POTENTIAL FOR SUSTAINABLE POULTRY PRODUCTION BASED ON LOCAL CHICKEN BREEDS AND REGIONAL PROTEIN PLANTS

Dissertation

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SUMMARY

During the 20th century, poultry production evolved to a highly specific sector with special chicken lines for egg production on the one hand and meat production on the other. While of broilers both sexes can be used for meat production, until now the male chicks of layer lines are killed on the first day of life because of their low growth potential and the associated economic disadvantages. This practice raises strong ethical concerns and will be forbidden in Germany from 2022 on. Research on alternative solutions is going on, e.g., sex determination in the egg, fattening of the male layer chicks and the use of dual-purpose breeds. Dual-purpose breeds are suitable for both, meat and egg production. In former times, many local breeds have been used this way, but today such breeds are mainly kept as a hobby. Since no selection on performance traits is taking place, the laying and fattening ability of local breeds is low compared to specialized commercial lines. Crossbreeding could be a means to increase the performance of local breeds by heterosis and position effects. Moreover, since the mass market is dominated by commercial hybrids, many local breeds are threatened to become extinct and with them potentially precious genetic resources could get lost. Agricultural use could enhance the chances of these breeds to survive, but for that purpose sufficient performance is necessary.

In **Chapters 2-4** two local chicken breeds, Bresse Gauloise (BG) and Vorwerkhuhn (VH), and the commercial layer line White Rock (WR) will be evaluated regarding their meat and egg production. In addition, crossbreds of the respective genotypes have been tested to investigate the effect of crossbreeding on performance enhancement. The crosses were: Bresse Gauloise male x White Rock female (BWR), Vorwerkhuhn male x Bresse Gauloise female (VBG), Vorwerkhuhn male x White Rock female (VWR).

To cover the demand for protein in animal feedstuff, huge amounts of soybeans and its products are imported to Europe from the Americas. Concerns regarding the sustainability of the use of soybeans are growing due to the cultivation practice and the huge amount of genetically modified seeds. Alternatives to imported soybean meal are regional protein plants, as for example faba beans (*Vicia faba L.*). Unfortunately, faba beans contain antinutritional factors (ANF), for example Vicin and Convicin (VC), that limit their use in animal nutrition. However, breeding activities led to the reduction of ANF in some varieties. In the present study, the feeding of diets with 20% faba beans with either high or reduced VC contents was compared to a standard soybean meal based diet.

The aim of the present study was to test a production system based on local chicken breeds and regional faba beans instead of soy. Such a system could contribute to the avoidance of culling day-old male chicks, preservation of poultry genetic resources and enhanced sustainability in feeding, at least in a niche market. Separated by cockerels and hens, feeding experiments have been conducted to investigate the performance levels of the above-mentioned chicken genotypes, the influence of faba bean feeding and interactions between genotypes and diets.

Chapter 2 discusses the weight gain, feed intake and valuable parts at slaughtering of the cockerels of the above-mentioned genotypes. Of the purebreds, the meat-type BG achieved the fastest growth and as crossing partner it enhanced the fattening performance of VH and WR. While the BWR and VBG showed similar growth, the VWR reached the target weight of 2 kg approximately two weeks later. Results for carcass yield, breast and leg percentage were similar for all genotypes. The feeding of faba beans had no adverse effects on the fattening performance and health of the cockerels.

The laying curves of the hens, egg weights and eggshell quality traits are shown in **Chapter 3,** further egg quality traits are discussed in **Chapter 4**. The laying performance of purebred WR was highest (83.7%), but the BWR also achieved a high production level (80.4%) and a mean egg weight of 58 g. The VWR showed a high peak production but a low persistency resulting in a mean laying performance of 71.1%. Although differences existed, the egg quality of all genotypes was comparable to that of commercial chicken. The feeding of vicin-rich faba beans led to a slight decrease in egg weights, but the vicin-poor faba beans showed no impact on the hen's performance or on egg quality parameters.

In **Chapters 2** and **3** it was confirmed that the performance and feed efficiency of the local breeds and their crosses is clearly lower than that of specialized commercial lines. Especially the low feed efficiency raises further questions regarding the environmental sustainability of these production system that have to be balanced against the advantage of preserving poultry genetic resources. The use of regionally grown faba beans and other legumes can enhance the sustainability of the poultry production without performance losses.

Taking the results of **Chapters 2-4** into account, the BWR cross turned out to be the most promising genotype regarding dual-purpose use, because of its comparably high performance in fattening and laying. In addition, the VBG showed an improvement in both categories compared to purebred VH, which is relevant for the conservation and use of local breeds. The crossbreeding of a meat-type with a layer-type breed could be transferred to other local breeds to enhance their performance level from one generation to the next. The feeding of 20% VC-poor faba beans had no negative impact on the parameters measured in the present study, while the VC-rich variety led to lower egg weights.

Overall, the study has shown that the use of local chicken breeds or rather crosses thereof in combination with regional protein feed proves to be a possible production system for niche markets.

ZUSAMMENFASSUNG

Im Verlauf des 20. Jahrhunderts hat sich die Geflügelproduktion zu einem hochspezialisierten Sektor mit speziellen Linien zur Eierzeugung auf der einen und Fleischproduktion auf der anderen Seite entwickelt. Während zur Fleischproduktion beide Geschlechter der Mastlinien genutzt werden können, werden die männlichen Küken der Legelinien aufgrund ihrer mangelnden wirtschaftlichen Effizienz am ersten Lebenstag getötet. Dieses Vorgehen ruft starke ethische Bedenken hervor und wird in Deutschland ab 2022 verboten. An alternativen Lösungen wird zurzeit geforscht, z.B. an der Geschlechtsbestimmung im Ei, der Mast der männlichen Tiere der Legelinien und der Nutzung von Zweinutzungsrassen. Zweinutzungsrassen eignen sich sowohl zur Fleisch- als auch zur Eierzeugung. Vor der Etablierung der industriellen Produktionssysteme wurden viele alte Rassen auf diese Weise genutzt, während sie heutzutage hauptsächlich als Hobby gehalten werden. Da keine Selektion auf Leistung stattfindet, ist die Lege- und Mastleistung verglichen mit spezialisierten kommerziellen Linien gering. Kreuzungszucht könnte ein Weg sein, um die Leistung lokaler Rassen von einer Generation zur nächsten durch Heterosis- und Positionseffekte zu verbessern.

Da der Massenmarkt von kommerziellen Linien dominiert wird, sind viele lokale Rassen vom Aussterben bedroht und mit ihnen könnten wertvolle genetische Ressourcen verloren gehen. Eine landwirtschaftliche Nutzung könnte das Überleben dieser Rassen sichern, allerdings wäre dafür ein hinreichendes Leistungsniveau notwendig.

In den **Kapiteln 2-4** werden zwei lokalen Rassen, Bresse Gauloise (BG) und Vorwerkhuhn (VH), und die kommerzielle Legelinie White Rock (WR) hinsichtlich ihrer Fleisch- und Eierproduktion beurteilt. Zusätzlich wurden Kreuzungstiere dieser Genotypen geprüft um den Effekt von Kreuzungszucht zur Leistungssteigerung zu bestimmen. Die Kreuzungsgenotypen waren: Bresse Gauloise Hahn x White Rock Henne (BWR), Vorwerkhuhn Hahn x Bresse Gauloise Henne (VBG), Vorwerkhuhn Hahn x White Rock Henne (VWR).

Um den Bedarf an Protein für die Tierernährung zu decken, werden große Mengen an Sojabohnen und –produkten aus Nord- und Südamerika nach Europa importiert. Aufgrund

der Art der Kultivierung und des hohen Anteils an genetisch verändertem Saatgut nehmen die Bedenken über die Nachhaltigkeit der Nutzung von Sojabohnen zu. Alternativen zu Soja sind regionale Eiweißpflanzen, zum Beispiel Ackerbohnen (*Vicia faba L.*). Allerdings enthalten Ackerbohnen antinutritive Inhaltsstoffe (ANF) wie Vicin und Convicin (VC), die ihren Einsatz in der Tierernährung einschränken. Züchterische Bemühungen haben allerdings zur Reduktion antinutritiver Inhaltsstoffe in einigen Sorten geführt. In der vorliegenden Studie wird die Fütterung von 20% Ackerbohnen mit hohen und reduzierten VC-Gehalten mit einer Standardfütterung auf Basis von Sojabohnenschrot verglichen.

Ziel der Arbeit war es, ein Produktionssystem, das die Nutzung lokaler Hühnerrassen mit der Fütterung von Ackerbohnen anstelle von Soja kombiniert, zu testen. Solch ein System könnte, zumindest in einer Nische, zum Verzicht auf das Töten der männlichen Eintagsküken, zum Erhalt genetischer Ressourcen und zu mehr Nachhaltigkeit in der Fütterung beitragen. Es wurden deshalb mit den oben genannten Genotypen getrennte Fütterungsversuche für Hähne und Hennen durchgeführt, um das Leistungspotenzial der einzelnen Genotypen, den Einfluss der Ackerbohnenfütterung auf die Tiere und die Wechselwirkungen zwischen Genotyp und Futter zu bestimmen.

In **Kapitel 2** werden die Gewichtsentwicklung, Futteraufnahme und der Anteil wertvoller Teilstücke der männlichen Tiere dargestellt. Von den Reinzuchttieren wuchsen die BG am schnellsten und verbesserten als Kreuzungspartner die Mastleistung von VH und WR. Während die BWR und VBG ein vergleichbares Wachstum zeigten, erreichten die VWR das Zielgewicht von 2 kg etwa zwei Wochen später. Die Ergebnisse für Ausschlachtung, Brust- und Beinanteil lagen dicht beieinander, aber deutlich unter den für kommerzielle Broiler angegeben Werten. Die Fütterung mit 20% Ackerbohnen hatte keine nachteiligen Auswirkungen auf die Leistung und Gesundheit der Hähne.

