back to management issues in the hatcheries.

For both fertility and hatchability in trial II, only small differences were observed between the groups with standard feed and reduced dietary levels of Ca and P. The fertility and hatchability data in trial IV split by group were only available for six weeks and not for the whole production period. For both factors, the differences between the groups, as in trial II, were small and indicated no effect of the different feeding regimes on the fertility and hatchability of the eggs. An exception to this observation was found for PW 20 in trial IV, with a particularly low hatchability for the group with Ca/P reduced feed, a particularly high hatchability for the group with standard feed, and a difference of 23.3%. These values and this difference did not fit the results of the other weeks and were probably caused by a mistake in the documentation.

For fertility, similar results were published by Potter et al. (1974) with dietary Ca levels between 0.99% and 3.33% and total P levels between 0.64% and 0.82%. For these levels, no variation in fertility was visible in the turkey breeder hens in this trial. Other trials with breeders by Moyle et al. (2012) and Hudson and Wilson (2003) have linked the level of fertility to the condition of the male breeders. An influence of the male breeders certainly cannot be excluded; therefore, fertility is not further discussed in this evaluation.

Concerning hatchability, Potter et al. (1974) conducted a trial with different levels of dietary Ca and observed a lower hatchability of fertile eggs at a level of 0.99% compared with dietary Ca levels of 1.77% or higher. Focusing on the dietary levels of P, Manley et al. (1980) reported a decrease in hatchability for dietary levels of 0.3% total P compared with a level of 0.5% total P for turkey breeder hens. The authors also suggested an even higher level of total P to ensure higher hatchability. Different observations, however, were made by Potter et al. (1974) with higher dietary total P levels between 0.64% and 0.82% (no change in Ca concentration). In this trial, the hatchability was not affected by the different levels of dietary P. Moreover, Godwin et al. (2005) and Slaugh et al. (1989) reported no effect of different dietary levels of total P (0.55%, 0.35%, and 0.17%) or av. P (0.70%, 0.50%, 0.30%, and 0.15%).

Overall, the results of these trials and the present study suggest that reductions in dietary Ca level from 2.90% to 2.80 or 2.60% and in dietary P level from 0.36% to 0.30% or 0.24% (av. P) or from 0.65% to 0.50% (total P) have no effect on the fertility and hatchability of hatching eggs, and also that a greater reduction may be practicable.

5.4.6 Weight of progeny

The weights of the hatchlings in trial II all throughout the trial period exhibited a tendency toward a higher mean weight in the group with standard feed compared with the group with Ca/P reduced feed. Although this difference was significant for PWs 17, 18, and 22, an influence from the treatments is quite improbable because of the few significant results.

Furthermore, the weight data from the hatchlings in trial IV indicated no visible difference between the groups with standard feed and reduced dietary levels of Ca and P. All differences were nonsignificant.

Overall, no negative effect on the weight of the progeny was observed due to a reduction in the dietary Ca level from 2.90% to 2.80 or 2.60% as well as a reduction in the dietary level of P from 0.36% to 0.30% or 0.24% (av. P) or from 0.65% to 0.50% (total P).

Similar results were published by Godwin et al. (2005) from a trial with turkey breeder hens using different dietary levels of total P (0.55%, 0.35%, and 0.17%, each with and without the addition of phytase). No difference in the weight of hatchlings was observed throughout the 28 weeks of laying.

In addition, it is striking that the group with standard feed in trial II exhibited a tendency toward a higher mean poult weight as well as a higher mean egg weight. This leads to the assumption that poult weight might be influenced by egg weight. A single value comparison with a particular poult weight belonging to a specific egg weight was not conducted in this work, and therefore, this hypothesis cannot be proven. Nonetheless, the correlation (according to Pearson) of mean egg weight to mean poult weight was 0.96 for both groups, which confirmed this hypothesis. A positive correlation (0.65) was also published by Applegate and Lilburn (1996) with turkey hens. The correlation in the present work was probably higher because of the use of mean weight; it does not show the real correlation but it does indicate a trend. In an older trial with laying hens, Halbersleben and Mussehl (1922) even found a constant relationship between egg weight and chick weight at hatching, with poult weight being 64% of the egg weight of the unhatched egg. In the present work, the proportion of the mean hatching weight of the poults from the mean weight of the eggs varied between 64.4% and 68.3% (mean: 66.7%), which also indicated a fairly constant relationship. In trial IV, analyses of the relation between egg weight and poult weight were omitted due to the small amount of data.

5.4.7 Conclusion on the effect of dietary levels of Ca and P on the performance of turkey breeder hens

The evaluation of laying performance, egg weight, culled eggs on the farm, fertility and hatchability, and weight of progeny indicated no positive or negative effect of a reduction of the dietary level of Ca in the laying period from 2.90% to 2.80 or 2.60% and of a reduction of the dietary level of P from 0.36% to 0.30% or 0.24% (av. P) or from 0.65% to 0.50% (total P).

The only effect found concerned the egg shell. That is, the group with a reduced dietary level of Ca and P tended to exhibit a superior egg shell quality. Unfortunately, this tendency did not reflect in better hatchability or fewer lost eggs. Additionally, this result is highly uncertain due to the small sample size.

Nevertheless, this study overall indicated no negative effect of a reduction in the dietary levels of Ca and P on the aforementioned values. Moreover, the data from the discussed literature suggest that an even greater reduction is possible without a negative effect on the performance of turkey breeder hens.

5.5 Effects on the concentration of calcium and phosphorus in manure

In trials I and III (rearing periods) and in both groups, the percentages of dry matter were highly similar (68.6% to 69.0%; Table 31). Therefore, it can be inferred that the use of Ca/P reduced feed had no influence on the proportion of dry matter in the rearing period of turkey breeder hens. In trials II and IV (laying periods), the overall percentages of dry matter were lower (trial II: 48.8% to 51.4%, trial IV: 39.0% to 41.7%) compared with the trials in the rearing period (trial I: 68.8% to 69.0%, trial III: 68.6% to 68.7%); therefore, the manure was wetter in the laying periods. The reason for this could lie in the use of different bedding materials. While wood shavings and straw were combined in the rearing period, only straw was used in the laying period. When comparing both trials in the laying period, wetter manure was found in trial IV, which might have been related to location or seasonal effects as trial IV started in February in cold and wet conditions while trial II started in May with mostly dry weather conditions. For the rearing periods (trials I and III), however, the location and weather seemed to have no influence on the condition of the manure. This may be explained by the forced ventilation in rearing while the barns in the laying periods also used shutters for ventilation.

Table 28: Means ± SD of the analyzed concentrations of dry matter, P, and Ca in manure samples (N = 10 per barn, trials I–IV)

Analyzed concentration (%)	Trial I (rearing)		Trial III (rearing)		Trial II (laying)		Trial IV (laying)	
	Standard feed	Ca/P reduced feed	Standard feed	Ca/P reduced feed	Standard feed	Ca/P reduced feed	Standard feed	Ca/P reduced feed
Dry matter	68.8 ± 1.2	69.0 ± 0.5	68.6 ± 11.3	68.7 ± 11.3	48.8 ± 6.7	51.4 ± 7.2	41.7 ± 6.6	39.0 ± 3.3
P (related to dry matter)	2.0 ± 0.4	2.0 ± 0.7	1.4 ± 0.2	1.5 ± 0.3	1.4 ± 0.3	1.4 ± 0.3	1.2 ± 0.4	1.2 ± 0.5
Ca (related to dry matter)	3.5 ± 0.7	3.3 ± 0.9	3.7 ± 1.0	2.3 ± 0.6	5.0 ± 1.1	5.1 ± 1.0	4.8 ± 1.6	4.3 ± 1.4

Furthermore, the mean proportion of P in manure was identical for both groups in trial I (2.0%), which indicates that the Ca/P reduced feed had no reducing effect on the amount of P in manure. This was confirmed by the calculation of the nutrient balance, which revealed a larger amount of P in manure in the group with Ca/P reduced feed. This was probably caused by the concentrations of total P in Ca/P reduced feed being nearly the same or even higher in some feeding phase (see also Section 5.1.1).

In trials II–IV, the amount of P was similar for both groups within each trial as well as when comparing the trials with each other (1.2% to 1.5%). In all three trials, the calculation of the nutrient balance indicated a reduction (trial II: 92 kg, trial III: 137 kg, trial IV: 218 kg) for the group with Ca/P reduced feed (Table 29). A comparison of these values must be handled with care because of the different trial designs (see also Section 3.8). However, this theoretically lower level of P could not be found in the manure. This led to the hypothesis that the reductions in dietary P in the trial period were too small to realize a measurable reduction in the manure from all throughout the rearing/laying period. Calculated as percentages of the total amount in the rearing/laying period (rough calculation), the differences were between 0.07% and 0.38% (Table 30).

This hypothesis was supported by the evaluation of the concentrations of Ca in manure. In trial I, the nutrient balance indicated a total reduction of 338 kg in the group with Ca/P reduced feed compared with the group with standard feed. This resulted in a significant reduction of the concentration of Ca in manure from 3.5% to 3.3%. For the other rearing trial (trial III), the calculation of the nutrient balance even suggested a reduction of 672 kg of Ca, which led to an even higher as well as significant reduction of the concentration of Ca in manure (3.7% to 2.3%). The difference of those values as a percentage of the total amount of manure was 0.52% (trial I) or 0.42% (trial III).

The concentrations of Ca in manure in trials II and IV (laying periods) overall were higher than the concentrations of Ca in the rearing periods (Table 28), probably due to the higher concentrations of Ca in the feed. All throughout these two trials and the groups within them, the concentration of Ca remained at a quite similar level (trial II: 5.0% to 5.1%, trial IV: 4.3% to 4.8%). The calculation of the nutrient balance suggested a reduction of 235 kg of Ca in trial II and of 116 kg in trial IV (Table 29); expressed as percentages of the total amount of manure this equated to 0.34% and 0.20%, respectively.

By comparing all calculated percentages of Ca and P from the total amount of manure, it was observed that only nutrient percentages of 0.42% or higher resulted in a significant difference in the analyzed concentrations of that nutrient in manure. All percentages of 0.38% or lower from the total amount of manure did not result in a significant difference in the analyzed concentrations in manure in the present study. This led to the hypothesis that dietary levels of Ca and P must be reduced to lower levels and/or a longer time of the rearing/laying period to observe significant reductions in the manure. Nevertheless, the detected relation between the calculated values (percentage of the total amount of manure) and significant difference in manure may be a causal relationship but may also be a random coincidence.

Table 29: Calculated amounts of P and Ca in manure out of the nutrient balances in trials I–IV (different length of trial periods, numbers of animals, and feeding regimens)

Input/output in kg	Trial I (rearing)		Trial III (rearing)		Trial II (laying)		Trial IV (laying)	
	Standard feed	Ca/P reduced feed)	Standard feed	Ca/P reduced feed)	Standard feed	Ca/P reduced feed)	Standard feed	Ca/P reduced feed)
Calculated P in manure	610	658	1,147	1,010	540	448	359	141
Calculated Ca in manure	1,009	671	1,911	1,234	1,739	1,504	1,220	1,104

Table 30: Calculation of differences in Ca and P between both treatments out of the nutrient balance (expressed as percentages of the total amount of manure; trials I–IV)

	Trial I	Trial III	Trial II	Trial IV
Amount of manure per year per 1,000 turkeys*	50.4	50.4	60.0	60.0
Duration of the whole rearing/laying period (number of weeks, with and without the trial period, including a one-week service period)	31	31	29	29
Number of periods per year	1.7	1.7	1.8	1.8
Mean number of animals per barn	2,177	5,382	2,054	1,703
Amount of manure per rearing/laying period	65,410	161,708	68,730	56,985
Calculated difference of P between the groups out of the nutrient balance (Table 29)	48	137	92	218
… expressed as a percentage of the total amount of manure	0.07%	0.08%	0.13%	0.38%
Calculated difference of Ca between the groups out of the nutrient balance (Table 29)	338	677	235	116
… expressed as a percentage of the total amount of manure	0.52%	0.42%	0.34%	0.20%

* Source: German fertilizer ordinance, Annex 9, Table 9 (Bundesministerium für Ernährung und Landwirtschaft, 2017)

5.6 Evaluation of the requirement recommendations and target levels from breeding companies

As the turkey breeders used in this work were BUT 6 hens, the evaluation focused on the target levels from *Aviagen Turkeys Ltd* for this particular strain (see Section 2.2.3). In addition, the target concentrations from the NRC, PAN, and Leeson and Summers were also considered (see Section 2.2.2).

The oldest of these target values were published by the NRC in 1994 based on the requirements of NPP. As the NRC uses the terms “NPP” and “available P” interchangeably (despite this not being correct; see Section 2.2.1), the published levels of NPP were assumed to be av. P as used in this work (Plumstead et al., 2007; Rodehutscord, 2001). For av. P, the concentrations used in this work were 11% to 47% below the recommendations by the NRC. Focusing on the used concentration of Ca, the concentrations in these trials were between 30% above the recommendations by the NRC and 47% below them. The recommendations from Leeson and Summers and PAN were both published in 2005. The level of av. P used in this work varied between 9% and 66% below the recommendation of Leeson and Summers and between 11% and 69% below the recommendation of PAN. For Ca, however, the concentrations used in this work varied between 4% above and 39% below the recommendations of Leeson and Summers and between 40% above and 39% below the recommendations of PAN.