Die Legeleistung der Hennen, Eigewichte und Parameter der Eischalenqualität werden in **Kapitel 3** gezeigt, während weitere Eiqualitätsparameter in **Kapitel 4** diskutiert werden. Wie erwartet war die Legeleistung der WR am höchsten (83.7%), aber auch die BWR erreichten ein hohes Leistungsniveau (80.4%) zusammen mit mittleren Eigewichten von 58 g. Die VWR zeigten eine hohe Spitzenproduktion aber geringe Persistenz, was in einer mittleren

Legeleistung von 71.1% resultierte. Obwohl Unterschiede vorhanden waren, war die Eiqualität aller Genotypen vergleichbar mit der kommerzieller Legehennen. Die Fütterung vicin-reicher Ackerbohnen führte zu einer leichten Abnahme der Eigewichte, während die vicin-armen Ackerbohnen keine Auswirkung auf die Leistung der Hennen oder die Eiqualitätsparameter hatten.

In den **Kapiteln 2** und **3** bestätigte sich, dass die Leistung und Futtereffizienz der lokalen Rassen und ihrer Kreuzungen deutlich niedriger ist als die der spezialisierten kommerziellen Genotypen. Besonders die geringe Futtereffizienz wirft weitere Fragen in Bezug auf die ökologische Nachhaltigkeit dieses Produktionssystems auf, die gegen den Vorteil der Erhaltung genetischer Ressourcen abgewogen werden müssen. Die Nutzung regional erzeugter Ackerbohnen und anderer Leguminosen kann hierbei die Nachhaltigkeit der Geflügelproduktion ohne Leistungseinbußen verbessern.

Unter Berücksichtigung der Ergebnisse der **Kapitel 2-4** erscheint die BWR-Kreuzung aufgrund ihrer vergleichsweise hohen Lege- und Mastleistung am vielversprechendsten für die Zweinutzung. Aber auch die VBG zeigten in beiden Nutzungsrichtungen eine Verbesserung im Vergleich zu reinen VH, was für die Erhaltung und Nutzung lokaler Rassen von Bedeutung ist. Der Ansatz der Kreuzungszucht einer fleischbetonten mit einer legebetonten Rasse könnte auf andere Rassen übertragen werden, um die Leistung der F1-Generation zu verbessern. Die Fütterung von 20% vicin-armen Ackerbohnen hatte keine negativen Auswirkungen auf die gemessenen Parameter, während die vicin-reiche Variante zu geringeren Eigewichten führte.

Zusammenfassend hat die Studie gezeigt, dass die Kombination aus lokalen Hühnerrassen, beziehungsweise Kreuzungen daraus, mit regionalem Eiweißfutter ein mögliches Produktionssystem für Nischenmärkte darstellt.

CHAPTER 1

General Introduction

Poultry production

Within the last century, a highly specialized poultry production system evolved with a strict separation of egg and meat production, and today commercial poultry breeding lays in the hands of a few globally acting concerns. As breeding for high laying performance and high fattening performance at the same time is genetically impossible [1,2], specialized genetic lines for either egg or meat production have been developed. These lines are not directly used for the production of eggs or meat, but hybrids based on three to four of these purebred lines with the aim to increase the performance level of the animals further by taking advantage of heterosis and position effects of the parents (**Figure 1.1**).

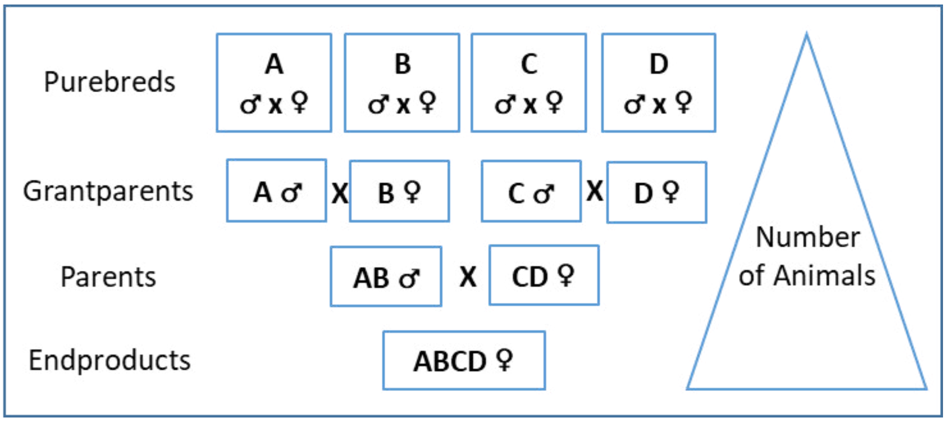

Figure 1.1. Production scheme of laying hen breeding, modified after Preisinger (2016)[2]

Purebreds and grandparent stocks are in the possession of the above mentioned companies, which sell solely parent animals including management guidelines to multipliers [2]. Often also the associated industries belong to this vertically integrated system, which is dominated by the private companies [3].

Commercial broilers are able to reach final weights of 2 kg in less than five weeks using only 1.5 kg feed per kg of weight gain [4,5]. The production performance of laying hens varies according to the length of their production period. Lohmann Brown Classic hens produce 356 eggs in 80 weeks of life, with a feed consumption of 2.2 kg feed per kg egg mass [6], hens of the origin ISA brown lay 466 eggs until 100 weeks of age, using 2.14 kg of feed per kg egg mass [7]. White layers lay even 363 eggs in 80 [8] respectively 480 eggs in 100 weeks [9].

The killing of day-old male chicks and alternative approaches

The male chicks of the layer lines are of no economic value in the described production system and have until now been culled on their first day of life [10]. In 2020 the number of affected chicks in Germany alone was more than 40 million [11], which were killed via carbon dioxide and used as feed for zoo animals or pets [1]. As consequence of increasing concerns regarding ethical reasonability and animal welfare of this procedure, Germany is going to ban the killing of day-old chicks from the year 2022 on [12]. Possible alternatives to the killing of day-old chicks that are currently being investigated are introduced in the following subchapters.

In-ovo sex determination

In-ovo sex determination aims at determining the chicken's sex during brooding in order to sort out eggs with male embryos and therefore to avoid the killing of day-old chicks. Different techniques are currently under development [10] and some of them are already used by retail firms [13]. The clear disadvantage of the currently used methods is the relatively late application during incubation. Until now, exact knowledge about the onset of pain perception of the embryo is missing, but due to the development of the nervous system, pain perception of the embryo cannot be excluded after day 7 of incubation [14]. It can be supposed that in-ovo methods will nevertheless become the solution for the mass market in future. However, they are not an acceptable alternative for all production systems, for example in organic agriculture [15] and they are not yet available for the mass market.

Fattening the brothers of laying hens

Looking for alternatives to the killing of day-old male chicks, several studies regarding the fattening of these chicks have been conducted. In general, the males of the layer lines showed inferior growth performance and feed efficiency than conventional broilers [16,17]. To avoid the economic and ecological disadvantages, Ammer et al. [18] tried to use extensive feedstuff in fattening, but the sustainability was only scarcely improved. As well, caponization, i.e. surgical removal of the testicles in male chickens, did not improve the fattening performance and feed efficiency in a reasonable way [19]. Moreover, in Germany, caponization is not allowed with respect to the German animal protection law [20]. Another concept was pursued by Koenig et al. [21], who shortened the fattening period and produced poussins. Poussins are defined as carcasses of maximum 650 g, respectively maximum 750 g and not older than 28 days [22]. Due to the shorter life time, the feed efficiency was improved compared to longer growth periods, but the marketing of this special product might be difficult.

Dual-purpose chickens

Dual-purpose genotypes are another possible alternative to refrain from killing of day-old male chicks. Originally all chicken breeds have been used for both, egg and meat production, as eggs were collected and surplus animals have been slaughtered for self-supply. During the last century the husbandry of local or fancy breeds evolved to a hobby and breeding was focused on appearance for exhibitions [23]. The absence of selection on performance traits led to the low performance levels of these breeds regarding laying and fattening performance compared to specialized lines, as demonstrated by Hahn et al. [24] and Lange [25]. These authors studied the performance of the local breeds Australorp, Bielefelder, New Hampshire and Rhode Island compared with the control Lohmann Brown. The males were characterized by a low feed efficiency, erratic growth and minor fattening performance. The Bielefelder cockerels performed best [24]. In the laying experiments the late onset of laying (hens older than 200 days), the low laying performance and egg weights as well as the low feed efficiency compared with the commercial hybrid were unfavorable [25]. In a more recent study, Lambertz et al. [26] tested the performance of Bresse Gauloise and crossbreds with New Hampshire hens regarding their dual-purpose potential. Especially the Bresse Gauloise showed satisfying performance to produce in an economically feasible way. Other studies came to comparable results considering the growth performance of local breeds [27–29], namely the cockerels of local breeds lag behind commercial broilers and, as well, commercial dual-purpose genotypes in terms of growth speed, valuable carcass parts and feed efficiency. Although the fattening performance of the local breeds might have been higher than that of laying hen cockerels, the associated hens are supposed to be inferior regarding laying performance and so the economic viability of layer lines is still higher [27]. Hocking et al. [30] and Moula et al. [31] compared the laying performance and egg quality of local breeds with commercial laying hens. Commercial layers laid more and heavier eggs than the local breeds, but the quality of eggs was not disadvantageous in local breeds. The main difference is the composition of eggs as the commercial lines showed higher percentages of albumen while the local breed's eggs had higher proportions of yolk.

With the growing public interest in the termination of chick culling and the use of dual-purpose breeds some years ago, breeding companies started to breed their own dual-purpose products by crossing sires of broiler genotypes with (dwarf) hens of layer lines [32]. For Lohmann Dual, Icken [33] showed that the higher fattening performance, compared with Lohmann Brown cockerels, cannot compensate for the lower laying performance of the hens regarding economic viability and resource efficiency.

Although all these results are well known, research on local breeds for dual-purpose use is going on. In the absence of available alternatives, finding a valuable dual-purpose genotype still seems to be an option to cope with social and legal requirements. Performance enhancement by means of breeding would last several generations, whereas crossbreeding could increase the performance level of animals from one generation to the next [34]. Heterosis and position effects of the parents are well known mechanisms from commercial hybrid breeding [2] and were demonstrated for local breeds by Götze and von Lengerken with crosses of Italian chicken, Sussex, New Hampshire, Marans and a Landrace [35]. The New Hampshire x Marans and New Hampshire x Landrace crosses achieved 80% of the laying performance of the control hybrids. Positive heterosis effects for laying rate and egg quality were also observed by Tixier-Boichard et al. [36], who crossed Fayoumi chicken with commercial brown layers. A crossbreeding scheme already used in practice on a small scale is the crossing of Vorwerkhuhn cocks with commercial White Rock hens [37]. The hybrid offspring, called Kollbecksmoor Huhn, shows an increased laying performance and the income from egg selling is used to support the conservation breeding activities.

Poultry genetic resources

Todays' commercial lines trace back to just a small number of breeds. In the case of white layers this is the Single Comb White Leghorn and for brown layers these are the Rhode Island Red, Australorp, White Plymouth Rock and New Hampshire [38]. Broilers descend among others from White Plymouth Rock and Cornish [3]. On the contrary a high number of local and fancy breeds exists, whereof many are threatened to get extinct [3]. In Germany more than 100 normal sized and dwarf chicken breeds are approved and of the traditional local German breeds nineteen are categorized as extremely or strongly at risk to get extinct [39,40]. As demonstrated by the SYNBREED chicken diversity panel, genetic diversity in the chicken is generally high, however, the commercial chicken lines show a considerable decline of genetic diversity [41,42]. The value of genetic diversity is not explicit to quantify, but it might be helpful for future breeding programs and reacting to future changes of environmental conditions or new diseases [42,43]. Moreover old local breeds can be seen as cultural heritage [42]. Different conservation strategies are in use, since breeds can be kept *in situ* (region of origin) or *ex situ* and *in vivo* or *in vitro* [44]. *In vitro* means the cryo-conservation of semen or germplasm in gene banks, where it can be stored unlimited and be thawed, when there is a need. However, adaption to future changes in environmental conditions or diseases is not taking place this way [45]. The *in vivo* or *on-farm* conservation of local chicken breeds is mainly done by fancy breeders with less or without commercial interest in the chicken. Whereas for other farm animal species financial support is paid to breeders to motivate the husbandry of endangered breeds, this is not common for poultry [44]. Nevertheless, in the long term, agricultural use including economic benefit would increase the chances of local breeds to survive, especially because many fanciers are older people and the interest in younger people for such a hobby is low [46]. The 'Initiative zur Erhaltung alter Geflügelrassen e.V.' (initiative for the conservation of old poultry breeds) set itself to preserve endangered poultry breeds via supporting breeders and founding breeding communities. Currently conservation flocks for the breeds Vorwerkhuhn, Bresse Gauloise (white), Gelbe Ramelsloher and Mechelner exist [34]. The parental generations of

Vorwerkhuhn and Bresse Gauloise for the present study were provided by members of this initiative.

Vorwerkhuhn

The Vorwerkhuhn chicken breed was developed at the beginning of the 20th century in Northern Germany by Oscar Vorwerk via crossbreeding of the breeds Lakenvelder, Buff Orpingtons, Ramelsloher and Andalusians. His breeding goal was a local chicken with a special appearance (**Figure 1.2**). The plumage color is yellow with black pattern. Cocks weigh 2.5 – 3 kg and hens 2 – 2.5 kg. The laying performance is up to 180 eggs per year with eggs of a yellowish shell color [47,48]. Currently the breed is classified as 'under observation' by the Central Documentation on Animal Genetic Resources in Germany (TGRDEU) regarding the population size. In 2020 the number of registered breeding animals was 993 cocks and 4558 hens [49].

Figure 1.2. Vorwerkhuhn hen (left) and cock (right).

(Source: J. Fellner, DARE, Goettingen University)

Bresse Gauloise

The origin of Bresse chickens is the South of Burgundy county in France [50]. They show a white plumage with red comb and blue legs (**Figure 1.3**). The name 'Bresse' is only allowed for chickens from certified farms in the Bresse region. There the fattening follows special rules and the carcass is marketed under protected designation of origin (PDO). Outside the Bresse region the breed has to be called 'Bresse Gauloise'. The Bresse Gauloise is a dual-purpose chicken with a laying performance of 180-200 eggs in the first year [51]. The cocks reach a weight of 2.6 – 3 kg at an age of 16 weeks [51].

Figure 1.3. Bresse Gauloise cock (right) and hens

(Source: J. Fellner, DARE, Goettingen University)

The European protein gap

The term 'European protein gap' describes the difference between the need of protein for animal feedstuff and its production. The level of self-sufficiency of the European Union (EU) with protein-rich feed amounts only 30%, i.e. about 70% of the protein-rich feed components needed for farm animal nutrition have to be imported [52,53]. In the 2020/2021 season, these imports amounted to 15.4 million metric tons of soybeans, which were imported into the EU mainly from the U.S. and Brazil [54]. The demand of imported protein exposes the European farmers to global price volatilities and makes them dependent on production systems they cannot influence.

Possible solutions to close or at least minimize the protein gap are an increase in the cropping of regional protein plants or the use of alternative protein sources, as for example insects, synthetic amino acids or meat and bone meal [55]. While the use of meat and bone meal was banned for the production of farm-animal feed in the course of the BSE crisis [56], synthetic amino acids are not allowed in organic farming [55]. The use of insect protein is currently topic of ongoing research but until now not used routinely in Europe [57]. Therefore, the increased cultivation of regional protein crops is currently the most pursued strategy.

In 2010 only 3% of European cropland have been used to grow legumes, which goes along with a low level of effort in breeding progress, innovations and research in the last decades [53]. The major reason for farmers not to grow legumes, is the low competitive ability compared to cereals and other crops because of instable yield and the market price [58]. To counteract this development, the German Federal Ministry of Food and Agriculture prepared a strategy to encourage research activities and cultivation of regional legumes [59].

Soy

The soybean (*Glycine max*) was domesticated in the 11th century BC in China [60]. While it is also used as human food, mainly in Africa and Asia, the majority is today used for animal feed production [61]. The worldwide area cropped with soybeans amounted 122.58 million ha in the season 2019/20 and yielded 335.35 million tons of soybeans [62]. The main growing countries are the US and Brazil. In the EU, 0.94 million ha have been planted with soybeans in 2020 and yielded 2.67 million tons with Italy as leading country [62]. In Germany, the cropped area is growing, mainly in the southern parts, because the plant needs warm conditions and sufficient water supply. Beneficial compared to other legumes is the possibility of closer crop rotations and higher prices at retail [63].

Soybeans are fat-rich legumes and used for oil production. The by-product of oil extraction, soybean meal, is used large scale in animal nutrition because of its high protein content of about 50% and its almost ideal amino acid composition with a high lysine content and satisfactory contents of the sulphur containing amino acids [64]. The two sulphur containing amino acids methionine and cysteine and lysine are the first three limiting amino acids in chicken as sulphur is needed amongst others for feather formation. Nevertheless, the soybean contains several antinutritive factors as are trypsin inhibitors, lectins and saponins, but they are usually deactivated by heat treatment (toasting) of the soybeans [64].

Concerns about the sustainability of soybean cultivation arise of different reasons: the cultivation in South America is directly or indirectly linked to the deforestation of rainforest and the giant monocultures reduce biodiversity even more and increase soil erosions [61,65,66]. Furthermore, over the half of the worlds' soybean area is planted with genetically modified soybeans, carrying traits for herbicide resistance [61].

Regional grain legumes

Grain legumes or pulses are belonging to the family of *fabaceae*, subfamily *faboideae*. In Germany, peas (*Pisum sativum*), lupines (*Lupinus angustifolius*) and faba beans (*Vicia faba L.*) are grown for agricultural purposes, together with an increasing area of soybeans (**Figure 1.4**).

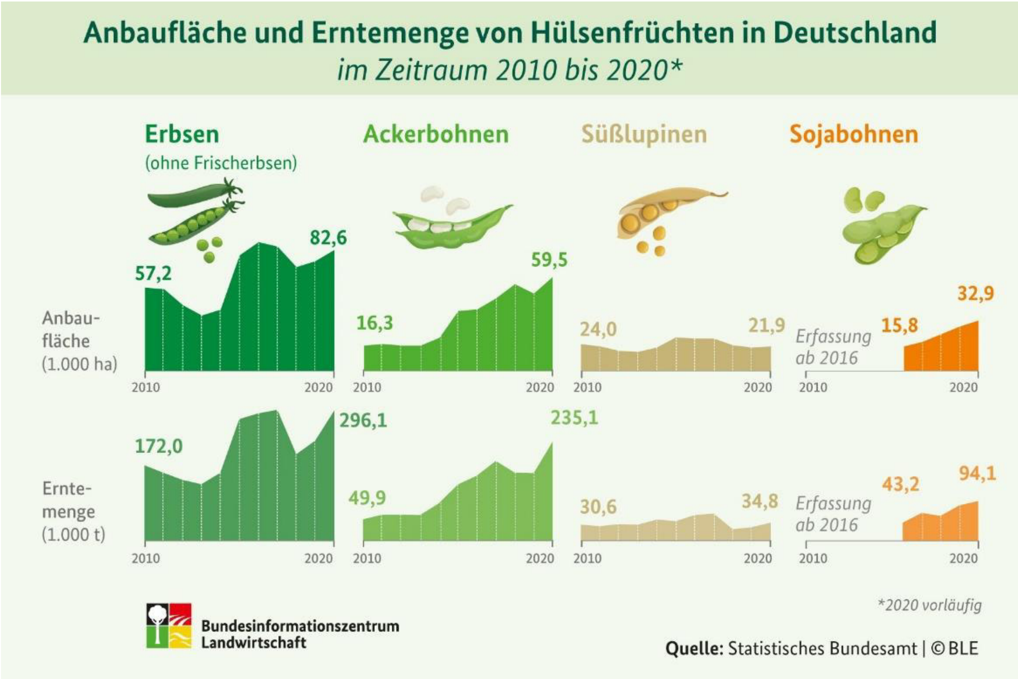

Figure 1.4. Cropped area and yield of legumes in Germany from 2010 to 2020. For peas, faba bean, sweet lupines and soybeans (from left to right) the cropped area (in 1000 ha; top) and yield (in 1000 t; bottom) are represented.