Table 31: Differences in the calculated Ca and av. P concentrations in this study compared with the recommendations from Leeson and Summers (2005), NRC (1994), and PAN (2005)

	Ca		**P**	
	Minimal reduction	**Maximal reduction**	**Minimal reduction**	**Maximal reduction**
Leeson and Summers (2005)	+4%	−39%	−9%	−66%
NRC (1994)	+30%	−47%	−11%	−47%
PAN[1] (2005)	+40%	−39%	−11%	−69%

[1] As ciited in Jeroch et al. (2019, pp. 343, 345)

This study did not report any negative effect of reductions between 9% and 69% compared with the recommendations, which suggested that the recommendations for P may easily be revised downwards. In addition, research stations should clarify their usage of av. P and aim to relate their positions to the standard protocol of the WPSA in a stepwise manner even more to achieve greater comparability. For the target levels from *Aviagen Turkeys Ltd* given for turkey breeder

BUT 6 hens as well as for the practical use of recommendations, however, concentrations given based on pcdP seem to be unusual today because of a lack of infrastructure. To use this system in practical surroundings, raw materials should first of all be tested for their level of pcdP. Nonetheless, the parallel use of a clearly defined level of av. P and (if accessible) the level of pcdP should be a goal for the near future to become even more familiar with the system of pcdP. This would enable even greater comparability among recommendations provided for the concentration of P in turkey breeder as well as other poultry diets.

For Ca, however, the concentrations from the NRC, PAN, and Leeson and Summers in some periods suggest the use of even lower concentrations compared with those used throughout this work. This indicates that the target levels of Ca from *Aviagen Turkeys Ltd* for turkey breeder hens might be revised downwards and that trials focusing on the effect of a reduction in the dietary level of Ca should be more ambitious next time.

5.7 Conclusion

Throughout all four trials of the present study, no negative effects on the performance or physical development of turkey breeder hens were observed between the groups with standard feed and the group with reduced dietary levels of Ca from 1.00–1.10% to 0.56–0.80% and of P from 0.48–0.61% to 0.35–0.50% (av. P) or from 0.70–0.80% to 0.50–0.60% (total P) in rearing and reductions in the dietary levels of Ca from 2.90% to 2.80% or 2.60% and of P from 0.36% to 0.30% or 0.24% (av. P) or from 0.65% to 0.50% (total P) in laying. In fact, bone stability was slightly superior for the group with reduced dietary levels of Ca and P, and egg shell also demonstrated a tendency to be of higher quality in this group.

A quite clear effect was observed in the concentration of minerals in manure using reduced levels of Ca. A reduction in the usage in feed was also reflected in a lower outcome in manure, but only if the period was long enough and the height effective enough. Tiny reductions in the dietary levels of Ca and P did not exhibit an effect on the concentrations of those minerals in manure.

It can be concluded that a lower usage of P and Ca in the feed of turkey breeder hens is possible and results in no disadvantages. A reduction of P may also be combined with the use of phytase to ensure an even lower level of P. This reduction in the use of P will save P resources, thus contributing to the sustainability of agriculture as well as helping to reduce feed costs.

As this work did not indicate any negative effects of reductions of P and Ca to those dietary levels, greater reductions seem possible and must be tested in further trials. In further trials, turkey breeder males should be examined to determine the possibility of a reduction of the dietary levels of Ca and P in their feed as well as to clarify the influence of males on fertility and hatchability. Nevertheless, the use of safety margins on top of the recommended levels for breeder hens seems excessive and should be reconsidered.

6 Summary

Phosphorus (P) is an essential mineral in feed for livestock and has finite resources all over the world. The aim of this study was to obtain an idea about the reduction potential of P in the diets of turkey breeders. As the metabolism of P is interlinked with the metabolism of calcium (Ca), Ca was also examined. Therefore, the requirements of P and Ca were studied using a factorial approach. As data about the requirements of these minerals in turkey breeders is limited, the present study mostly used data from other poultry species. Thus, this study can be viewed as an approach to building new resilient data for turkey breeder hens.

The results of the factorial approach were embedded in a feeding program for turkey breeder hens during rearing and laying with two different treatments. One group was fed a standard feed (practical diet used before the trial), while the other group was fed a Ca/P reduced feed. In total, four trials were conducted: trials I and III focused on the rearing period while trials II and IV focused on the laying period. All trials were observed independently but the hens from trial I were used also in trial II afterwards for studying long-term effects. In each trial, body weight and feed intake were measured throughout the trial period. Bone mineralization was studied in trial I, including analyses of bone ash as well as Ca and P in the bone ash from the tibia of fallen and culled animals. The egg components were studied in eggs from trial II, including analyses of the percentages of albumen, egg yolk, and egg shell; Ca in albumen; P in albumen; Ca in egg yolk; P in egg yolk; water in egg shell; Ca in egg shell; and P in egg shell. In trials II and IV, laying performance, egg weights, number (and causes) of culled eggs, fertility, hatchability, as well as body weight and fitness of the hatchlings were additionally observed. At the end of each trial, the concentrations of dry matter, Ca, and P in manure were analyzed and a nutrient balance was calculated to classify the results.

Body weight development exhibited significant differences between the treatments in eight out of 30 weeks in trial I (five weeks had a higher mean weight in the group with standard feed; three weeks had a higher mean weight in the group with Ca/P reduced feed); three out of 28 weeks in trial II (two weeks had a higher mean weight in group with Ca/P reduced feed; one week had a higher mean weight in the group with standard feed); four out of 30 weeks in trial III (higher mean weight in the group with Ca/P reduced feed); and two weeks out of 28 in trial IV (higher mean weight in the group with Ca/P reduced feed).

The feed intake data also fluctuated, with three out of 30 weeks exhibiting a significant difference in trial I (higher mean feed intake in the group with Ca/P reduced feed) and one week with a significant difference in trial II (higher mean feed intake in the group with standard feed). The observations throughout all of the trials revealed the tendency for a higher mean feed intake in the groups with Ca/P reduced feed.

A significant difference concerning bone mineralization was not observed. Moreover, the results of laying performance, fertility, and hatchability exhibited no differences between the treatments.

Regarding the egg weight in trial II, there were eight weeks with a significant difference between the treatments (seven weeks with a higher mean egg weight in the group with standard feed; one week with a higher mean egg weight in the group with Ca/P reduced feed). In trial IV, only one week exhibited a significant difference between the treatments (higher mean egg weight in the group with Ca/P reduced feed).

Regarding the weight of hatchlings in trial II, three weeks had a significant difference between the groups (higher mean weight in the group with standard feed). In trial IV, no significant difference was observed for any week all throughout the trial period.

The analyses of egg components revealed significant differences between the treatments according to the percentages of albumen and egg yolk and the calculated value of total Ca in the egg (including the shell).

In trials I and III, significant differences existed between the treatments in the concentration of Ca in manure, with a lower level in the group with Ca/P reduced feed.

This study concluded that a reduction in the dietary levels of Ca from 1.00–1.10% to 0.56–0.80% and of P from 0.48–0.61% to 0.35–0.50% (av. P) or 0.70–0.80% to 0.50–0.60% (total P) in rearing and a reduction of the dietary levels of Ca from 2.90% to 2.80% or 2.60% and of P from 0.36% to 0.30% or 0.24% (av. P) or from 0.65% to 0.50% (total P) in laying in the feed of turkey breeder hens are possible and did not result in disadvantages. As the present study also compared the results with required recommendations and target levels from breeding companies, it was also able to conclude that these levels are obsolete and should be adjusted downwards.

7 Zusammenfassung

Phosphor (P) is ein essentielles Mineral in der Fütterung von landwirtschaftlichen Nutztieren, stammt aber auch aus erschöpflichen Quellen auf der ganzen Welt. Ziel dieser Studie war es daher das Potential einer Reduktion von P in der Fütterung von Putenelterntierhennen auszuloten. Da die Metabolismen von P von Calcium (Ca) sich gegenseitig beeinflussen, wurde auch Ca mit betrachtet. Die Bedürfnisse von P und Ca wurden mittels faktorieller Bedarfsermittlung bestimmt. Dabei stammten die meisten Daten von anderen Geflügelarten, da die Datenlage in Bezug auf Puten und besonders Putenelterntiere stark limitiert ist. Daher versteht sich diese Studie als eine Annäherung und der Versuch neue belastbare Daten zu dieser Thematik zu schaffen.

Die Ergebnisse der faktoriellen Bedarfsanalyse wurden anschließend in ein Futterprogramm für Putenelterntierhennen in Aufzucht- und Legephase eingebettet. Dabei wurde jeweils eine Gruppe der Tiere mit dem praxisüblichen Standardfutter versorgt, eine zweite identische Gruppe bekam ein Futter mit einem reduziertem Ca- und P-Gehalt. Insgesamt wurden vier Versuche durchgeführt: Versuch I und III in der Aufzuchtphase, Versuch II und IV in der Legephase. Alle Versuche wurden unabhängig voneinander betrachtet, Versuch II erhielt dabei jedoch die Tiere aus Versuch I (um mögliche Langzeiteffekte beobachten zu können). In jedem Versuch wurde das Körpergewicht und die Futteraufnahme über den gesamten Versuchszeitraum erfasst. Die Mineralstoffgehalte der Tibia-Knochen wurde an Knochen von gefallenen und notgetöteten Tieren aus Versuch I untersucht. Die Analysen umfassten dabei den Anteil an Knochenasche sowie die Anteile von Ca und P in der Knochenasche. Die Zusammensetzung und Inhaltsstoffe der Eier wurde an Eiern aus Versuch II bestimmt und umfassten die Bestimmung der Anteile von Eiweiß, Eidotter und Eischale sowie Ca im Eiweiß, P im Eiweiß, Ca im Eidotter, P im Eidotter, Wasser in der Eischale, Ca in der Eischale und P in der Eischale. In Versuch II und IV wurde zusätzlich auch noch die Legeleistung, die Eigewichte, die Menge (und Gründe) aussortierter Eier, die Befruchtungsrate, die Schlupfrate und das Gewicht und die Fitness der Küken über die gesamte Versuchsperiode erfasst. Am Ende jedes Versuches wurde der Mist im Hinblick auf seine Konzentration von Trockensubstanz sowie Ca und P analysiert. Anschließend wurde zusätzlich eine Nährstoffbilanz beider Mineralien berechnet um die Ergebnisse einordnen zu können.

Die Gewichtsentwicklung der Hennen zeigte signifikante Unterschiede zwischen beiden Fütterungsgruppen in acht von insgesamt 30 Wochen in Versuch I (fünf mit einem höheren mittleren Körpergewicht in der Gruppe mit Standardfutter, drei mit einem höheren mittleren Körpergewicht in der Gruppe mit Ca- und P-reduziertem Futter), in drei von insgesamt 28 Wochen in Versuch II (zwei mit einem höheren mittleren Körpergewicht in der Gruppe mit Standardfutter, eine mit einem höheren mittleren Körpergewicht in der Gruppe mit Ca- und P-reduziertem Futter), vier von insgesamt 30 Wochen in Versuch III (höheres mittleres Körpergewicht in der Gruppe mit Ca- und P-reduziertem Futter) und zwei von insgesamt 28 Wochen in Versuch IV (höheres mittleres Körpergewicht in der Gruppe mit Ca- und P-reduziertem Futter).

Auch die Futteraufnahme in diesem Versuch zeigte ein unklares Bild. In Versuch I zeigte sich in drei unterschiedlichen Wochen ein signifikanter Unterschied zwischen beiden Fütterungsgruppen (höhere Futteraufnahme in der Gruppe mit Ca- und P-reduziertem Futter). In Versuch II konnte nur in einer Woche ein signifikanter Unterschied gefunden werden (höhere Futteraufnahme in der Gruppe mit Standardfutter). Die Betrachtung aller Versuche zeigte einen Trend zu einer höheren Futteraufnahme in der Gruppe mit Ca- und P-reduziertem Futter.

Die Analysen der Knochenzusammensetzung zeigten keinen Unterschied zwischen beiden Fütterungsgruppen. Auch die Ergebnisse in Bezug auf Legeleistung, Befruchtungsrate und Schlupfrate zeigten keine Unterschiede zwischen den beiden Gruppen.

Die Ergebnisse zum Eigewicht zeigten acht Wochen mit einem signifikanten Unterschied zwischen beiden Fütterungsgruppen (sieben mit einem höheren mittleren Eigewicht in der Gruppe mit Standardfutter, eine mit einem höheren mittleren Eigewicht in der Gruppe mit Ca- und P-reduziertem Futter). In Versuch IV zeigte nur eine Gruppe diesen Unterschied (höheres mittleres Eigewicht in der Gruppe mit Ca- und P-reduziertem Futter).

Das Gewicht der Küken in Versuch II zeigte drei Wochen mit einem signifikanten Unterschied zwischen beiden Fütterungsgruppen (höheres mittleres Kükengewicht in der Gruppe mit Standardfutter). In Versuch IV konnten kein signifikanter Unterschied zwischen den beiden Gruppen beobachtet werden.

Die Analyse der Eiinhaltsttoffe zeigte einen signifikanten Unterschied zwischen beiden Gruppen in Bezug auf den Gehalt von Eiweiß, Eidotter und dem berechneten Gesamtanteil von Ca im Ei (inklusive Eischale).

Versuch I und III zeigten einen signifikanten Unterschied zwischen beiden Fütterungsgruppen bei der Konzentration von Ca im Mist (niedrigere Konzentration in der Gruppe mit Ca- und P-reduziertem Futter).

Es kann geschlussfolgert werden, dass eine Reduktion der Konzentration von Ca von 1,00-1,10% zu 0,56-0,80% und eine Reduktion der Konzentration von P von 0,48-0,61% zu 0,35-0,50% (verfügbarer P) bzw. 0,70-0,80% zu 0,50-0,60% (Gesamt-P) im Futter von Putenhennen im Verlauf der Aufzucht zu keinen Auswirkungen auf die Tiere führte. Auch eine Reduktion der Konzentration von Ca von 2,90% zu 2,80% oder 2,60% und eine Reduktion der Konzentration von P von 0,36% zu 0,30% oder 0,24% (verfügbarer P) bzw. 0,65% zu 0,50% (Gesamt-P) im Futter von Putenelterntierhennen in der Legephase zeigte keine negative Auswirkung auf die Entwicklung und Leistung der Tiere in der vorliegenden Studie. Der Vergleich der vorliegenden Ergebnisse mit Versorgungsempfehlungen und Zielvorgaben von Zuchtunternehmen zeigte, dass diese Werte veraltet sind und einer Überarbeitung und Korrektur hin zu niedrigeren Werten bedürfen.

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Appendix

Table A.- 1: Nutrient concentrations for standard feed and Ca/P reduced feed for all used feed types in trials I and II (target levels)

Feed type	ME (MJ/kg)	Crude protein (%)	Crude fat (%)	Starch (%)	Sugar (%)	Ca (%)	Total P (%)	Av. P[1] (%)
PA 1	11.40	26.59	5.20	28.35	5.12	1.40	1.00	0.64
PA 3	11.51	23.11	4.40	34.45	4.43	1.30	0.95	0.60
PA 4	11.70	18.37	4.02	42.09	3.55	1.10	0.80	0.49
PA 5	11.70	15.20	3.49	46.56	2.96	1.10	0.80	0.48
PA 5 Plus	11.71	15.50	4.80	43.47	3.16	1.05	0.70	0.36
JP 1	11.71	12.20	4.08	48.47	2.58	1.00	0.70	0.37
JP 2	11.71	10.50	4.04	50.35	2.31	1.00	0.70	0.36
Layer standard	11.70	17.00	5.91	39.87	3.26	2.90	0.65	0.37
PA4 P-	11.71	18.20	4.01	42.35	3.58	0.81	0.82	0.50
PA5 P-	11.70	15.20	3.47	46.65	3.07	0.67	0.74	0.42
PA5 Plus P-	11.70	15.50	4.80	43.49	3.26	0.63	0.75	0.41
JP1 P-	11.70	12.20	3.86	48.94	2.64	0.65	0.75	0.40
JP2 P-	11.70	11.18	3.68	50.41	2.50	0.57	0.69	0.40
Layer P-	11.70	17.09	5.69	40.32	3.26	2.80	0.57	0.35
Layer P- II	11.73	17.06	5.41	41.12	3.25	2.61	0.49	0.24

Table A.- 2: Nutrient concentrations for standard feed and Ca/P reduced feed for all used feed types in trials III and IV (target levels)

Feed type	ME (MJ/kg)	Crude protein (%)	Crude fat (%)	Starch (%)	Sugar (%)	Ca (%)	Total P (%)	Av. P[1] (%)
PA 1	11.40	26.59	5.20	28.35	5.12	1.40	1.00	0.64
PA 3	11.51	23.11	4.40	34.45	4.43	1.30	0.95	0.60
PA 4	11.70	18.37	4.02	42.09	3.55	1.10	0.80	0.49
PA 5	11.70	15.20	4.09	44.24	3.13	1.10	0.70	0.34
PA 5 Plus	11.70	15.50	4.50	42.52	3.27	1.05	0.60	0.26
JP 1	11.70	12.20	4.39	46.76	2.72	1.00	0.60	0.25
JP 2	11.70	10.50	4.52	48.07	2.48	1.00	0.60	0.24
Layer standard	11.70	17.00	5.69	39.30	3.26	2.90	0.65	0.32
PA5 P-	11.70	15.20	3.98	45.54	3.14	0.67	0.60	0.28
PA5 Plus P-	11.70	15.50	4.55	42.52	3.35	0.63	0.50	0.17
JP1 P-	11.70	12.20	4.43	46.91	2.78	0.65	0.50	0.17
JP2 P-	11.70	10.50	4.57	48.27	2.56	0.56	0.50	0.16
Layer P-	11.70	17.00	5.71	39.37	3.28	2.80	0.50	0.24

[1] As calculated from the feed mill

Table A.- 3: Ingredients for standard feed and Ca/P reduced feed for all used feed types in trials I and II (target levels)

Ingredients/ Feed type	Wheat (%)	Corn (%)	Soya (%)	Wheat bran (%)	Rapeseed meal (%)	Barley (%)	Ca carbonate (%)	Soya oil (%)	Vegetable fat (%)	MCP (%)	Premix [1] (%)	Luprocid (%)	Soy meal extract (%)	L-Lysine (%)	Methionine (%)	Threonine (%)
PA 1	22.17	20.00	40.14	0.00	0.00	0.00	2.00	1.29	0.00	2.52	1.00	0.50	10.00	0.09	0.22	0.00
PA 3	28.56	25.00	34.38	0.00	0.00	0.00	1.83	1.17	0.00	2.52	1.00	0.50	5.00	0.00	0.13	0.00
PA 4	54.70	14.00	20.49	1.45	0.00	0.00	1.50	1.24	0.00	1.95	1.00	0.50	3.00	0.04	0.12	0.00
PA 5	70.46	7.00	11.64	1.60	2.87	0.00	1.49	1.30	0.00	2.02	1.00	0.50	0.00	0.09	0.04	0.00
PA 5 Plus	58.40	12.00	12.53	6.98	3.00	0.00	1.62	1.00	1.48	1.35	1.00	0.50	0.00	0.04	0.10	0.00
JP 1	70.14	10.50	2.61	7.65	3.00	0.00	1.48	0.74	0.75	1.48	1.00	0.50	0.00	0.10	0.04	0.00
JP 2	76.07	8.00	0.00	8.83	1.00	0.00	1.63	0.50	0.75	1.54	1.00	0.50	0.00	0.13	0.06	0.00
Layer standard	57.21	8.76	19.52	0.00	0.00	0.00	6.59	1.80	1.80	1.37	1.00	0.50	2.00	0.02	0.15	0.03
PA4 P-	54.35	14.00	20.09	3.00	0.00	0.00	0.71	1.20	0.00	1.99	1.00	0.50	3.00	0.04	0.12	0.00
PA5 P-	64.45	12.00	11.21	4.46	3.00	0.00	0.50	1.12	0.00	1.63	1.00	0.50	0.00	0.09	0.04	0.00
PA5 Plus P-	57.81	12.00	11.96	9.23	3.00	0.00	0.43	0.94	1.47	1.50	1.00	0.50	0.00	0.05	0.10	0.00
JP1 P-	69.65	10.50	2.16	9.48	3.00	0.00	0.48	0.69	0.75	1.64	1.00	0.50	0.00	0.11	0.04	0.01
JP2 P-	74.52	8.00	0.00	11.81	1.00	0.00	0.42	0.50	0.75	1.35	1.00	0.50	0.00	0.11	0.04	0.00
Layer P-	57.21	8.76	19.40	0.00	0.00	0.00	6.57	1.80	1.55	1.00	1.00	0.50	2.00	0.02	0.15	0.03
Layer P- II	58.61	8.76	19.00	0.00	0.00	0.00	6.21	1.50	1.55	0.66	1.00	0.50	2.00	0.02	0.15	0.03

[1] Premix: vitamin A (12,000 IU), vitamin D3 (5,000 IU), vitamin E (100 IU) copper sulfate (15 mg), iron (40 mg), manganese (120 mg), zinc oxide (90 mg), selenium (0.20 mg), additional at standard feed: phytase EC 3.1.3.8 (500 FTU)

Table A.- 4: Ingredients for standard feed and Ca/P reduced feed for all used feed types in trials III and IV (target levels)

Ingredients/ Feed type	Wheat (%)	Corn (%)	Soya (%)	Wheat bran (%)	Rapeseed meal (%)	Barley (%)	Ca carbonate (%)	Soya oil (%)	Vegetable fat (%)	MCP (%)	Premix [1] (%)	Luprocid (%)	Soy meal extract (%)	L-Lysine (%)	Methionine (%)	Threonine (%)
PA 1	22.17	20.00	40.14	0.00	0.00	0.00	2.00	1.29	0.00	2.52	1.00	0.50	10.00	0.09	0.22	0.00
PA 3	28.56	25.00	34.38	0.00	0.00	0.00	1.83	1.17	0.00	2.52	1.00	0.50	5.00	0.00	0.13	0.00
PA 4	54.70	14.00	20.49	1.45	0.00	0.00	1.50	1.24	0.00	1.95	1.00	0.50	3.00	0.04	0.12	0.00
PA 5	53.41	18.00	9.91	7.57	5.00	0.00	1.67	1.60	0.00	1.21	1.00	0.50	0.00	0.09	0.04	0.00
PA 5 Plus	49.58	15.00	10.14	9.00	6.00	4.00	1.84	1.20	0.84	0.76	1.00	0.50	0.00	0.06	0.09	0.00
JP 1	56.63	16.00	1.93	13.56	3.00	3.00	1.65	0.90	0.90	0.76	1.00	0.50	0.00	0.10	0.06	0.03
JP 2	50.87	22.00	0.00	16.30	0.00	4.76	1.82	0.80	0.95	0.79	1.00	0.50	0.00	0.14	0.07	0.00
Layer standard	49.10	15.00	17.06	0.00	5.50	0.00	6.70	1.40	2.11	1.08	1.00	0.50	0.34	0.05	0.13	0.02
PA5 P-	53.41	18.00	9.91	8.83	5.00	0.00	0.85	1.60	0.00	0.77	1.00	0.50	0.00	0.09	0.04	0.00
PA5 Plus P-	49.58	15.00	10.14	10.41	6.00	4.00	0.93	1.20	0.84	0.26	1.00	0.50	0.00	0.06	0.09	0.00
JP1 P-	56.63	16.00	1.93	14.62	3.00	3.00	1.03	0.90	0.90	0.33	1.00	0.50	0.00	0.10	0.06	0.03
JP2 P-	50.87	22.00	0.00	17.73	0.00	4.76	0.85	0.80	0.95	0.33	1.00	0.50	0.00	0.14	0.07	0.00
Layer P-	49.10	15.00	17.06	0.46	5.50	0.00	6.70	1.40	2.11	0.63	1.00	0.50	0.34	0.05	0.13	0.02

[1] Premix: vitamin A (12,000 IU), vitamin D3 (5,000 IU), vitamin E (100 IU) copper sulfate (15 mg), iron (40 mg), manganese (120 mg), zinc oxide (90 mg), selenium (0.20 mg), additional at standard and Ca/P reduced feed: phytase EC 3.1.3.8 (500 FTU)

Table A.- 5: Target values for weight development in the rearing period by *Moorgut Kartzfehn Turkey Breeder GmbH* (2020)

Week	Target weight (kg)
7	2.20
8	2.75
9	3.34
10	3.96
11	4.60
12	5.25
13	5.90
14	6.55
15	7.18
16	7.80
17	8.40
18	8.97
19	9.52
20	10.04
21	10.54
22	11.00
23	11.44
24	11.84
25	12.23
26	12.58
27	12.91
28	13.22
29	13.50
30	13.50

Table A.- 6: Target values for laying performance, fertility, and hatchability in the laying period by *Moorgut Kartzfehn Turkey Breeder GmbH* (2020) (PW = production week)

PW	Laying performance (settable eggs) (%)	Fertility (%)	Hatchability (%)
1	49.6	86.3	79.4
2	69.2	91.5	85.1
3	71.2	93.0	86.9
4	70.9	93.4	87.7
5	71.4	93.7	88.3
6	71.9	93.9	88.8
7	72.3	94.0	89.1
8	72.4	94.0	89.1
9	72.2	93.8	88.9
10	71.8	93.7	88.4
11	71.2	93.5	88.0
12	70.5	93.3	87.7
13	69.6	93.2	87.4
14	68.7	93.1	87.2
15	67.7	93.0	87.1
16	66.7	92.8	86.9
17	65.8	92.6	86.6
18	64.9	92.4	86.2
19	64.1	92.1	85.7
20	63.3	91.9	85.2
21	62.6	91.6	84.8
22	61.8	91.4	84.3
23	60.9	91.2	83.8
24	60.0	90.9	83.3
25	59.1	90.6	82.7
26	58.3	90.1	81.9
27	57.6	89.6	80.9
28	56.6	88.8	79.8
29	55.3	88.0	78.6
30	54.0	87.1	77.5

Table A.- 7: Mean differences between the calculated and analyzed levels of Ca and total P in the feed samples (trials I–IV)