Legumes are protein rich seeds which have been used for feed and food since thousands of years. They are characterized by a low starch content and several antinutritive agents [67]. As a special characteristic, legumes form symbioses with Rhizobium bacteria to fix nitrogen from atmosphere and soil, which allows them to grow in poor soils. In agricultural crop rotations, legume plants can provide this nitrogen to the following crop, leading to reduced need for fertilizers and increasing yield. The integration of legumes in cereal dominated rotations can interrupt the spreading of cereal specific pests and diseases and increase biodiversity in fauna and flora, e.g. by providing nectar to insects [68,69]. Because of this, legumes have always been integrated in the crop rotation of ecological farms.

Faba bean

Faba beans (*Vicia faba L.*, **Figure 1.5.**) were cultivated in the temperate zones of the northern hemisphere since thousands of years and were used as food and feed. Today the main producing countries are China, Ethiopia and the UK [70]. Different types of faba beans were distinguished according to the size of the seeds: the large-seeded *Vicia faba major* or broad bean and the small-seeded *Vicia faba minor*.

Figure 1.5. Faba bean flowering (left; Source: W. Link, Goettingen University), ripe (top right; Source: www.landwirtschaftskammer.de), dry (down right; Source: www.saaten-union.de)

Faba bean seeds are rich in protein and lysine and therefore generally suitable to replace soy in poultry nutrition [71,72]. However, as with other pulses, the content of methionine and cysteine is deficient. Furthermore, seeds of faba beans contain different antinutritional substances, of most importance are tannins and the glycopyranosides vicin and convicin, that limit the use in animal nutrition [73]. Vicin and convicin (together abbreviated as VC) are heat-stable and located in the cotyledon, which makes deactivation and removal difficult. According to Duc et al. [74] the VC contents vary between 6-14 g/kg, but can be reduced 10-20fold by integration of the vc^- allele [75]. The origin of the allele was a spontaneous mutation and the inheritance is monogenic additive [75].

Tannins reduce the digestibility of protein, energy and starch. As they are located in the hulls, they can be removed by dehulling of the seeds [73]. Moreover, tannins can be minimized by means of breeding as the specific zt1 or zt2 alleles lead to a massive reduction of the tannin content [74]. Both alleles are recessive and genetically independent of each other. They control the tannin content and the flower color (pure white flowers) and influence nutrients in a different way, e.g. zt2 leading to higher protein content of the seed [74,76,77]. Commercial varieties with lowered VC and tannin contents exist, but at least in Germany the majority of tested faba bean varieties is still traditional [78]. Faba beans are mainly grown to be fed on farm.

Vicin and Convicin

Vicin and convicin are causative agents of the human disease favism. They are converted to the redox aglycones divicine and isouramil by enzymes present in the bean itself and in the digestive tract of humans. Divicine and isouramil in turn oxidize glutathione (GSH) of the red blood cells. In healthy people the GSH will be regenerated by glucose-6-phosphate-dehydrogenase (G6PD), but people with a deficient G6PD will come down with a severe anemia because of massive erythrocyte degeneration [73,79].

Regarding the effect of VC on poultry, results from literature are ambiguous. The reasons might be that VC was fed in different concentrations, as pure substance or as part of the faba bean; the beans or the feed were treated in different ways, i.e., dehulling, heat treatment, pelleting, micronization; presence of other antinutritional factors; supplementation or not of limiting amino acids; the genotype and age of the chickens. As a consequence, the results from the different studies are only comparable to a limited extend.

VC was identified by Olaboro et al. [80,81] as “egg-weight depressing factor”. Fed as 1% pure substance it led to reduced egg weights, yolk weights and laying performance. The authors hypothesized that divicine and isouramil influence somehow metabolic processes, leading to less deposition of lipids in the yolk, which leads to smaller eggs. Muduuli et al. [82] fed 0.5% and 1.0% crude VC to laying hens and recognized as well reduced egg and yolk weights, an increased frequency of blood spots in the eggs and reduced fertility and hatchability. They assumed that VC destroys granulosa cells of the ovum. Laudadio [83] and Dänner [84] fed 24% and 5-30% faba beans respectively with differing VC contents and did not observe the reduction of egg weights as described above. Laying performance was reduced in some studies at different faba bean levels [80,85,86], however, other authors did not observe a performance loss [83,84,87–89]. Feeding 10%, 20% and 30% of VC-poor and VC-rich faba beans, Halle [85] observed markedly increased mortalities in the groups fed 20% and 30% VC-rich and 30% VC-poor faba beans.

In Broiler nutrition the information about the impact of VC in the feed is less contradictory. Feeding up to 20 % faba beans in the diet did not impair the weight development of broilers, as described in several research studies [90–92]. Even 30% and more were unproblematic in some cases [93–97], whereas other authors [90–92] observed reduced growth performance at levels of more than 20% faba beans. Dal Bosco et al. [98] observed reduced growth rates and increased mortality in the starter period feeding 16% extruded faba beans compared to soybean based feed to slow-growing broiler chicks. As there were lower levels of the essential amino acids Methionine, Cysteine and Threonine in the feed, it is not clear, whether the effect was caused by VC or a lack of amino acids. An influence on the blood count as described for humans was observed by Lessire et al. [87], who showed reduced GSH levels and hemolysis under the feeding of VC-rich diets in laying hens.

The aim and objectives

Alternative ways of poultry production are of interest to avoid the killing of day-old male chicks and to preserve genetic resources. The use of regional protein plants in the feed could increase the sustainability of such a production system. The main aim of the thesis was therefore to characterize the performance of two local chicken breeds, one commercial layer line and crossbreds thereof as well as to examine the effect of feeding faba beans with different VC levels on these genotypes. Practical recommendations will then be derived based on these investigations. Therefore, the first objective was to evaluate the fattening performance of the male chickens of the six genotypes while feeding them diets with 20% vicin-rich or 20% vicin-poor faba beans compared to soybean meal. The second objective was to identify the laying performance and egg quality of the respective hens, which as well were fed diets containing 20% faba beans. For both sexes, first the purebred local chicken breeds Bresse Gauloise and Vorwerkhuhn as well as the commercial line White Rock have been tested. In a second experiment crossbreds of Bresse Gauloise cock × White Rock hen, Vorwerkhuhn cock × Bresse Gauloise hen and Vorwerkhuhn cock × White Rock hen were evaluated to generate information about the value of crossbreeding for increasing the performance level of the respective local breeds.

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CHAPTER 2

Growth Performance of Local Chicken Breeds, a High-Performance Genotype and Their Crosses Fed with Regional Faba Beans to Replace Soy

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Simple Summary: The culling of day-old male chicks and the ecological impact of high soy imports from overseas as animal feed are intensively discussed by the Western European agricultural sector and society. One possible approach to mitigate these problems could be the use of dual-purpose chickens for meat and egg production in combination with a predominant use of regionally grown protein plants. In the present study the suitability of six different chicken genotypes for fattening was evaluated while feeding them two different faba bean varieties. No adverse effects of the faba bean feeding on the performance and the health of the birds could be detected.

Abstract: The faba bean (*Vicia faba* L.) is a native protein crop and considered a promising alternative to soybeans. Due to its anti-nutritive substances such as vicin and convicin (VC) its use in animal nutrition has been restricted. In the present study, two consecutive experiments were conducted to analyse the effects of feeding 20% faba beans, which differ in their VC content on fattening performance and slaughter traits of different chicken genotypes. In a first trial, purebred male chickens of the local breeds Bresse Gauloise and Vorwerkhuhn as well as of a high-performance White Rock line were tested. In a second trial, crossbreds of them were evaluated: Vorwerkhuhn x Bresse Gauloise, Vorwerkhuhn x White Rock, Bresse Gauloise x White Rock. Daily weight gain and feed intake were recorded until slaughter at approximately 2100 g. At slaughter the final live weight, carcass yield and the weights of the valuable parts (breasts and legs) were measured. For the genotypes studied, no adverse or undesirable effects of both VC−rich and VC−poor faba beans in the feedstuff were detected regarding body weight development, carcass quality, and fattening parameters. Furthermore, there was no indication that the birds' health was impaired.

Keywords: carcass traits; faba bean; growth; local breeds; vicin

Introduction

Since the genetic correlation between growth rate and egg production is negative, in commercial poultry farming, predominantly crosses of specialized lines are used that have been selected either for a high laying performance or for a high growth rate and muscularity [1]. Therefore, in the layer industry the male chicks of layer hybrids are culled on the first day of life because of their low fattening performance. In Germany alone this accounts for more than 42 million chicks per year, given a sex ratio of 50% females and 50% males [2], which is problematic in terms of animal welfare, legal and ethical aspects, and social acceptance. In addition to methods of sex determination "in ovo", which are currently under development, and extended laying periods [1,3], fattening of male layer chicks is considered to be a possibility for overcoming the killing of day-old chicks. In this context, the fattening of male chickens up to a maximum weight of 650 g, then called poussins [4], the fattening of the cocks of heavy layer hybrids or the use of dual-purpose-breeds [5,6] have been examined. All these studies have shown that the layer cockerels are clearly inferior to commercial broilers in terms of fattening performance and are consequently economically unprofitable under current production and market conditions. The use of local breeds could be an alternative solution to serve niche markets in a regional context. Previous to the industrialisation of poultry production in the middle of the last century, these breeds had been used for both, egg and meat production. Nowadays, local breeds are mostly kept by hobby breeders, and selection is focused more on phenotypic appearance than on growth or laying performance. Although the performance level of these local breeds is unfavourable compared to commercial layers and broilers [7,8], crosses of these breeds may perform better due to heterosis effects, as has already been shown by Götze and von Lengerken [8] with hybrids of different local breeds.