Trial	Feed	N	Ca (g/kg) (%)	Total P (g/kg) (%)	Trial	Feed	N	Ca (g/kg) (%)	Total P (g/kg) (%)
Trial I	PA 4	5	+1.1 (+9%)	+0.3 (+4%)	**Trial III**	PA 4	-	---	---
	PA4 P-	4	+2.4 (+23%)	0.0		PA4 P-	-	---	---
	PA 5	3	+0.5 (+4%)	0.0		PA 5	1	+10.7 (+49%)	−0.3 (−4%)
	PA5 P-	3	+3.3 (+33%)	−0.1 (−1%)		PA5 P-	1	+4.3 (+39%)	−0.8 (−15%)
	JP 1	2	−0.6 (−6%)	+0.4 (+5%)		JP 1	1	+0.1 (+1%)	−0.4 (−7%)
	JP1 P-	2	+6.5 (+50%)	−0.5 (−7%)		JP1 P-	1	+4.2 (+39%)	−0.4 (−9%)
	PA5 Plus	2	+2.9 (+31%)	−0.5 (−6%)		PA5 Plus	1	+1.8 (+15%)	+0.9 (+13%)
	PA5 Plus P-	5	+0.1 (+1%)	+0.3 (+4%)		PA5 Plus P-	1	+5.2 (+45%)	+2.2 (+31%)
	JP2	15	+2.0 (+17%)	−0.1 (−2%)		JP2	1	+0.6 (+6%)	−0.8 (−15%)
	JP 2 P-	11	+2.9 (+33%)	+0.1 (+1%)		JP 2 P-	1	+1.4 (+20%)	+2.1 (+30%)
Trial II	Layer standard	10	−1.0 (−4%)	−0.3 (−5%)	**Trial IV**	Layer standard	1	−3.0 (−12%)	−0.6 (−10%)
	Layer P-	9	−3.5 (−14%)	0.0		Layer P-	1	−1.2 (−4%)	+0.7 (+12%)
Ø Trial I			**+2.0 (+19%)**	**0.0**	**Ø Trial III**			**+3.5 (+30%)**	**+0.3 (+5%)**
Ø Trial II			**−2.3 (−9%)**	**−0.2 (−3%)**	**Ø Trial IV**			**−2.1 (−8%)**	**0.0**
Ø Standard feed			**+1.0 (+7%)**	**−0.1 (−1%)**					
Ø Ca/P reduced feed			**+1.1 (+7%)**	**+0.1 (+1%)**					
Ø total			**+1.1 (+7%)**	**0.0**					

Table A.- 8: Analyzed levels of ME and crude protein in the feed samples (trials I–IV)

Trial	Feed	N	ME (MJ/kg) (%)	Crude protein (g/kg) (%)	Trial	Feed	N	ME (MJ/kg) (%)	Crude protein (g/kg) (%)
Trial I	PA 4	5	12.3	169	**Trial III**	PA 4	-	---	---
	PA4 P-	4	12.3	170		PA4 P-	-	---	---
	PA 5	3	12.7	148		PA 5	1	12.1	142
	PA5 P-	3	12.4	140		PA5 P-	1	12.0	153
	JP 1	2	12.4	128		JP 1	1	12.2	125
	JP1 P-	2	12.2	130		JP1 P-	1	12.4	119
	PA5 Plus	2	12.4	148		PA5 Plus	1	12.1	147
	PA5 Plus P-	5	12.3	157		PA5 Plus P-	1	12.1	146
	JP2	15	12.3	115		JP2	1	12.0	98
	JP 2 P-	11	12.4	121		JP 2 P-	1	12.4	110
Trial II	Layer standard	10	12.2	171	**Trial IV**	Layer standard	1	12.2	15.7
	Layer P-	9	12.2	169		Layer P-	1	12.1	16.4

Table A.- 9: Mean differences between the calculated and analyzed levels of ME and crude protein in the feed samples (trials I–IV)

Trial	Feed	N	ME (MJ/kg) (%)	Crude protein (g/kg) (%)	Trial	Feed	N	ME (MJ/kg) (%)	Crude protein (g/kg) (%)
Trial I	PA 4	5	+0.6 (+5%)	−13.4 (−8%)	**Trial III**	PA 4	-	---	---
	PA4 P-	4	+0.6 (+5%)	−12.3 (−7%)		PA4 P-	-	---	---
	PA 5	3	+1.0 (+8%)	−4.0 (−3%)		PA 5	1	+0.4 (+3%)	−10.0 (−7%)
	PA5 P-	3	+0.7 (+5%)	−12.3 (−9%)		PA5 P-	1	+0.3 (+3%)	+1.0 (+1%)
	JP 1	2	+0.7 (+5%)	+6.0 (+5%)		JP 1	1	+0.5 (+4%)	+3.0 (+2%)
	JP1 P-	2	+0.5 (+4%)	+7.5 (+6%)		JP1 P-	1	+0.7 (+6%)	−3.0 (−3%)
	PA5 Plus	2	+0.7 (+5%)	−7.0 (−5%)		PA5 Plus	1	+0.4 (+3%)	−8.0 (−5%)
	PA5 Plus P-	5	+0.6 (+5%)	+1.6 (+1%)		PA5 Plus P-	1	+0.4 (+3%)	−9.0 (−6%)
	JP2	15	+0.6 (+5%)	+5.8 (+5%)		JP2	1	+0.3 (+3%)	−7.0 (−7%)
	JP 2 P-	11	+0.7 (+6%)	+8.9 (+7%)		JP 2 P-	1	+0.7 (+6%)	+5.0 (+5%)
Trial II	Layer standard	10	+0.5 (+4%)	+1.2 (+1%)	**Trial IV**	Layer standard	1	+0.5 (+4%)	−13.0 (+8%)
	Layer P-	9	+0.5 (+4%)	−1.8 (−1%)		Layer P-	1	+0.4 (+3%)	−6.0 (−4%)
Ø Trial I			**+0.7 (+5%)**	**+0.8 (+1%)**	**Ø Trial III**			**+0.5 (+4%)**	**−3.5 (−3%)**
Ø Trial II			**+0.5 (+4%)**	**−0.4 (−0%)**	**Ø Trial IV**			**+0.5 (+4%)**	**−9.5 (−6%)**
Ø Standard feed			**+0.6 (+5%)**	**−0.5 (−0%)**					
Ø Ca/P reduced feed			**+0.6 (+5%)**	**+0.1 (+0%)**					
Ø total			**+0.6 (+5%)**	**−0.1 (0%)**					

Table A.- 10: Weight development by weekly means ± SD of N = 25 weighed hens per barn (trial I) (significant differences per barns are marked with different letters behind the means, significant differences per group with * in the last column)

Week	Comparison by barn				Comparison by group		
	Barn 2A (standard feed)	Barn 2B (standard feed)	Barn 3A (Ca/P reduced feed)	Barn 3B (Ca/P reduced feed)	Ø Barn 2A + 2B (standard feed)	Ø Barn 3A + 3B (Ca/P reduced feed)	Significant difference between groups
7	1.5 ± 0.2	1.6 ± 0.2	1.5 ± 0.2	1.5 ± 0.2	1.5 ± 0.2	1.5 ± 0.2	
8	1.9 ± 0.3	1.8 ± 0.3	1.8 ± 0.2	1.8 ± 0.2	1.8 ± 0.3	1.8 ± 0.2	
9	2.5 ± 0.3	2.4 ± 0.3	2.4 ± 0.3	2.3 ± 0.3	2.5 ± 0.3	2.4 ± 0.3	
10	3.1 ± 0.4	3.1 ± 0.3	3.0 ± 0.3	2.9 ± 0.3	3.1 ± 0.4	3.0 ± 0.3	*
11	3.9 ± 0.4	3.9 ± 0.5	3.8 ± 0.4	3.8 ± 0.5	3.9 ± 0.4	3.8 ± 0.4	*
12	4.5 ± 0.6	4.4 ± 0.4	4.5 ± 0.7	4.4 ± 0.5	4.5 ± 0.5	4.4 ± 0.6	
13	5.0 ± 0.5 [a,b]	5.4 ± 0.5 [a]	4.9 ± 0.5 [b]	5.0 ± 0.5 [a,b]	5.2 ± 0.5	5.0 ± 0.5	*
14	5.6 ± 0.6	5.6 ± 0.6	5.5 ± 0.9	5.7 ± 0.5	5.6 ±0.6	5.6 ± 0.7	
15	6.5 ± 0.4	6.6 ± 0.5	6.4 ± 0.5	6.6 ± 0.6	6.5 ± 0.4	6.5 ± 0.6	
16	7.0 ± 0.9	7.0 ± 0.7	6.8 ± 0.9	7.0 ± 0.8	7.0 ± 0.8	6.9 ± 0.8	
17	7.7 ± 0.8 [a,b]	7.6 ± 0.8 [a]	7.6 ± 0.6 [a]	8.2 ± 0.7 [b]	7.6 ± 0.8	7.9 ± 0.7	
18	8.0 ± 0.6	8.0 ± 0.8	8.2 ± 0.6	8.4 ± 0.5	8.0 ± 0.7	8.3 ± 0.6	*
19	8.7 ± 0.8 [a]	8.8 ± 0.9 [a]	9.6 ± 0.9 [b]	9.5 ± 0.7 [b]	8.8 ± 0.9	9.5 ± 0.8	*
20	9.3 ± 0.8	9.8 ± 0.8	9.6 ± 0.7	9.9 ± 0.9	9.6 ± 0.8	9.8 ± 0.8	
21	10.2 ± 0.6 [a]	9.7 ± 0.6 [b]	10.0 ± 0.8 [a,b]	10.1 ± 0.6 [a,b]	9.9 ± 0.7	10.1 ± 0.7	
22	9.7 ± 0.9 [a]	10.5 ± 0.8 [b]	10.4 ± 0.7 [b]	10.4 ± 0.8 [b]	10.1 ± 0.9	10.4 ± 0.8	
23	10.6 ± 0.8	11.2 ± 0.8	10.9 ± 0.7	11.0 ± 0.8	10.9 ± 0.9	10.9 ± 0.7	
24	11.7 ± 1.0	11.6 ± 0.8	11.7 ± 0.8	11.6 ± 0.9	11.6 ± 0.9	11.7 ± 0.9	
25	12.3 ± 0.7 [a]	12.3 ± 0.6 [a]	11.8 ± 0.4 [b]	12.1 ± 0.5 [a,b]	12.3 ± 0.6	12.0 ± 0.5	*
26	12.0 ± 0.8	12.1 ± 0.7	11.8 ± 0.7	11.9 ± 0.8	12.0 ± 0.7	11.8 ± 0.7	
27	12.8 ± 0.7	12.9 ± 0.8	12.9 ± 0.7	13.1 ± 0.7	12.9 ± 0.7	13.0 ± 0.7	
28	12.7 ± 0.6 [a,b]	13.2 ± 0.6 [a]	12.4 ± 0.9 [b]	12.8 ± 0.9 [a,b]	13.0 ± 0.6	12.6 ± 0.9	*
29	12.9 ± 1.0	13.4 ± 0.7	12.9 ± 0.7	13.3 ± 0.7	13.1 ± 0.9	13.1 ± 0.7	
30	13.2 ± 0.8 [a]	13.3 ± 0.8 [a,b]	13.5 ± 1.2 [b]	13.8 ± 1.2 [a,b]	13.2 ± 0.8	13.7 ± 1.2	*

Table A.- 11: Weekly mean feed intake per barn and per group (kg) (trial I)

Week	Comparison by barn				Comparison by group		
	Barn 2A (standard feed)	**Barn 2B (standard feed)**	**Barn 3A (Ca/P reduced feed)**	**Barn 3B (Ca/P reduced feed)**	**Ø Barn 2A + 2B (standard feed)**	**Ø Barn 3A + 3B (Ca/P reduced feed)**	**Significant difference between groups**
7	0.13	0.11	0.12	0.14	0.12	0.13	
8	0.16	0.15	0.14	0.16	0.15	0.15	
9	0.20	0.19	0.18	0.20	0.19	0.19	
10	0.22	0.21	0.19	0.22	0.21	0.21	
11	0.27	0.24	0.22	0.24	0.25	0.23	
12	0.25	0.23	0.24	0.28	0.24	0.26	
13	0.26	0.23	0.27	0.30	0.25	0.28	
14	0.31	0.29	0.33	0.33	0.30	0.33	
15	0.32	0.30	0.32	0.32	0.31	0.32	
16	0.33	0.30	0.37	0.35	0.31	0.36	
17	0.37	0.34	0.42	0.40	0.35	0.41	*
18	0.40	0.37	0.43	0.41	0.38	0.42	*
19	0.34	0.32	0.36	0.34	0.33	0.35	
20	0.32	0.29	0.35	0.33	0.30	0.34	*
21	0.33	0.31	0.35	0.32	0.32	0.33	
22	0.33	0.32	0.38	0.36	0.32	0.37	
23	0.35	0.35	0.40	0.35	0.35	0.38	
24	0.37	0.34	0.39	0.35	0.37	0.37	
25	0.38	0.34	0.41	0.36	0.38	0.38	
26	0.35	0.33	0.41	0.34	0.37	0.37	
27	0.36	0.31	0.40	0.33	0.37	0.37	
28	0.39	0.34	0.41	0.37	0.39	0.39	
29	0.37	0.31	0.36	0.34	0.34	0.34	