In chicken production, the high performance level of selected specialised broilers and layers, respectively, requires high protein content in animal feedstuff, which is usually achieved by using soybeans as a source of protein. About 2.5 million tons of soybeans are imported annually for feed production to Germany [9], which is seen critically by the German society due to the environmental impact of soy cultivation in South America and the high proportion of genetically modified soy [10,11]. Therefore, in Germany efforts are being made to reduce soy imports and to accelerate the cultivation of native protein crops [12].

Faba beans (*Vicia faba* L.) are considered a suitable alternative for poultry feed [13]. However, the endogenous glycosides vicin and convicin (together abbreviated as VC) are considered problematic (anti-nutritive), and thus limit the use of faba beans in human and animal nutrition. In humans, vicin and convicin are converted to the redox aglycones divicine and isouramil. These metabolites cause anaemia in people who are deficient in glucose-6-phosphate-dehydrogenase, the enzyme that physiologically detoxifies these substances. This genetic disposition in humans leads to the so-called favism [14]. It is known that a mutation of the VC− gene locus of the faba bean reduces the VC content in the faba bean seeds substantially [15], but there is currently no appropriate screening method for selection for low VC content available, and marker-assisted selection is presently only beginning. The difficulties to select for low VC content cause that only few low-VC varieties are available, for example the variety *Tiffany* [16].

The influence of faba beans in general and of VC in particular on the health and performance of chickens is not clear, and the results of different studies vary greatly. In laying hens, Olaboro et al. [17] showed VC to be responsible for reducing egg weights, which has been confirmed in other studies [18,19]. Experiments with commercial laying hens from Halle [20] fed with different concentrations of faba beans led to increased mortality and reduced laying performance. The recommended maximum levels of faba beans without adverse effects on the health of broilers vary between 125 g/kg [21] and 310 g/kg feed [22]. Causes for this wide variation can be found in the different experimental approaches of the studies cited.

Another main anti-nutritional factor besides VC is tannin. This substance has been shown to reduce the digestibility of protein as well as apparent metabolizable energy (AME_n), with an additive effect of tannins with VC [23]. However, no effect of tannins on fattening performance was found [24,25]. In addition to the anti-nutritional factors, the amino-acid content of the faba bean has to be considered. Faba beans are deficient in sulphur-containing amino acids [13], which can be compensated by adding methionine to the diet in order to reduce performance losses [26]. Furthermore, not only the composition of the faba beans, but also the preparation of the feed has an influence on the acceptance by and growth performance of chickens. Ivarsson and Wall [27], as well as Gous [28], showed that the consumption of pelleted feed led to higher body weights compared to a mash-fed group. Similarly, Wilson and McNab [26] described a positive effect of autoclaving in comparison to the feeding of raw faba beans. There are two possible explanations for the positive effect of heat treatment on chicken performance. One is the destruction of heat-labile anti-nutritional factors, the other is a better availability of nutrients after heating.

Summarising the findings from all these studies, the safe amount of faba beans in the diet depends on many factors, that might occur together and interfere with each other in their effects on the animals. Furthermore, in contrast to commercial chicken lines, it is not known if and to what extent the feeding with faba beans influences the performance parameters of local chicken breeds.

The main objective of this study is to characterize the fattening performance of two purebred local breeds in comparison to a commercial line and crossbreds thereof, and the effect of feeding faba beans with different VC content on the performance traits of these genotypes.

Materials and Methods

Ethical Declaration

The experiment was performed in accordance with the German Animal Welfare Law and approved by the Lower Saxony State Office for Consumer Protection and Food Safety (LAVES) (reference number 33.9-42502-04-17/2622).

Stock and Husbandry

The experimental design applied in this research work is shown in Figure 2.1. According to the objectives of this study, two local breeds, the Vorwerkhuhn (VH) and Bresse Gauloise (BG), were included. The VH chicken breed is a German dual-purpose breed with an egg yield of up to 170 eggs per year and a cock weight of 2.2 kg at 16 weeks of age [29]. BG originates from the Bresse region in France and is marketed there as a delicacy with protected designation of origin (PDO). They have a laying performance of 240 eggs per year and the cocks weigh around 2.5 kg at 16 weeks of age [30]. For comparison with these local breeds, White Rock brown layer parent stocks (WR) from Lohmann Tierzucht GmbH were used. The cross of Vorwerkhuhn cocks and White Rock hens is known as Kollbecksmoorhuhn. This crossbreeding scheme was established in 2005 and has been used by breeders of the “Vorwerkerhaltungszucht” for more than 10 years to produce hybrid offspring for niche market production. These crosses have a higher productivity than the purebred VH chickens and support the conservation breeding activities [31].

For experiment A, day-old WR chicks were provided by Lohmann Tierzucht GmbH (Cuxhaven, Germany), while BG and VH chicks were reproduced from parent stocks at the Institute of Farm Animal Genetics of the Friedrich-Loeffler-Institut (Mariensee, Germany). After hatch, all chicks were marked with individual wing tags and reared for the first three weeks under the same conditions at the Institute of Animal Welfare and Animal Husbandry of the Friedrich-Loeffler-Institut in Celle, Germany. During the first three weeks of life, that is, before the beginning of the experiment, all chicks were fed the same commercial starter diet (11.4 MJ AMEn/kg DM, 180.0 g/kg crude protein, 26.1 g/kg crude fat, 37.5 g/kg crude fibre, 56.0 g/kg crude ash, 7.8 g/kg calcium, 4.7 g/kg phosphorous).

In the fourth week of life, 120 male chicks of BG and WR each and 94 male chicks of the VH breed were transferred to the Department of Animal Sciences of Goettingen University. The lower number of VH chicks was caused by low hatchability. The animals were divided into 12 pens per genotype (10 animals/pen for BG and WR, 7–8 animals/pen for VH) resulting in four replicates for each feeding treatment.

In experiment B, crossbreeds of the respective breeds (Vorwerkhuhn males x Bresse Gauloise females: VBG, Vorwerkhuhn males x White Rock females: VWR, and Bresse Gauloise males x White Rock females: BWR) were subjected to the same feeding regime as the purebred individuals. The feeding treatment in Goettingen started one week earlier than in experiment A, at the age of three weeks. Similar to the previous experiment, 120 animals of each cross were divided into 12 pens per genotype, again resulting in four replicates per feeding treatment.

The animals had ad libitum access to feed and water. The pens had a floor space of 2 × 1.5 m and were covered with wood shavings. They were equipped with perch, feeder and automatic cup-drinker. The room temperature was lowered from 22 °C at the beginning of the experiments to 20 °C from the 5th week of life. The light duration was 16 hours per day.

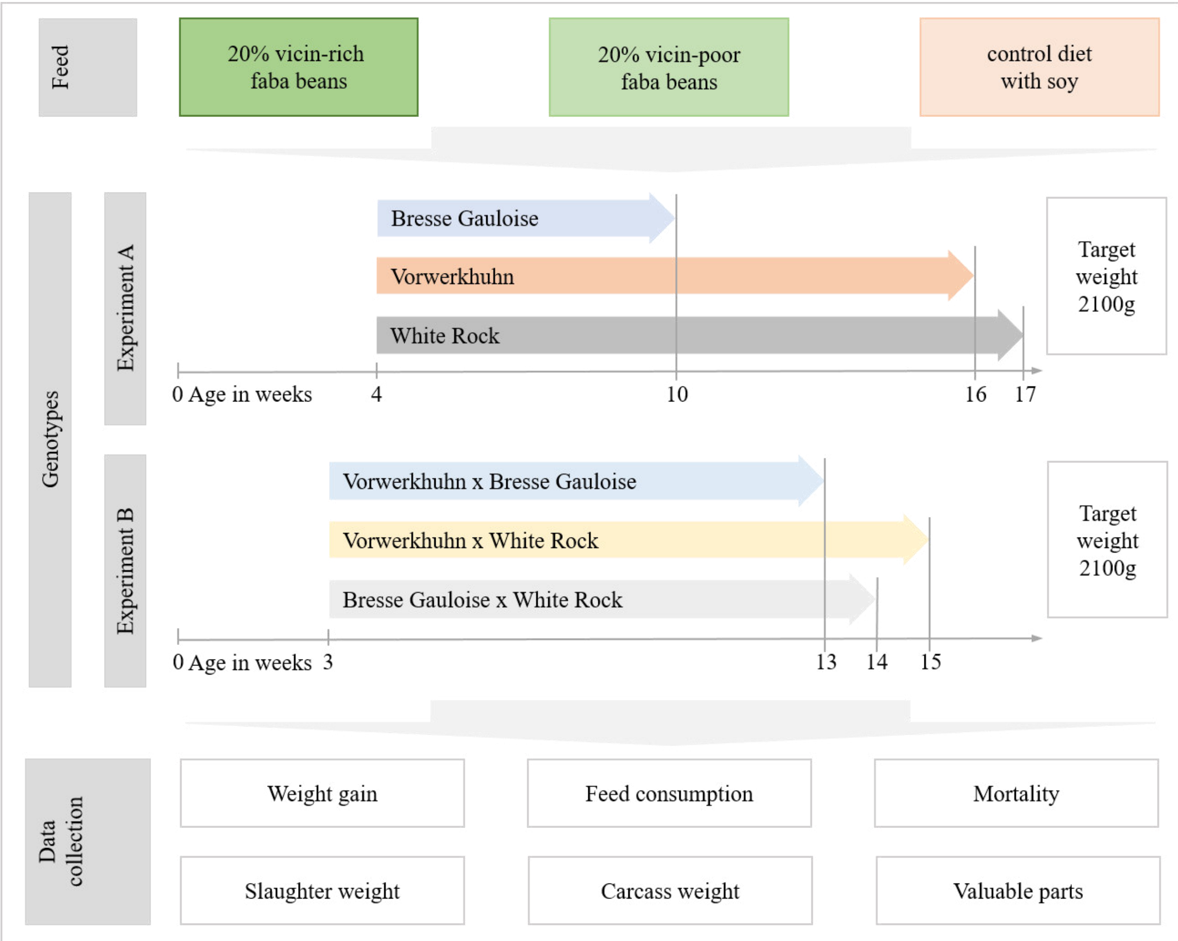

Figure 2.1. Schematic representation of the feeding treatments, genotypes, fattening period and collected data.

Feeding Treatment

The animals of the two experiments were subjected to three different feeding treatments. While two diets contained faba beans as an alternative source of protein, the third diet was a soybean-based standard feed as control (soy). To investigate the influence of anti-nutritive substances on performance and health parameters, one of the experimental diets contained 20% of the VC−rich faba bean variety *Fuego* (VC+) and the other 20% of the VC−poor variety *Tiffany* (VC−). To meet the nutritional requirements of the chickens without soybean meal, 28.6% blue sweet lupines (*Lupinus angustifolius cv. Boruta*) and 10.5% peas (*Pisum sativum cv. Astronaute*) were also added to the two experimental diets. The feed was offered pelleted. The composition of the three different diets was calculated based on the recommendations of the German Society for Nutritional Physiology (GfE) and is presented in Table 2.1.

The VC content of the diets was measured with HPLC and the results showed that the VC content in the VC+ diets was 0.138% in experiment A and 0.136% in Experiment B. The VC content in the VC− diets was 0.022% and 0.016% in experiments A and B, respectively.

Dry matter, ash, crude protein, crude fibre, crude fat, neutral detergent fibre (NDF), starch and sugar were analysed according to VDLUFA (Association of German Agricultural Analytic and Research Institutes) methods [32] in the laboratory facilities of the Institute of Animal Nutrition of the Friedrich-Loeffler-Institut, Braunschweig, Germany. Tannin content was quantified with the AOAC Official Method 952.02 in the same laboratory.

Fatty acids were analysed according to ASU L 13.00-27/3 + −46 and ISO 12966-3/4 and amino acids according to VDLUFA III 4.11.1 and 4.11.5 (both SYNLAB Analytics & Services GmbH, Jena, Germany). The amounts of saturated, monounsaturated and polyunsaturated fatty acids were calculated there based on the results of the fatty acid analysis**.** The nitrogen-corrected apparent metabolizable energy content was calculated on the basis of the chemical analysis with the WPSA formula [33].

Table 2.1. Composition of diets and chemical analysis.

Item	Experiment A			Experiment B		
	Soy	VC+	VC−	Soy	VC+	VC−
Ingredient (%)						
Wheat	30.0	8.00	8.00	30.0	8.00	8.00
Corn	36.0	25.2	25.2	36.0	25.2	25.2
Soybean meal	24.4			24.4		
Blue sweet lupine cv. Boruta		28.6	28.6		28.6	28.6
Field pea cv. Astronaute		10.5	10.5		10.5	10.5
Field bean cv. Fuego		20.2			20.2	
Field bean cv. Tiffany			20.2			20.2
Grass meal	5.6	0.1	0.1	5.6	0.1	0.1
Soybean oil	0.2	2.7	2.7	0.2	2.7	2.7
Dicalcium phosphate	1.3	2.2	2.2	1.3	2.2	2.2
Calcium carbonate	1.0	0.7	0.7	1.0	0.7	0.7
Cattle salt (NaCl)	0.3	0.4	0.4	0.3	0.4	0.4
DL-Methionine	0.2	0.4	0.4	0.2	0.4	0.4
Broilerpremix[1]	1.0	1.0	1.0	1.0	1.0	1.0
Chemical analysis						
Dry matter abs (%)	90.0	90.3	90.1	89.7	90.1	90.2
Ash (g/kg DM)	67.6	64.5	64.9	67.3	64.3	67.0
Crude protein (g/kg DM)	211.6	220.5	228.3	213.0	213.1	214.3
Crude fat (g/kg DM)	29.7	56.2	58.7	33.5	67.0	67.3
Crude fibre (g/kg DM)	43.8	60.4	68.5	45.2	72.0	74.0
NDF (g/kg DM)	123.3	124.2	132.7	148.5	128.9	136.6
Starch (g/kg DM)	472.2	423.7	402.6	480.2	416.6	413.1
Sugar (g/kg DM)	40.7	35.2	33.0	37.0	33.5	32.3
SFA (g/100g fat)	17.4	15.2	15.4	13.6	14.9	14.6
MUFA (g/100g fat)	22.7	26.6	26.6	24.4	26.7	26.8
PUFA (g/100g fat)	59.9	58.2	58.0	62.1	58.3	58.6
Methionine (%)	0.49	0.48	0.43	0.46	0.50	0.49
Cysteine (%)	0.30	0.27	0.29	0.29	0.29	0.30
Lysine (%)	0.97	1.01	1.07	0.90	1.08	1.09
Threonine (%)	0.71	0.66	0.69	0.68	0.69	0.69
Vicin (%)	0.005	0.095	0.016	0.00	0.094	0.013
Convicin (%)	0.003	0.043	0.006	0.00	0.042	0.003
VC (Vicin + Convicin; %)	0.008	0.138	0.022	0.00	0.136	0.016
Tannin (mg/g)	4.22	4.48	4.01	3.74	3.39	3.89
Calculated energy content						
AME_n (MJ/kg)	14.1	14.3	14.1	12.9	13.0	12.9

VC+: VC-rich faba bean diet, VC−: VC-poor faba bean diet, DL-Methionine: racemic mixture of dextrorotary and laevorotary Methionine, NDF: neutral detergent fibre, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, AME_n: nitrogen-corrected apparent metabolizable energy; [1] vitamin-mineral premix provided per kg of diet: Fe, 32 mg; Cu, 12 mg; Zn, 80 mg; Mn, 100 mg; Se, 0.4 mg; I, 1.6 mg; Co, 0.64 mg; retinol, 3.6 mg; cholecalciferol, 0.088 mg; tocopherol, 40 mg; menadione, 4.5 mg; thiamine, 2.5 mg; riboflavin, 8 mg; pyridoxine, 6 mg; cobalamin, 32 µg; nicotinic acid, 45 mg; pantothenic acid, 15 mg; folic acid, 1.2 mg; biotin, 50 µg; choline chloride, 550 mg.

Data Collection

To determine body weight development, all animals were weighed individually on a weekly basis over the experimental period. Feed consumption was measured weekly by weighing the amount of feed offered to the animals and the amount of remaining feed on a pen basis.
At the end of the experiment, a mixed sample of all feed bags from each of the diets was analysed in order to determine the chemical composition, the VC and Tannin content, fatty acid composition and the amino acids Methionine, Cysteine, Lysine and Threonine (Table 2.1).
Health status and mortality were checked daily. Mortality was recorded and the bodies of the deceased animals were examined for pathological changes and anatomical disorders.
The animals were slaughtered at a target weight of approximately 2100 g to assess fattening and slaughter performance. Therefore, the slaughter age of the breeds varied between 10 to 17 weeks of life (Figure 2.1). After 14 hours of fasting, the animals were brought to the poultry slaughterhouse of the Department of Animal Sciences of Goettingen University.
Before slaughter, the weight of each animal was determined. The birds were electrically stunned and killed by neck cut. After scalding and plucking, the feet were removed and the carcasses eviscerated and rinsed. Until dissection on the following day, they were chilled at 1 °C. After storage for 24 hours, the carcasses were weighed (without head, innards and feet) and dissected according to a standardized procedure. The weights of breast fillets (*M. pectoralis supf.*, without skin) and legs (thigh + drumstick) were recorded. Carcass yield was calculated by determining the carcass weight as a percentage of live weight for each animal. The percentages of breast and leg were calculated as the portion of the respective part on the carcass weight.
Not all genotypes were slaughtered exactly at the planned target weight of 2100 g. The capacity of the laboratory facilities allowed only slaughtering one genotype per week, so that in case two genotypes in one experiment had a similar weight development, they had to be slaughtered in two consecutive weeks. This was the case with VH and WR in experiment A, and with VBG and BWR in experiment B. Limited laboratory capacity was also the reason the BG had to be slaughtered at an earlier time point and before reaching the target weight.

Statistics

Due to the different slaughter ages, the statistical analysis of the evaluated parameters was performed separately for each genotype. The statistical analysis of the weight gain data was first started by applying an initial model as a 4th-order polynomial growth function and then fitted using the backward selection approach. The initial model is represented as follows:

$$y_{ijkl} = \mu + F_i + b_s W_{ij} + \sum_{v=1}^{4} b_{rv} \left(A_{ij}\right)^v + \sum_{v=1}^{4} b_{tv} F_i \left(A_{ij}\right)^v + p_k + e_{ijkl} \qquad (2.1)$$

where y_{ijkl} is the weekly weight, μ is the general mean, F_i is the fixed effect of treatment (feeding group), b_s is the fixed regression coefficient of the pre-treatment weight (W_{ij}), b_{rv} are the fixed regression coefficients up to the fourth polynomial degree of age (A_{ij}), b_{tv} are the fixed regression coefficients of the interaction between treatment and age, p_k is the random effect of the pen and e_{ijkl} is the random error. Using a backward selection, the non-significant regression coefficients of different polynomial degrees were removed from the initial model by applying F-statistics [34]. All statistical analyses were carried out using the procedure ‘mixed’ of the statistical program SAS (SAS 9.3., SAS Institute Inc., Cary, NC, USA). The final models for the respective genotypes are presented in Table 2.2. Least square means, which are adjusted means at average value of the considered covariates age and initial weight, were estimated by applying the least squares means (LSMEANS) statement. Significant differences between least square means were tested using Tukey-Kramer post-hoc tests by the PDIFF (p-value difference) option in the LSMEANS statement. Standard errors of least square means were calculated as described by Littell et al. [35].