Table A.- 12: Analyzed content of manure in trial I (original single values)

Barn (group)	Location of extraction	Dry matter (%)	P_2O_5 (%)	CaO (%)	P_2O_5 (%) (related to dry matter)	CaO (%) (related to dry matter)
2A (standard feed)	front/left	71.21	3.28	3.35	4.61	4.71
	front/middle	71.51	4.18	3.69	5.85	5.16
	front/right	65.22	2.25	4.61	3.45	7.07
	middle/left	67.92	3.01	2.91	4.43	4.27
	middle/middle	69.52	3.08	2.84	4.44	4.09
	middle/right	72.10	3.69	3.43	5.12	4.75
	back/left	63.04	2.68	2.53	4.25	4.01
	back/middle	73.26	2.79	3.22	3.81	4.40
	back/right	68.27	3.73	3.58	5.46	5.24
	Average	**69.12**	**3.19**	**3.35**	**4.60**	**4.86**
2B (standard feed)	front/left	63.58	2.97	2.86	4.67	4.50
	front/middle	43.39	1.50	1.49	3.45	3.44
	front/right	63.74	2.26	2.08	3.55	3.26
	middle/left	67.49	4.16	4.28	6.17	6.34
	middle/middle	72.35	3.55	3.20	4.91	4.42
	middle/right	70.55	3.08	2.82	4.29	3.92
	back/left	77.27	3.92	3.65	5.08	4.73
	back/middle	82.59	2.89	3.22	3.50	3.90
	back/right	75.55	3.42	3.17	4.41	4.08
	Average	**68.50**	**3.08**	**2.97**	**4.45**	**4.29**
3A (Ca/P reduced feed)	front/left	79.12	3.11	2.07	3.94	2.61
	front/middle	71.15	1.99	1.69	2.80	2.37
	front/right	65.42	4.30	2.74	6.57	4.18
	middle/left	80.00	0.79	0.85	0.99	1.07
	middle/middle	72.20	3.58	3.07	4.95	4.25
	middle/right	59.50	2.38	2.05	4.00	3.44
	back/left	72.55	3.18	5.25	4.38	7.24
	back/middle	68.14	3.30	2.41	4.84	3.53
	back/right	63.67	4.81	2.98	7.56	4.68
	Average	**70.19**	**3.05**	**2.57**	**4.45**	**3.71**
3B (Ca/P reduced feed)	front/left	75.43	4.28	2.62	5.67	3.47
	front/middle	54.56	2.3	2.41	4.22	4.41
	front/right	69.23	4.17	2.55	6.03	3.68
	middle/left	69.86	4.06	2.81	5.82	4.02
	middle/middle	70.87	2.34	1.61	3.31	2.27
	middle/right	54.09	2.85	2.19	5.27	4.05
	back/left	58.17	2.42	1.72	4.16	2.95
	back/middle	66.9	2.70	1.68	4.03	2.52
	back/right	73.68	2.90	2.64	3.93	3.58
	Average	**65.87**	**3.11**	**2.25**	**4.72**	**3.44**

Table A.- 13: Boxplot elements for the analyses of P in manure in trial I (related to dry matter; N = 9 per barn)

	2A	2B	3A	3B	Standard feed	Ca/P reduced feed
Minimum (%)	1.5	1.5	0.4	1.4	1.5	0.4
1st Quantile (%)	1.9	1.6	1.7	1.8	1.7	1.7
Median (%)	1.9	1.9	1.9	1.8	1.9	1.9
3rd Quantile (%)	2.2	2.1	2.2	2.5	2.2	2.4
Maximum (%)	2.6	2.7	3.3	2.6	2.7	3.3

Table A.- 14: Boxplot elements for the analyses of Ca in manure in trial I (related to dry matter; N = 9 per barn)

	2A	2B	3A	3B	Standard feed	Ca/P reduced feed
Minimum (%)	2.9	2.3	0.8	1.6	2.3	0.8
1st Quantile (%)	3.1	2.8	1.9	2.1	2.9	1.9
Median (%)	3.4	2.9	2.5	2.6	3.1	2.5
3rd Quantile (%)	3.7	3.2	3.0	2.9	3.4	3.0
Maximum (%)	5.1	4.5	5.2	3.1	5.1	5.2

Table A.- 15: Boxplot elements for the analyses of bone ash (N = 24 per group with standard feed, N = 29 per group with Ca/P reduced feed)

	Bone ash (g)		P in bone ash (%)		Ca in bone ash (%)	
	Standard feed	**Ca/P reduced feed**	**Standard feed**	**Ca/P reduced feed**	**Standard feed**	**Ca/P reduced feed**
Minimum	51.3	52.5	16.2	16.6	32.6	34.5
1st Quantile	55.9	57.5	17.1	17.2	34.2	34.9
Median	59.6	60.2	17.3	17.2	34.8	35.4
3rd Quantile	61.3	61.5	17.4	17.4	35.8	35.6
Maximum	62.7	64.0	17.8	17.9	36.5	36.6

Table A.- 16: Weight development by weekly means ± SD of N = 50 weighed hens per barn (trial II) (significant differences per barn are marked with different letters behind the means, while significant differences per group are marked with * in the last column)

PW	Comparison by barn				Comparison by group		
	Barn 1 (standard feed)	Barn 2 (standard feed)	Barn 3 (Ca/P reduced feed)	Barn 4 (Ca/P reduced feed)	Ø Barn 1+2 (standard feed)	Ø Barn 3+4 (Ca/P reduced feed)	Significant difference between groups
1	13.2 ± 0.7	13.2 ± 0.8	13.2 ± 0.8	13.0 ± 0.7	13.2 ± 0.8	13.1 ± 0.8	
2	13.2 ± 0.7	13.1 ± 0.8	13.3 ± 0.8	13.1 ± 0.7	13.2 ± 0.7	13.2 ± 0.7	
3	13.0 ± 0.9	12.6 ± 0.9	13.0 ± 0.8	12.7 ± 0.7	12.8 ± 0.9	12.9 ± 0.8	
4	12.5 ± 0.8	12.4 ± 0.8	12.9 ± 0.8	12.6 ± 1.0	12.5 ± 0.8	12.7 ± 0.9	*
5	12.5 ± 1.6	12.5 ± 1.2	12.1 ± 0.8	12.2 ± 1.0	12.5 ± 1.4	12.2 ± 0.9	*
6	12.5 ± 1.0	12.1 ± 0.9	12.7 ± 0.8	12.2 ± 0.8	12.3 ± 0.9	12.4 ± 0.8	
7	12.3 ± 0.7	12.1 ± 0.8	12.2 ± 0.9	12.4 ± 0.6	12.2 ± 0.7	12.3 ± 0.8	
8	12.3 ± 0.8	12.0 ± 0.7	12.1 ± 0.7	12.1 ± 0.7	12.1 ± 0.8	12.1 ± 0.7	
9	12.1 ± 0.8	12.1 ± 0.8	12.0 ± 0.9	11.8 ± 0.7	12.1 ± 0.8	11.9 ± 0.8	
10	11.8 ± 1.0	11.9 ± 0.7	12.0 ± 0.9	11.6 ± 0.8	11.8 ± 0.9	11.8 ± 0.9	
11	12.1 ± 0.9	11.9 ± 0.6	12.2 ± 0.7	11.6 ± 0.7	12.0 ±0.8	11.9 ± 0.7	
12	12.1 ± 0.8	12.0 ± 0.7	12.2 ± 0.6	12.1 ± 0.9	12.0 ± 0.8	12.1 ± 0.8	
13	11.9 ± 0.9	11.9 ± 0.9	12.2 ± 0.8	11.8 ± 0.8	11.9 ± 0.9	12.0 ± 0.8	
14	11.9 ± 0.8	11.9 ± 0.9	11.9 ± 1.0	11.9 ± 0.8	11.9 ± 0.9	11.9 ± 0.9	
15	12.1 ± 1.0	12.3 ± 1.0	12.4 ± 1.0	12.1 ± 0.7	12.2 ± 1.0	12.2 ± 0.9	
16	12.0 ± 0.8	12.2 ± 1.0	12.2 ± 0.8	12.1 ± 1.1	12.1 ± 0.9	12.14 ± 1.0	
17	12.2 ± 1.1	12.5 ± 0.9	12.4 ± 0.9	12.1 ± 1.0	12.4 ± 1.0	12.2 ± 1.0	
18	12.3 ± 0.8 $^{a.b}$	12.0 ± 0.8 ^{a}	12.4 ± 0.8 $^{a.b}$	12.6 ± 0.8 ^{b}	12.2 ± 0.8	12.5 ± 0.8	*
19	12.3 ± 0.7	12.4 ± 0.9	12.3 ± 0.8	12.3 ± 0.7	12.3 ± 0.8	12.3 ± 0.7	
20	12.3 ± 0.8	12.6 ± 1.2	12.4 ± 0.9	12.6 ± 0.9	12.5 ± 1.0	12.5 ± 0.9	
21	12.6 ± 0.9	12.5 ± 1.0	12.6 ± 0.9	12.7 ± 0.9	12.5 ± 0.9	12.6 ± 0.9	
22	12.6 ± 1.1	12.5 ± 0.8	12.7 ± 0.9	12.8 ± 0.8	12.6 ± 1.0	12.7 ± 0.9	
23	12.9 ± 0.8	12.5 ± 1.0	12.8 ± 0.9	12.9 ± 1.0	12.7 ± 0.9	12.8 ± 0.9	
24	12.6 ± 1.1	12.7 ± 0.9	12.8 ± 1.1	12.7 ± 0.9	12.7 ± 1.0	12.7 ± 1.0	

Table A.- 17: Weekly mean feed intake per barn and per group (kg) (trial II)

	Comparison by barn					Comparison by group	
PW	**Barn 1 (standard feed)**	**Barn 2 (standard feed)**	**Barn 3 (Ca/P reduced feed)**	**Barn 4 (Ca/P reduced feed)**	**Ø Barn 1+2 (standard feed)**	**Ø Barn 3+4 (Ca/P reduced feed)**	**Significant difference between groups**
1	0.25	0.25	0.24	0.24	0.25	0.24	
2	0.24	0.25	0.24	0.25	0.25	0.25	
3	0.22	0.24	0.23	0.24	0.23	0.23	
4	0.22	0.22	0.22	0.22	0.22	0.22	
5	0.23	0.24	0.23	0.23	0.23	0.23	
6	0.23	0.24	0.23	0.23	0.24	0.23	
7	0.25	0.25	0.24	0.24	0.25	0.24	
8	0.23	0.25	0.25	0.25	0.24	0.25	
9	0.26	0.27	0.25	0.25	0.26	0.25	
10	0.27	0.27	0.26	0.26	0.27	0.26	
11	0.27	0.28	0.26	0.27	0.28	0.26	
12	0.29	0.30	0.28	0.28	0.29	0.28	
13	0.30	0.30	0.29	0.30	0.30	0.29	
14	0.30	0.30	0.30	0.30	0.30	0.30	
15	0.29	0.31	0.29	0.29	0.30	0.29	
16	0.30	0.31	0.30	0.30	0.30	0.30	
17	0.30	0.31	0.31	0.30	0.31	0.30	
18	0.29	0.29	0.30	0.29	0.29	0.30	
19	0.31	0.31	0.29	0.30	0.31	0.30	
20	0.31	0.31	0.31	0.30	0.31	0.31	
21	0.31	0.32	0.31	0.31	0.32	0.31	
22	0.31	0.30	0.31	0.30	0.31	0.30	
23	0.32	0.31	0.28	0.30	0.32	0.29	*
24	0.31	0.30	0.30	0.29	0.31	0.30	

Table A.- 18: Development of net laying performance (settable eggs) as weekly averages (trial II)

	Comparison per barn				**Comparison by group**	
PW	**Barn 1 (standard feed)**	**Barn 2 (standard feed)**	**Barn 3 (Ca/P reduced feed)**	**Barn 4 (Ca/P reduced feed)**	**Ø Barn 1+2 (standard feed)**	**Ø Barn 3+4 (Ca/P reduced feed)**
1	51.2	54.4	49.3	51.5	52.8	50.4
2	70.8	73.7	70.1	72.1	72.3	71.1
3	70.6	75.3	73.9	67.1	73.0	70.5
4	70.2	69.8	69.4	67.9	70.0	68.6
5	69.0	71.6	68.6	68.7	70.3	68.7
6	69.2	72.6	71.1	70.8	70.9	70.9
7	72.1	75.3	72.5	72.7	73.7	72.6
8	71.9	76.3	72.4	73.6	74.1	73.0
9	72.6	76.0	72.2	73.6	74.3	72.9
10	73.1	75.7	72.4	73.1	74.4	72.7
11	71.4	75.8	72.7	72.3	73.6	72.5
12	70.6	74.7	71.5	71.8	72.6	71.7
13	70.5	74.7	71.7	72.1	72.6	71.9
14	69.3	73.7	70.1	70.7	71.5	70.4
15	68.3	72.1	70.5	69.8	70.2	70.1
16	68.4	71.7	69.3	69.8	70.0	69.5
17	67.0	71.5	68.3	69.2	69.3	68.8
18	67.4	69.8	67.9	68.2	68.6	68.0
19	68.4	69.8	69.2	69.4	69.1	69.3
20	67.6	70.6	68.2	68.9	69.1	68.6
21	66.3	70.7	67.1	68.2	68.5	67.7
22	65.4	67.7	65.2	66.8	66.6	66.0
23	65.6	64.8	59.1	65.1	65.2	62.1
24	63.8	66.7	52.9	63.0	65.2	57.9