For assessing the daily gain, the weight gain of the whole experimental period was divided by the number of fattening days and analysed using a linear mixed model with the feeding group as fixed and the pen as random effect. The analysis of daily feed intake (DFI) was performed using a similar statistical approach as for the growth. Therefore, the weekly feed intake per pen was transformed to daily feed intake per animal. The model is shown below, where y_{ijkl} is the daily feed intake and the other variables as explained above:

$$y_{ijkl} = \mu + F_j + \sum_{v=1}^{4} b_{rv} \left(A_{ij}\right)^v + \sum_{v=1}^{4} b_{tv} F_i \left(A_{ij}\right)^v + p_k + e_{ijkl} \qquad (2.2)$$

The feed conversion ratio (FCR) was calculated by dividing the amount of feed consumed during the experiment through the weight gain over the same period. Statistical analysis was done using a linear mixed model with the treatment group as fixed and the pen as random effect. For the analysis of slaughtering parameters, the following linear mixed model was used:

$$y_{ijkl} = \mu + F_i + b_r W_j + p_k + e_{ijkl} \qquad (2.3)$$

where y_{ijkl} is the respective parameter (carcass weight, carcass yield, breast and leg weight and percentage), μ is the overall mean, F_i is the treatment (feeding group), b_r is the regression coefficient of the pre-experimental weight (W_j), p_k is the random effect of the pen and e_{ijkl} is the random error.

For the time frame from 4 to 10 weeks of life, weight data of all six genotypes was available, so that a genotype comparison was done for this period. The weight at week 4 served as pre-treatment weight and was set as co-variable. The following model was used:

$$\begin{aligned} y_{ijklm} = \mu + B_i + F_j + b_s W_{ijk} + b_r A_{ijk} + b_r A_{ijk}^2 + B_i F_j + b_r B_i A_{ijk} \\ + b_r B_i F_j A_{ijk} + p(E)_l + e_{ijklm} \end{aligned} \qquad (2.4)$$

where y_{ijklm} is the weekly weight, μ is the general mean, B_i is the fixed effect of genotype, F_j is the fixed effect of treatment (feeding group), b_s is the fixed regression coefficient of the pre-treatment weight (W_{ijk}), b_r is the fixed regression coefficient of the age (A_{ijk}), $p(E)_l$ is the random effect of the experiment nested in pen and e_{ijklm} is the random error. Additionally, for FCR a genotype comparison for the period from week 4 to 10 was performed. As fixed effects the genotype, the treatment group and their interaction were included in the model.

Table 2.2. Significance of sources of variation in the analysis of weight development.

Genotype	Effect	Type III Sum of Squares	
		F Statistic	p Value
BG	FG	0.98	0.4128
	Age	0.80	0.3721
	Age ^{2}	5.42	0.0202
	Age ^{3}	5.40	0.0205
	Start	465.46	<0.0001
VH	FG	1.11	0.3396
	Age	1490.51	<0.0001
	Age ^{2}	197.13	<.0001
	Age × FG	2.73	0.0656
	Start	524.32	<0.0001
WR	FG	2.24	0.1406
	Age	54.46	<0.0001
	Age ^{2}	339.34	<0.0001
	Age ^{3}	405.57	<0.0001
	Age × FG	17.64	<0.0001
	Start	17.64	<0.0001
VBG	FG	4.23	0.0277
	Age	16.96	<0.0001
	Age ^{2}	14.71	0.0001
	Age ^{3}	23.08	<0.0001
	Age ^{4}	32.76	<0.0001
	Age × FG	4.11	0.0166
	Start	255.28	<0.0001
VWR	FG	5.54	0.0161
	Age	8.71	0.0032
	Age ^{2}	3.82	0.0509
	Age ^{3}	10.43	0.0013
	Age ^{4}	19.87	<0.0001
	Age × FG	18.35	<0.0001
	Start	439.35	<0.0001
BWR	FG	10.94	0.0034
	Age	8.75	0.0032
	Age ^{2}	6.33	0.0120
	Age ^{3}	11.72	0.0006
	Age ^{4}	18.48	<0.0001
	Start	236.78	<0.0001

BG: Bresse Gauloise; VH: Vorwerkhuhn; WR: White Rock; VBG: VH male × BG female; VWR: VH male × WR female; BWR: BG male × WR female; FG: feeding group; Age: age in weeks; Age ^{2,3,4}: age to the power of 2, 3, 4, Start: pre-experimental weight.

Results

Weight Gain

The development of bodyweight under the influence of feeding treatments is shown in Figure 2.2A. For experiment A, the animals of the BG breed were slaughtered in the 10th week of life. At this time, the live weight of the control group (soy) was 1883 g, while the weights of VC+ and VC− groups were 1888 g and 1905 g, respectively. Group VH was slaughtered in week 16 and the birds reached a final body weight of 2164 g (soy), 2139 g (VC+), and 2196 g (VC−). The WR chickens weighed 2308 g (soy), 2279 g (VC+), and 2233 g (VC−) when slaughtered in the 17th week of life.

For experiment B, the birds of the VBG group reached the target weight of 2100 g in the 13th week of life, as the final weights were 2114 g (soy), 2124 g (VC+), and 2122 g (VC−). Group BWR was slaughtered with 14 weeks and the weights were 2195 g, 2299 g and 2271 g in the soy, VC+ and VC− groups respectively. The VWR animals reached the slaughter weight in their 15th week of life. The respective weights for soy, VC+ and VC− were 2081 g, 2042 g and 2052 g.

The daily weight gains for all genotypes are presented in Table 2.3. The highest values were achieved by BG and the lowest by VH and WR. However, no significant effect of the feeding group on daily weight gain was found for the genotypes studied. The effect of the different variables on the body weight development of the different genotypes is presented in Table 2.2. The statistical model for the growth curves of the BG and WR fitted best with a third order polynomial degree of the age. For VH only a model with significant effects of the first and second order polynomial degree for the parameter age was selected. For the crossbreds VBG, VWR and BWR, a model with the fourth-order polynomial degree was found to be significant. While in BG and BWR no significant interactions between the fixed effect of the feeding treatment and the regression parameters of age could be detected, a significant effect of the interaction between the fixed effect of the feeding treatment and the linear regression term of age was observed in the genotypes VH, WR, VBG and VWR. Since the effect of the feeding treatment on weight development was only marginal, these interaction effects are negligible with respect to the extent of weight development (Figure 2.2A).

A significant effect of the feeding treatment is indicated for the crosses VBG and VWR (Table 2.2). However, this is neither reflected in the bodyweight development (Figure 2.2A), nor in the bodyweights adjusted by the mean of age (Figure 2.2B). The differences observed at the beginning of the experiment between the respective feeding groups decreased over time. These small differences were detected by the test statistics, but vanished when the whole period was taken into account. For the crossbred BWR, however, significant differences in weight development between the feeding treatments were found during the entire experiment. The soy-fed BWR group showed a significantly lower weight gain compared to the two faba bean groups. The difference between the groups was −105 g (Soy vs. VC+) and −77 g (Soy vs. VC−), respectively. This difference is also reflected in the mean bodyweights adjusted by the mean of age (Figure 2.2B).

The comparison of the genotypes from week five to ten showed differences between the respective genotypes, which became clearer with increasing age (Figure 2.3A). In week ten the BG showed the significantly highest weights with 1823 g, followed by the two BG-crosses VBG and BWR (1580 g, 1536 g), which also differed significantly at this time point. The VH were with 1342 g significantly lighter than the BG and its crosses but heavier than the WR (1286 g). The VWR birds' weights (1312 g) were in between VH and WR. The LS-means adjusted for the mean of age (Figure 2.3B) showed a similar picture but the difference between VBG and BWR was not statistically significant in this case.

Adjusted by the mean of age, the difference between the soybean and faba bean groups was statistically significant (Figure 3C), with the difference amounting to −19 g (soy vs. VC−) and −21 g (soy vs. VC+). Within the respective genotypes no significant differences between the feeding groups could be determined.