Table A.- 19: Development of total laying performance as weekly averages (trial II)

	Comparison by barn				Comparison by group	
PW	**Barn 1 (standard feed)**	**Barn 2 (standard feed)**	**Barn 3 (Ca/P reduced feed)**	**Barn 4 (Ca/P reduced feed)**	**Ø Barn 1+2 (standard feed)**	**Ø Barn 3+4 (Ca/P reduced feed)**
1	76.0	77.3	77.2	77.1	76.6	77.2
2	81.5	84.0	81.7	83.1	82.8	82.4
3	80.2	82.1	81.1	80.6	81.1	80.8
4	76.2	76.8	75.5	74.8	76.5	75.1
5	74.7	76.9	74.2	74.7	75.8	74.4
6	74.8	76.6	75.7	75.9	75.7	75.8
7	75.8	79.2	75.9	76.5	77.5	76.2
8	75.8	79.2	75.7	76.9	77.5	76.3
9	75.2	78.5	75.4	76.3	76.9	75.9
10	75.2	78.2	75.1	75.1	76.7	75.1
11	73.8	77.9	74.8	74.7	75.8	74.7
12	72.7	76.5	73.7	74.1	74.6	73.9
13	72.7	76.5	73.5	74.2	74.6	73.8
14	71.1	75.2	72.2	73.1	73.2	72.6
15	70.9	73.4	72.3	72.0	72.2	72.1
16	70.3	73.3	71.2	71.6	71.8	71.4
17	68.8	73.1	70.2	71.1	70.9	70.6
18	69.6	72.2	71.0	70.1	70.9	70.5
19	70.4	72.4	71.5	71.7	71.4	71.6
20	70.6	72.8	70.5	71.5	71.7	71.0
21	68.5	72.7	69.1	70.7	70.6	69.9
22	68.1	70.1	67.8	69.3	69.1	68.6
23	67.9	67.3	61.5	67.7	67.6	64.6
24	66.1	68.6	54.9	65.3	67.3	60.1

Table A.- 20: Egg weight development by weekly means ± SD of N = 150 weighed eggs per barn (trial II) (significant differences per barn are marked with different letters behind the means, while significant differences per group are marked with * in the last column)

PW	Comparison by barn				Comparison by group		
	Barn 1 (standard feed)	**Barn 2 (standard feed)**	**Barn 3 (Ca/P reduced feed)**	**Barn 4 (Ca/P reduced feed)**	**Ø Barn 1+2 (standard feed)**	**Ø Barn 3+4 (Ca/P reduced feed)**	**Significant difference between groups**
1	83.5 ± 5.1	83.9 ± 5.3	83.4 ± 5.4	83.6 ± 6.2	83.7 ± 5.2	83.5 ± 5.8	
2	85.7 ± 4.7	86.5 ± 5.0	85.1 ± 4.9	85.6 ± 4.8	86.1 ± 4.8	85. ± 4.8	
3	86.5 ± 5.2	87.5 ± 5.4	87.0 ± 5.3	87.0 ± 5.2	87.0 ± 5.3	87.0 ± 5.3	
4	87.4 ± 5.5	87.9 ± 5.7	87.3 ± 4.9	86.7 ± 5.2	87.6 ± 5.6	87.0 ± 5.1	
5	87.5 ± 5.6	87.0 ± 4.9	86.7 ± 5.0	86.8 ± 4.9	87.2 ± 5.3	86.7 ± 5.0	
6	89.1 ± 5.1 [a]	87.8 ± 4.8 [a.b]	87.0 ± 4.7 [b]	88.0 ± 5.5 [a.b]	88.4 ± 5.0	87.5 ± 5.1	*
7	88.9 ± 5.7	88.9 ± 5.2	88.0 ± 5.0	88.0 ± 5.3	88.9 ± 5.4	88.0 ± 5.1	*
8	90.6 ± 5.7 [a]	87.9 ±5.4 [b]	88.9 ± 5.2 [b]	88.3 ± 5.1 [b]	89.3 ± 5.3	88.6 ± 5.3	
9	89.5 ± 5.4	89.9 ± 5.2	89.4 ± 5.4	90.0 ± 5.1	89.7 ± 5.7	89.7 ± 5.2	
10	91.1 ± 6.2	90.8 ± 6.0	89.8 ± 5.5	90.6 ± 5.2	90.9 ± 6.1	90.2 ± 5.3	
11	93.0 ± 6.1 [a]	92.3 ± 5.4 [a.b]	91.3 ± 5.2 [b]	91.2 ± 5.4 [b]	92.7 ± 5.7	91.2 ± 5.3	*
12	91.6 ± 5.0 [a.b]	93.1 ± 4.8 [a]	91.0 ± 5.5 [b]	90.8 ± 5.1 [b]	92.3 ± 5.0	90.9 ± 5.3	*
13	94.5 ± 6.0	95.3 ± 6.2	94.8 ± 5.6	94.8 ± 5.5	94.9 ± 6.1	94.8 ± 5.6	
14	94.7 ± 5.9 [a]	95.0 ± 5.9 [a]	92.1 ± 5.4 [b]	93.6 ± 6.0 [a.b]	94.8 ± 5.9	92.9 ± 5.8	*
15	95.2 ± 5.4 [a.b]	95.9 ± 6.1 [a]	94.1 ± 6.0 [b.c]	93.3 ± 5.8 [c]	95.6 ± 5.7	93.7 ± 5.9	*
16	94.0 ± 6.4	95.3 ± 5.7	94.2 ± 6.1	95.1 ± 5.8	94.6 ± 5.7	94.7 ± 5.9	
17	95.1 ± 6.2	96.7 ± 6.2	95.6 ± 5.1	95.0 ± 5.9	95.9 ± 6.2	95.3 ± 5.5	
18	95.2 ± 5.4	96.0 ± 6.0	96.3 ± 5.9	96.0 ± 6.0	95.6 ± 5.7	96.1 ± 5.9	
19	96.7 ± 6.1	96.2 ± 5.9	97.2 ± 5.8	95.9 ± 5.5	96.4 ± 6.0	96.6 ± 5.7	
20	96.9 ± 6.3	97.9 ± 6.7	97.1 ± 6.4	96.9 ± 5.4	97.4 ± 6.5	97.0 ± 5.9	
21	95.5 ± 5.6 [a]	96.9 ± 5.9 [a.b]	97.2 ± 5.3 [b]	97.8 ± 5.6 [a.b]	96.2 ± 5.8	97.5 ± 5.4	
22	99.0 ± 5.7 [a]	97.7 ± 5.9 [a.b]	97.9 ± 5.5 [a.b]	97.2 ± 5.7 [b]	98.3 ± 5.8	97.5 ± 5.6	
23	98.6 ± 6.1	97.5 ± 5.6	98.7 ± 5.5	98.8 ± 5.8	98.1 ± 5.8	98.8 ± 5.7	
24	98.8 ± 5.9	98.8 ± 6.0	97.8 ± 6.2	97.3 ± 5.7	98.8 ± 5.9	97.5 ± 5.9	*

Table A.- 21: Development of the percentage of dead embryos at screening (10–12 days after being placed in an incubator) in trial II (weekly averages)

PW	Standard feed (barn 1 + 2) (%)	Ca/P reduced feed (barn 3 + 4) (%)
1	4.5	4.3
2	3.7	3.3
3	3.0	3.0
4	3.0	3.0
5	2.5	2.3
6	2.6	2.2
7	2.6	2.7
8	3.0	2.8
9	2.8	3.5
10	2.4	2.6
11	3.1	3.3
12	3.1	3.2
13	3.2	3.0
14	2.7	2.8
15	3.1	2.7
16	2.6	2.8
17	2.9	3.0
18	3.2	3.2
19	3.5	3.5
20	3.2	3.0
21	4.0	3.8
22	4.1	5.2
23	5.8	5.3
24	4.0	4.7

Table A.- 22: Development of fertility of group with standard feed and group with Ca/P reduced feed in trial II (weekly averages)

PW	Standard feed (barn 1 + 2) (%)	Ca/P reduced feed (barn 3 + 4) (%)
1	82.8	88.5
2	90.1	92.0
3	92.5	92.8
4	93.6	93.8
5	93.9	94.1
6	94.4	94.2
7	94.4	94.7
8	94.4	94.5
9	94.4	93.3
10	93.4	93.4
11	93.2	93.0
12	93.1	93.1
13	93.2	94.2
14	94.2	93.9
15	93.9	94.2
16	94.6	94.5
17	93.9	94.1
18	93.9	93.7
19	93.0	93.5
20	93.2	93.3
21	92.4	92.8
22	92.0	90.7
23	90.6	90.2
24	91.4	90.3

Table A.- 23: Development of hatchability of group with standard feed and group with Ca/P reduced feed in trial II (weekly averages)

PW	Standard feed (barn 1 + 2) (%)	Ca/P reduced feed (barn 3 + 4) (%)
1	76.3	80.6
2	85.9	85.5
3	88.1	87.6
4	89.1	89.2
5	89.4	89.5
6	89.5	89.9
7	90.1	89.4
8	90.3	91.2
9	87.2	86.7
10	89.6	89.2
11	87.6	86.0
12	86.0	86.6
13	87.6	87.1
14	89.1	88.8
15	88.3	88.7
16	88.8	88.4
17	87.2	88.6
18	87.4	87.8
19	86.2	87.0
20	85.8	85.4
21	83.5	85.3
22	82.9	82.5
23	82.9	81.4
24	82.4	77.1

Table A.- 24: Development of the mean weight ± SD of N = 200 progeny per group per week (trial II) (significant differences are marked with * in the last column)

PW	Standard feed (barn 1 + 2) (g)	Ca/P reduced feed (barn 3 + 4) (g)	Significant difference
1	55.9 ± 3.1	55.5 ± 3.1	
2	56.2 ± 3.6	56.6 ± 3.5	
3	58.0 ± 3.9	58.1 ± 4.0	
4	57.6 ± 4.2	57.0 ± 3.8	
5	58.0 ± 4.4	58.0 ± 4.4	
6	59.3 ± 4.0	58.9 ± 4.2	
7	58.2 ± 4.3	57.6 ± 4.2	
8	60.4 ± 3.9	60.0 ± 3.9	
9	60.3 ± 4.2	59.9 ± 4.3	
10	62.1 ± 4.5	61.5 ± 4.8	
11	61.4 ± 4.8	61.4 ± 4.3	
12	61.0 ± 4.4	61.5 ± 4.8	
13	62.0 ± 4.3	61.7 ± 4.3	
14	61.6 ± 4.9	61.6 ± 4.2	
15	62.5 ± 4.1	62.0 ± 4.1	
16	62.9 ± 4.7	62.3 ± 4.1	
17	64.6 ± 4.4	63.4 ± 4.4	*
18	64.4 ± 4.5	61.9 ± 4.3	*
19	65.1 ± 5.1	64.8 ± 4.6	
20	65.8 ± 5.1	66.0 ± 4.7	
21	65.6 ± 4.6	65.4 ± 4.7	
22	67.0 ± 5.1	64.9 ± 4.8	*
23	66.3 ± 4.4	65.9 ± 4.7	
24	65.7 ± 4.3	66.2 ± 4.7	

Table A.- 25: Analyzed content of manure in trial II (single values)

Barn (group)	Location of ex-traction	Dry matter (%)	P_2O_5 (%)	CaO (%)	P_2O_5 (%) (related to dry matter)	CaO (%) (related to dry matter)
1 (standard feed)	Right side 1	59.75	1.66	3.64	2.78	6.09
	Right side 2	49.09	1.50	3.23	3.06	6.58
	Right side 3	51.00	1.69	3.89	3.31	7.62
	Right side 4	51.01	1.17	3.04	2.30	5.95
	Right side 5	59.89	2.21	4.82	3.69	8.05
	Right side 6	41.79	1.64	3.52	3.92	8.42
	Right side 7	56.97	2.26	4.65	3.97	8.17
	Right side 8	52.41	1.81	4.09	3.46	7.80
	Right side 9	51.45	1.84	3.84	3.58	7.47
	Right side 10	53.12	2.31	4.45	4.35	8.39
	Left side 1	48.18	1.74	3.72	3.60	7.72
	Left side 2	46.88	1.04	2.36	2.23	5.04
	Left side 3	56.07	1.41	2.94	2.51	5.25
	Left side 4	52.09	1.42	3.09	2.73	5.92
	Left side 5	51.37	1.50	3.00	2.93	5.86
	Left side 6	47.70	1.84	3.68	3.87	8.14
	Left side 7	55.74	1.84	4.03	3.3	7.23
	Left side 8	52.33	1.79	4.18	3.41	8.00
	Left side 9	53.1	1.84	3.75	3.47	7.07
	Left side 10	44.74	1.58	3.19	3.54	7.13
	Average	51.73	1.70	3.66	3.30	7.10
2 (standard feed)	Right side 1	44.81	1.45	2.94	3.23	6.56
	Right side 2	43.38	1.38	3.49	3.19	8.05
	Right side 3	45.13	1.93	3.71	4.28	8.22
	Right side 4	53.30	1.90	4.30	3.56	8.08
	Right side 5	42.22	0.98	2.40	2.31	5.69
	Right side 6	61.65	1.57	3.33	2.55	5.40
	Right side 7	50.82	1.53	3.60	3.02	7.09
	Right side 8	44.29	0.62	4.10	1.41	3.17
	Right side 9	46.34	1.86	3.70	4.00	7.97
	Right side 10	54.02	1.60	3.33	2.96	6.16
	Left side 1	45.24	1.56	3.45	3.45	7.63
	Left side 2	49.61	1.15	2.91	2.33	5.86
	Left side 3	34.59	0.40	0.99	1.17	2.87
	Left side 4	39.54	1.54	3.24	3.89	8.18
	Left side 5	40.35	1.59	3.88	3.95	9.62
	Left side 6	44.55	0.97	2.18	2.18	4.90
	Left side 7	53.76	2.20	4.86	4.10	9.04
	Left side 8	53.01	1.48	3.31	2.79	6.25
	Left side 9	34.95	1.54	3.01	4.40	8.61
	Left side 10	34.55	1.26	2.73	3.65	7.89
	Average	45.81	1.43	3.27	3.12	6.86

Barn (group)	Location of extraction	Dry matter (%)	P_2O_5 (%)	CaO (%)	P_2O_5 (%) (related to dry matter)	CaO (%) (related to dry matter)
3	Right side 1	47.33	1.69	3.69	3.56	7.80
(Ca/P reduced feed)	Right side 2	54.47	1.86	3.54	3.42	6.50
	Right side 3	50.74	1.88	3.97	3.70	7.83
	Right side 4	51.63	1.77	3.70	3.42	7.17
	Right side 5	49.54	1.29	2.81	2.60	5.67
	Right side 6	60.06	1.63	3.53	2.71	5.88
	Right side 7	46.92	0.73	1.89	1.55	4.01
	Right side 8	56.37	2.07	4.22	3.68	7.49
	Right side 9	46.13	1.47	3.50	3.20	7.58
	Right side 10	54.08	1.94	4.08	3.59	7.54
	Left side 1	35.43	1.13	2.76	3.20	7.79
	Left side 2	67.14	2.15	4.32	3.20	6.43
	Left side 3	60.67	1.95	3.64	3.21	6.00
	Left side 4	46.90	1.74	3.82	3.71	8.15
	Left side 5	45.53	1.51	3.62	3.33	7.96
	Left side 6	45.67	1.47	3.51	3.22	7.69
	Left side 7	44.30	1.65	3.62	3.72	8.17
	Left side 8	40.38	1.08	2.49	2.68	6.17
	Left side 9	51.61	2.23	5.01	4.32	9.70
	Left side 10	39.09	0.59	1.37	1.50	3.51
	Average	49.70	1.59	3.45	3.18	6.95
4	Right side 1	41.59	1.61	3.50	3.88	8.42
(Ca/P reduced feed)	Right side 2	56.47	1.27	3.82	2.25	6.76
	Right side 3	58.28	1.89	3.96	3.24	6.80
	Right side 4	61.60	1.90	4.01	3.09	6.53
	Right side 5	52.70	1.30	2.45	2.47	4.65
	Right side 6	55.86	2.08	4.31	3.73	7.72
	Right side 7	59.15	1.40	2.79	2.37	4.72
	Right side 8	51.49	1.67	3.76	3.25	7.30
	Right side 9	57.37	2.19	4.46	3.81	7.78
	Right side 10	60.37	2.27	4.66	3.76	7.72
	Left side 1	43.20	1.59	3.48	3.67	8.06
	Left side 2	51.81	2.12	4.36	4.09	8.42
	Left side 3	49.03	1.23	2.82	2.51	5.75
	Left side 4	50.54	1.51	3.32	2.98	6.57
	Left side 5	48.00	1.57	3.80	3.26	7.92
	Left side 6	55.73	2.00	3.94	3.59	7.07
	Left side 7	54.52	2.01	5.43	3.68	9.96
	Left side 8	39.70	1.40	3.19	3.52	8.04
	Left side 9	62.84	1.79	3.66	2.84	5.82
	Left side 10	52.72	1.79	3.95	3.40	7.49
	Average	53.15	1.73	3.78	3.27	7.18

Table A.- 26: Boxplot elements for the analyses of P in manure in trial II (related to dry matter; N = 20 per barn)

	Barn 1 (standard feed)	Barn 2 (standard feed)	Barn 3 (Ca/P reduced feed)	Barn 4 (Ca/P reduced feed)	Standard feed	Ca/P reduced feed
Minimum (%)	1.0	0.5	0.7	1.0	0.5	0.7
1st Quantile (%)	1.3	1.1	1.3	1.3	1.2	1.3
Median (%)	1.5	1.4	1.4	1.5	1.5	1.4
3rd Quantile (%)	1.6	1.7	1.6	1.6	1.6	1.6
Maximum (%)	1.9	1.9	1.9	1.8	1.9	1.9

Table A.- 27: Boxplot elements for the analyses of Ca in manure in trial II (related to dry matter; N = 20 per barn)

	Barn 1 (standard feed)	Barn 2 (standard feed)	Barn 3 (Ca/P reduced feed)	Barn 4 (Ca/P reduced feed)	Standard feed	Ca/P reduced feed
Minimum (%)	3.6	2.1	2.5	3.3	2.1	2.5
1st Quantile (%)	4.3	4.2	4.4	4.7	4.2	4.5
Median (%)	5.2	5.2	5.4	5.3	5.3	5.4
3rd Quantile (%)	5.7	5.8	5.6	5.7	5.8	5.6
Maximum (%)	6.0	6.9	6.9	7.1	6.9	7.1

Table A.- 28: Weight development by weekly means ± SD of N = 36 weighed hens per barn (trial III;) (significant differences per group with * in the last column)

Week	Barn 2 (standard feed)	Barn 3 (Ca/P reduced feed)	Significant difference per group
12	5.3 ± 0.4	5.5 ± 0.5	*
13	5.7 ± 0.5	5.9 ± 0.5	
14	6.5 ± 0.6	6.7 ± 0.6	
15	7.1 ± 0.7	7.2 ± 0.6	
16	7.8 ± 0.6	7.7 ± 0.6	
17	7.9 ± 0.8	8.2 ± 0.7	
18	8.3 ± 0.9	8.6 ± 0.7	
19	9.0 ± 0.7	9.2 ± 0.6	
20	9.4 ± 0.8	9.7 ± 0.8	
21	10.2 ± 0.6	10.6 ± 0.6	*
22	10.6 ± 0.5	10.7 ± 0.7	
23	11.2 ± 0.6	11.5 ± 0.9	
24	11.3 ± 0.7	11.9 ± 0.8	*
25	11.7 ± 0.6	11.9 ± 0.7	
26	11.6 ± 0.8	11.9 ± 0.6	*
27	12.3 ± 0.9	12.4 ± 0.7	
28	12.5 ± 0.8	12.4 ± 0.8	
29	13.2 ± 0.7	13.0 ± 0.8	

Table A.- 29: Analyzed content of manure in trial III (original single values)

Barn (group)	**Location of extraction**	**Dry matter (%)**	**P_2O_5 (%) (related to dry matter)**	**CaO (%) (related to dry matter)**
2 (standard feed)	Left side 1	74.73	2.86	3.13
	Left side 2	79.18	2.69	4.49
	Left side 3	51.87	3.00	4.97
	Left side 4	75.58	4.01	4.63
	Left side 5	63.39	3.10	7.95
	Right side 1	78.48	3.22	3.64
	Right side 2	76.85	3.76	5.30
	Right side 3	73.87	3.24	6.46
	Right side 4	64.61	2.91	6.35
	Right side 5	47.77	2.85	4.68
	Average	**68.63**	**3.16**	**3.47**
3 (Ca/P reduced feed)	Left side 1	73.88	3.30	4.31
	Left side 2	78.82	2.45	2.05
	Left side 3	51.92	4.14	3.95
	Left side 4	46.52	3.70	3.62
	Left side 5	61.89	2.82	2.50
	Right side 1	77.13	3.51	2.98
	Right side 2	73.4	3.23	4.43
	Right side 3	76.31	3.46	2.95
	Right side 4	71.75	4.73	3.50
	Right side 5	74.92	3.38	1.99
	Average	**68.65**	**3.47**	**3.23**

Table A.- 30: Boxplot elements for the analyses of P in manure in trial III (related to dry matter; N = 10 per barn)

Factor	**Barn 2 (standard feed)**	**Barn 3 (Ca/P reduced feed)**
Minimum (%)	1.2	1.1
1st Quantile (%)	1.3	1.4
Median (%)	1.3	1.5
3rd Quantile (%)	1.4	1.6
Maximum (%)	1.8	2.1

Table A.- 31: Boxplot elements for the analyses of Ca in manure in trial III (related to dry matter; N = 10 per barn)

Factor	Barn 2 (standard feed)	Barn 3 (Ca/P reduced feed)
Minimum (%)	2.2	1.4
1st Quantile (%)	3.2	1.9
Median (%)	3.4	2.3
3rd Quantile (%)	4.4	2.8
Maximum (%)	5.7	3.2

Table A.- 32: Weight development by biweekly means ± SD of N = 30 weighed hens per barn (trial IV) (significant differences per group are marked with * in the last column)

PW	Barn 1 (Ca/P reduced feed)	Barn 4 (standard feed)	Significant difference per group
12	12.3 ± 0.8	12.2 ± 0.8	
14	12.5 ± 1.0	12.1 ± 0.7	*
16	12.5 ± 0.6	12.3 ± 0.5	
18	12.5 ± 0.7	12.1 ± 0.7	*
20	12.8 ± 0.6	12.7 ± 0.7	
22	12.4 ± 0.5	12.5 ± 0.4	
24	12.4 ± 0.3	12.4 ± 0.3	
26	12.7 ± 0.6	12.8 ± 0.7	

Table A.- 33: Development of net laying performance (settable eggs) as weekly averages (trial IV)

PW	Barn 1 (Ca/P reduced feed)	Barn 4 (standard feed)
12	74.8	75.0
13	73.0	72.1
14	71.6	72.7
15	72.4	72.3
16	71.0	69.6
17	70.4	69.4
18	69.4	68.3
19	68.9	68.8
20	67.4	67.0
21	67.5	66.7
22	66.0	66.1
23	64.1	64.8
24	64.2	64.8
25	62.0	62.7
26	62.2	63.2

Table A.- 34: Development of total laying performance as weekly averages (trial IV)

PW	Barn 1 (Ca/P reduced feed)	Barn 4 (standard feed)
12	77.5	77.7
13	76.2	75.2
14	73.8	75.2
15	74.2	74.2
16	74.0	71.7
17	72.4	71.6
18	71.2	70.5
19	70.8	71.0
20	69.6	69.0
21	69.1	68.7
22	68.1	68.4
23	66.2	67.3
24	65.8	66.5
25	64.2	64.7
26	64.2	65.2

Table A.- 35: Egg weight development by weekly means ± SD of N = 150 weighed eggs per barn (trial IV) (significant differences per group are marked with * in the last column)

PW	Barn 1 (Ca/P reduced feed)	Barn 4 (standard feed)	Significant difference per group
14	92.6 ± 5.1	91.5 ± 4.9	
16	91.6 ± 4.9	89.4 ± 4.5	*
18	95.3 ± 6.0	95.7 ± 5.0	
20	95.6 ± 6.2	96.6 ± 6.0	
22	96.3 ± 5.9	96.0 ± 6.2	
24	96.8 ± 6.5	97.5 ± 6.0	
26	96.6 ± 5.9	98.0 ± 6.4	

Table A.- 36: Development of the percentage of dead embryos at screening (10–12 days after being placed in an incubator) of group with standard feed and group with Ca/P reduced feed in trial IV (weekly averages)

PW	Ca/P reduced feed (barn 1) (%)	Standard feed (barn 4) (%)	Uncertain (%)
12	3.4	3.5	
13			2.1
14			2.8
15			3.9
16			3.4
17			5.2
18			3.3
19	2.7	3.2	
20	3.3	3.0	
21	2.6	2.8	
22			2.7
23			3.5
24			4.4
25	4.2	4.3	
26	4.1	4.1	

Table A.- 37: Development of fertility of group with standard feed and group with Ca/P reduced feed in trial IV (weekly averages)

PW	Ca/P reduced feed (barn 1) (%)	Standard feed (barn 4) (%)	Uncertain (%)
12	91.6	92.4	
13			92.1
14			92.3
15			90.6
16			91.2
17			89.6
18			91.7
19	93.7	92.3	
20	91.1	93.1	
21	92.2	91.4	
22			89.4
23			89.9
24			86.4
25	90.8	89.4	
26	90.7	88.3	

Table A.- 38: Development of hatchability of group with standard feed and group with Ca/P reduced feed in trial IV (weekly averages)

PW	Ca/P reduced feed (barn 1) (%)	Standard feed (barn 4) (%)	Uncertain (%)
12	88.1	87.7	
13			88.4
14			87.5
15			85.9
16			85.2
17			78.2
18			86.1
19	88.8	85.5	
20	99.5	76.2	
21	85.5	84.8	
22			82.6
23			82.7
24			83.6
25	82.1	82.7	
26	82.4	80.5	

Table A.- 39: Analyzed content of manure in trial IV (single values)

Barn (group)	Location of extraction	Dry matter (%)	P_2O_5 (%)	CaO (%)	P_2O_5 (%) (related to dry matter)	CaO (%) (related to dry matter)
1 (Ca/P reduced feed)	Left side 1	34.21	0.85	1.87	2.47	5.48
	Left side 2	44.55	1.40	2.66	3.14	5.96
	Left side 3	40.99	1.55	2.61	3.77	6.37
	Left side 4	36.70	0.79	1.92	2.15	5.24
	Left side 5	40.56	1.46	2.69	3.59	6.63
	Right side 1	35.81	1.53	3.61	4.27	10.10
	Right side 2	42.26	1.01	2.61	2.40	6.18
	Right side 3	40.59	0.61	1.63	1.50	4.03
	Right side 4	35.86	1.32	2.41	3.68	6.73
	Right side 5	38.66	0.43	1.11	1.11	2.87
	Average	**39.02**	**1.10**	**2.31**	**2.81**	**5.96**
4 (standard feed)	Left side 1	36.21	1.12	2.36	3.10	6.53
	Left side 2	41.96	0.52	1.46	1.23	3.47
	Left side 3	50.84	1.10	2.81	2.17	5.53
	Left side 4	44.61	2.15	4.96	4.82	11.10
	Left side 5	47.41	1.27	3.74	2.69	7.88
	Right side 1	32.95	0.86	2.44	2.60	7.40
	Right side 2	31.89	0.52	1.36	1.62	4.27
	Right side 3	38.28	1.30	3.36	3.40	8.78
	Right side 4	43.30	0.91	2.16	2.10	5.00
	Right side 5	49.05	1.63	3.68	3.33	7.51
	Average	**41.65**	**1.14**	**2.83**	**2.71**	**6.75**

Table A.- 40: Boxplot elements for analyses of P in manure in trial IV (related to dry matter; N = 10 per barn)

Factor	Barn 1 (Ca/P reduced feed)	Barn 4 (standard feed)
Minimum (%)	0.5	0.5
1st Quantile (%)	1.0	0.9
Median (%)	1.2	1.2
3rd Quantile (%)	1.6	1.4
Maximum (%)	1.9	2.1

Table A.- 41: Boxplot elements for the analyses of Ca in manure in trial IV (related to dry matter; N = 10 per barn)

Factor	Barn 1 (Ca/P reduced feed)	Barn 4 (standard feed)
Minimum (%)	2.1	2.5
1st Quantile (%)	3.8	3.7
Median (%)	4.3	5.0
3rd Quantile (%)	4.7	5.6
Maximum (%)	7.2	7.9

Table A.- 42: Calculated concentrations of av. P and Ca for different scenarios compared with the used concentration (trial I)

Week (feeding phase)	**LW 7–10 (PA 4 P-)**		**LW 11–13 (PA 5 P-)**		**LW 14–15 (JP 1 P-)**		**LW 16–19 (PA 5 Plus P-)**		**LW 20–27 (JP 2 P-)**	
Scenario	av. P (%)	Ca (%)	av. P (%)	Ca (%)	av. P (%)	Ca (%)	av. P (%)	Ca (%)	av. P (%)	Ca (%)
Used concentration	0.50	0.80	0.42	0.67	0.41	0.65	0.40	0.63	0.35	0.56
Calculated concentration	0.45	0.74	0.42	0.61	0.41	0.55	0.39	0.46	0.35	0.27
Calculated concentration if …										
… body weight was −20%	0.36	0.60	0.33	0.49	0.33	0.44	0.31	0.37	0.28	0.21
… body weight was +20%	0.54	0.89	0.50	0.73	0.49	0.66	0.46	0.55	0.42	0.32
… feed intake was −20%	0.57	0.93	0.52	0.76	0.51	0.69	0.48	0.58	0.43	0.34
… feed intake was +20%	0.38	0.62	0.35	0.51	0.34	0.46	0.32	0.38	0.29	0.22
… body weight was −20% and feed intake was −20%	0.45	0.74	0.42	0.61	0.41	0.55	0.39	0.46	0.35	0.27
… body weight was −20% and feed intake was +20%	0.30	0.50	0.28	0.41	0.27	0.37	0.26	0.31	0.23	0.18
… body weight was +20% and feed intake was −20%	0.68	1.12	0.62	0.92	0.61	0.83	0.58	0.69	0.52	0.40
… body weight was +20% and feed intake was +20%	0.45	0.74	0.42	0.61	0.41	0.55	0.39	0.46	0.35	0.27
Minimum from all calculated values (rounded up to one decimal place)	0.40	0.50	0.30	0.50	0.30	0.40	0.30	0.40	0.30	0.20
Maximum from all calculated values (rounded up to one decimal place)	0.70	1.20	0.70	1.00	0.70	0.90	0.60	0.70	0.60	0.50

Table A.- 43: Calculated concentrations of av. P and Ca for different scenarios compared with the used concentration (trial II)

Week (feeding phase)	LW 30–47 (Layer P-)		LW 48–56 (Layer P- II)	
Scenario	av. P (%)	Ca (%)	av. P (%)	Ca (%)
Used concentration	0.30	2.80	0.24	2.60
Calculated concentration	0.24	2.66	0.24	2.63
Calculated concentration if …				
… egg weight was −20%	0.45	1.71	0.43	1.69
… egg weight was +20%	0.49	2.54	0.47	2.51
… feed intake was −20%	0.59	2.66	0.56	2.63
… feed intake was +20%	0.39	1.77	0.38	1.75
… egg weight was −20% and feed intake was −20%	0.56	2.14	0.54	2.11
… egg weight was −20% and feed intake was +20%	0.37	1.42	0.36	1.41
… egg weight was +20% and feed intake was −20%	0.61	3.18	0.59	3.14
… egg weight was +20% and feed intake was +20%	0.41	2.12	0.39	2.09
Minimum from all calculated values (rounded up to one decimal place)	0.30	1.50	0.30	1.50
Maximum from all calculated values (rounded up to one decimal place)	0.70	3.20	0.60	3.20

Table A.- 44: Calculated concentrations of av. P and Ca for different scenarios compared with the used concentrations (trial III)

Week (feeding phase)	**LW 11–13 (PA 5 P-)**		**LW 14–17 (JP 1 P-)**		**LW 18–19 (PA 5 Plus P-)**		**LW 20–27 (JP 2 P-)**	
Scenario	av. P (%)	Ca (%)	av. P (%)	Ca (%)	av. P (%)	Ca (%)	av. P (%)	Ca (%)
Used concentration	0.60	0.67	0.50	0.65	0.50	0.63	0.50	0.56
Calculated concentration	0.42	0.61	0.40	0.51	0.40	0.43	0.35	0.27
Calculated concentration if …								
… body weight was −20%	0.33	0.49	0.32	0.41	0.32	0.34	0.28	0.21
… body weight was +20%	0.50	0.73	0.48	0.61	0.47	0.51	0.42	0.32
… feed intake was −20%	0.52	0.76	0.50	0.63	0.49	0.53	0.43	0.34
… feed intake was +20%	0.35	0.51	0.33	0.42	0.33	0.35	0.29	0.22
… body weight was −20% and feed intake was −20%	0.48	0.61	0.48	0.51	0.50	0.43	0.46	0.27
… body weight was −20% and feed intake was +20%	0.32	0.41	0.32	0.34	0.33	0.28	0.31	0.18
… body weight was +20% and feed intake was −20%	0.62	0.92	0.60	0.76	0.59	0.64	0.52	0.40
… body weight was +20% and feed intake was +20%	0.42	0.61	0.40	0.51	0.40	0.43	0.35	0.27
Minimum from all calculated values (rounded up to one decimal place)	0.40	0.50	0.40	0.40	0.40	0.30	0.30	0.20
Maximum from all calculated values (rounded up to one decimal place)	0.70	1.00	0.60	0.80	0.60	0.70	0.60	0.50

Table A.- 45: Calculated concentrations of av. P and Ca for different scenarios compared with the used concentrations (trial IV)

Week (feeding phase)	**LW 43–57 (Layer P-)**	
Scenario	av. P (%)	Ca (%)
Used concentration	0.50	2.80
Calculated concentration	0.22	1.88
Calculated concentration if …		
… egg weight was −20%	0.46	1.52
… egg weight was +20%	0.51	2.25
… feed intake was −20%	0.60	2.36
… feed intake was +20%	0.40	1.57
… egg weight was −20% and feed intake was −20%	0.57	1.90
… egg weight was −20% and feed intake was +20%	0.38	1.26
… egg weight was +20% and feed intake was −20%	0.64	2.82
… egg weight was +20% and feed intake was +20%	0.42	1.88
Minimum from all calculated values (rounded up to one decimal place)	0.30	1.30
Maximum from all calculated values (rounded up to one decimal place)	0.70	2.90

Affidavit

Declaration in lieu of an oath on independent work

according to Sec. 18(3) sentence 5 of the University of Hohenheim's Doctoral

Regulations for the Faculties of Agricultural Sciences, Natural Sciences, and Business,

Economics and Social Sciences

1. This dissertation submitted on the topic of

Effects of a reduction of dietary levels of calcium and phosphorus on performance, bone minerals and mineral excretion of turkey breeder hens in the rearing and laying period

is work done independently by me.

2. I only used the sources and aids listed and did not make use of any impermissible assistance from third parties. In particular, I marked all content taken word-for-word or paraphrased from other works.

3. I did not use the assistance of a commercial doctoral placement or advising agency.

4. I am aware of the importance of the declaration in lieu of oath and the criminal consequences of false or incomplete declarations in lieu of oath.

I confirm that the declaration above is correct. I declare in lieu of oath that I have declared only the truth to the best of my knowledge and have not omitted anything.

Hatten, 1st August 2022

Place, Date

Signature

Curriculum vitae

Personal data

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Work experience

08/2021 to present: **University of Veterinary Medicine Hannover**
- Research associate
- Project „Wissenschaft und Innovation für Nachhaltige Geflügelhaltung“ (WING, *Science and Innovation for Sustainable Poultry Production*)

10/2018 to 07/2021: **Moorgut Kartzfehn Turkey Breeder GmbH**
- Research associate
- Planning and evaluation of feeding trials with turkey breeders

03/2016 to 09/2016: **HEBRICO GmbH in Walchum**
- Undergraduate assistant in the field of broiler fattening
- Collection of data and evaluation of feeding trials

04/2015 to 12/2015: **Chamber of Agriculture (Lower Saxony) in Oldenburg**
- Undergraduate assistant in the field of laying hens
- Project „Minimierung von Federpicken und Kannibalismus bei unkupierten Legehennen“ (*Minimization of featherpecking and cannibalism of non-docked laying hens*)

Education

02/2019 to present: **University of Hohenheim**
- Doctorate for a doctoral degree in agricultural science (Dr. sc. agr.)
- Institute of Animal Science, Dept. of Animal Nutrition
- Supervisors: Prof. Dr. Markus Rodehutscord, apl. Prof. Dr. Hans Schenkel (both: University of Hohenheim), Dr. Hartmut Meyer (Moorgut Kartzfehn)
- Research project: “*Potential effects of an adjustment from the content of calcium and phosphorus in feeding program for turkey parent stock hens in the rearing and laying period”*

10/2016 to 09/2018: **University of Göttingen**

- Master's degree study of agricultural science, focus: animal science
- Thesis: „Kartierung von Geschlechtsdimorphismen im Synbreed Chicken Diversity Panel" *(Mapping of sexual dimorphisms at the synbreed chicken diversity panel)*
- Degree: Master of Science „Agrarwissenschaften" (grade: 1.7)

10/2013 to 09/2016: **University of Göttingen**

- Bachelor's degree study of agricultural science, focus: animal science
- Thesis: „Nährstoffbilanzierung in der Broilermast bei Futterzusatz von aktivierter Pflanzenkohle" *(Nutrient balance in broiler fattening with the addition of activated vegetable carbon in feed)*
- Degree: Bachelor of Science „Agrarwissenschaften" (grade: 2.3)

Internships

02/2015 to 04/2015: **Lehr-, Versuchs- und Forschungszentrum (LVFZ) für Geflügelhaltung und Kleintiere *(Educational, experimental and technical centre for poultry and small livestock)* in Kitzingen**

- Practical work with laying hens, broilers, turkeys, broiler parent stock, quails, ducks, geese, pheasants, and rabbits
- Insights into all production processes from hatching to slaughter

08/2014 to 10/2014: **Chamber of Agriculture (Lower Saxony) in Oldenburg**

- Department of animal breeding, animal keeping, and research with animals
- Support of the consultation and education for cattle and cows, swine, horses, sheep, goats, and poultry

02/2014 to 04/2014: **Meyer family in Sandkrug (district Oldenburg)**

- Practical work with cows, swine, cattle, arable farming, and biogas production

Skills and interests

Languages:	German (mother language)
	English (fluent)
	Italian (fair)
IT:	Data evaluation with EXCEL, SPSS, SAS, and R
Hobbies:	Riding
Other:	German driving license B and BE

Hatten, 1st August 2022

Gickel

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