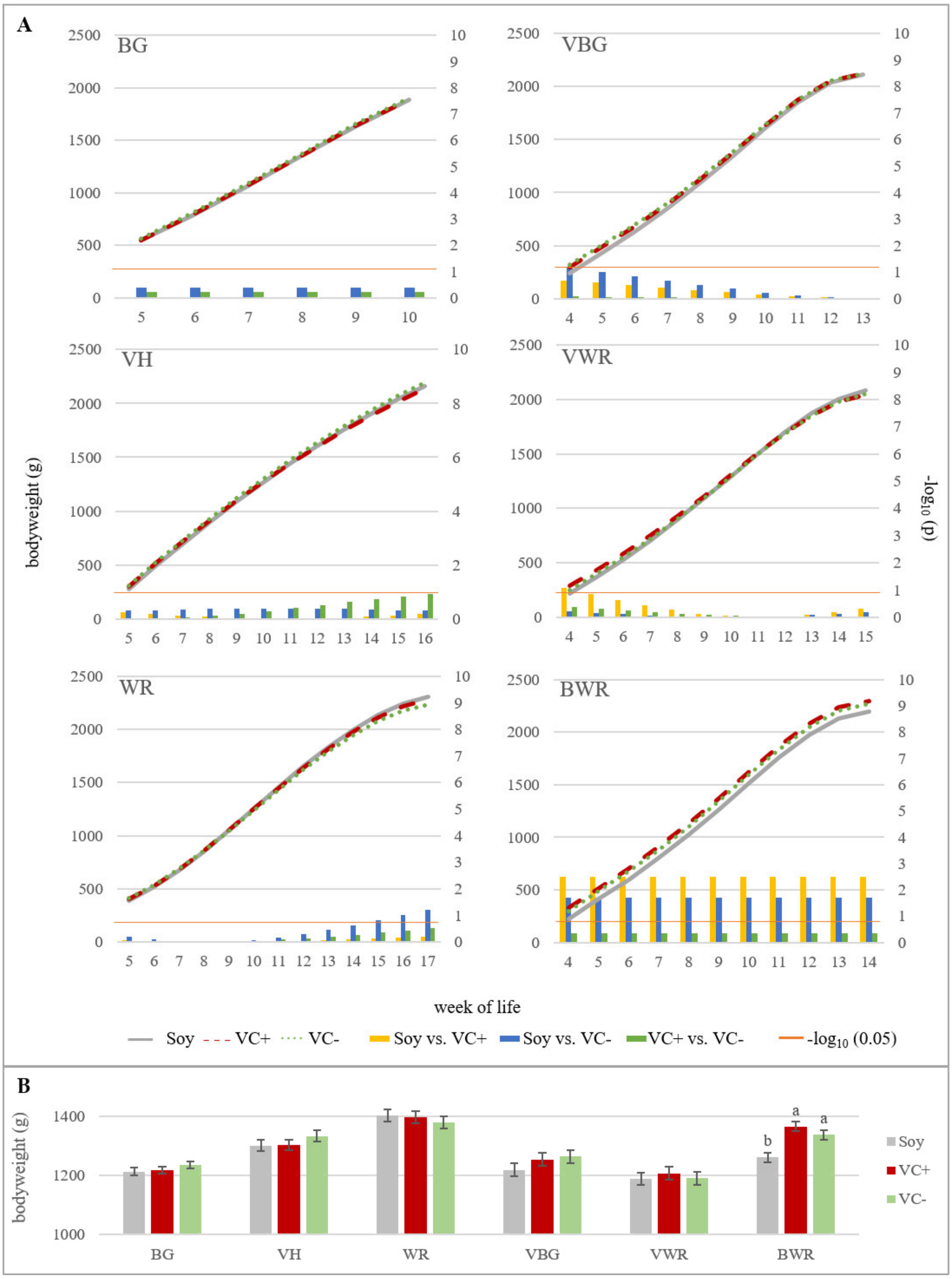

Figure 2.2. Effect of feeding treatment on bodyweight development within genotypes (**A**) and LSMEANS ± SE for the effect of feeding treatment within genotypes adjusted by the mean of age (**B**). In A, curves show bodyweight development for the respective feeding groups. The bar charts exhibit the differences between LS-means of the different combinations of feeding groups on a weekly base. The orange line represents the significance threshold of $p = 0.05$. Bars that cross this line imply significant differences between feeding groups in the respective week. In B bars with different letters within one genotype show significant differences ($p < 0.05$).

Table 2.3. Effect of feeding treatment on the daily weight gain (in g).

	Purebreds			Crossbreds		
	BG	**VH**	**WR**	**VBG**	**VWR**	**BWR**
Soy	34.9 ± 0.6	21.8 ± 0.6	22.1 ± 0.6	27.8 ± 0.5	23.2 ± 0.5	27.2 ± 0.6
VC+	35.3 ± 0.6	21.4 ± 0.6	22.1 ± 0.6	27.7 ± 0.5	22.5 ± 0.5	27.8 ± 0.6
VC−	35.7 ± 0.6	22.2 ± 0.6	21.2 ± 0.6	27.7 ± 0.5	23.0 ± 0.5	27.6 ± 0.6

Least square means ± SE. BG: Bresse Gauloise; VH: Vorwerkhuhn; WR: White Rock; VBG: VH male × BG female; VWR: VH male × WR female; BWR: BG male × WR female, VC+: VC−rich faba bean diet, VC−: VC−poor faba bean diet. Values in the same column with no superscript are not significantly different ($p > 0.05$).

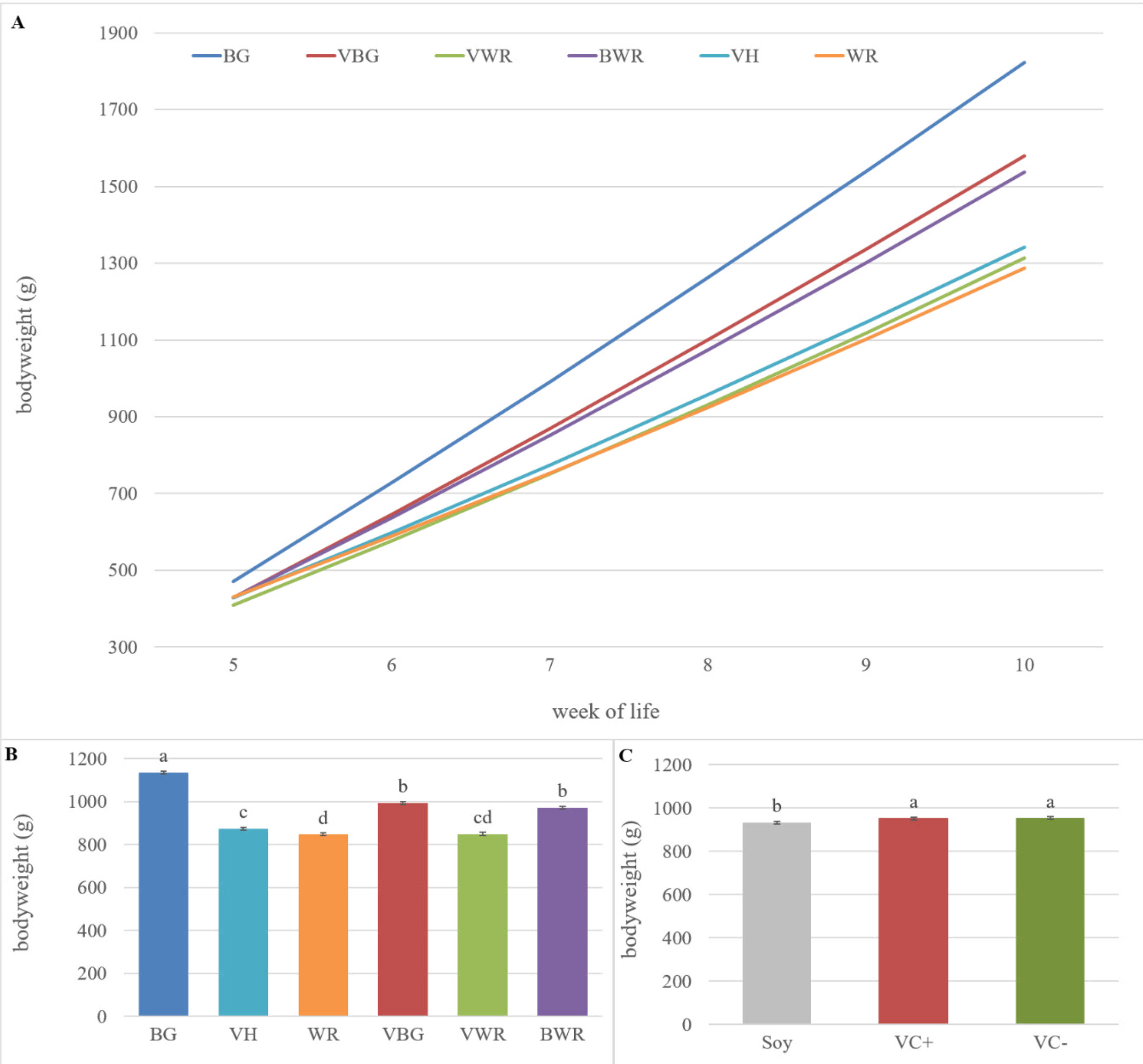

Figure 2.3. Comparison of the genotypes from week of life five to ten. (**A**) Effect of genotype on weight development. (**B**) LS-means ± SE for bodyweight of the respective genotypes adjusted by the mean of age. (**C**) LS-means ± SE for bodyweight of the different feeding groups adjusted by the mean of age. [a, b, c, d] Genotypes not sharing a letter differ at $p < 0.05$.

Mortality

The mortality in the present study was very low, of all 694 animals, only 5 animals died. The mortality rate in experiment A across all breeds was 0.6%. For the VH breed the mortality rate was 1.1% and for the WR it was 0.8%. There were no losses observed for BG. Towards the end of the experiment, which overlapped with the beginning of sexual maturity, aggressive behaviour of the cocks among each other occurred, especially in the breed VH, which resulted in picking injuries.

The overall mortality at the end of the fattening period in experiment B amounted to 0.8%, which was similarly low as in in the purebred animals of experiment A. While no animal losses were observed in the BWR breed, the mortality rate for the VBG breed was 1.7% and for the VWR breed 0.8%. There was no influence of the feeding treatment on the mortality of the respective genotypes ($p > 0.05$).

Feed Intake and Efficiency

The feed efficiency for the different feeding groups and genotypes for the whole experimental period is presented in Table 2.4. No significant differences were found between the feeding groups of the respective breeds.

A comparison between genotypes was only possible for the time period from week 5 to 10, where data from all genotypes was available (Table 2.5). No statistically significant differences of the FCR were found between the feeding groups in general and within the respective genotypes as could also be seen in the separate analysis of the genotypes over the entire experimental period. In contrast, between the genotypes significant differences were detected. The BG showed the best feed efficiency (2.56 kg/kg) compared to all other genotypes. The VBG had a FCR of 2.76 kg/kg, which was significantly better than that of the VWR (2.91 kg/kg). The BWR, VH and WR were in between these two genotypes with FCRs of 2.74 kg/kg, 2.82 kg/kg and 2.84 kg/kg, respectively; differences were not significant. The LS-mean values of the daily feed intake (DFI) of the different feeding treatments of the genotypes are shown in Figure 2.4. The differences between the feeding groups within each genotype were rather small. Considering the whole experimental period, no significant differences in the mean DFI could be detected. Only in VBG chicks, the DFI differed significantly between the soy and VC− groups in week 7 and 8, hence the DFI of the soy group was reduced by 8 g per day.

The statistical model for the development of daily feed intake with age had the best fit at the polynomial degree of fourth order of age in the genotypes WR, VWR and BWR. For VBG it had to be reduced to the third order, and for BG and VH a model with only the first and second polynomial degree of age was significant. Significant interactions between the fixed effect of feeding treatment and the regression parameter of age were found only in VBG and VWR. For these two genotypes, the interaction was significant up to the polynomial degree of third order of the age.

Table 2.4. Least square means (±SE) for the effect of feeding treatment on feed conversion ratio (FCR, in kg/kg) and daily feed intake (DFI, in g).

		Purebreds			Crossbreds		
		BG	**VH**	**WR**	**VBG**	**VWR**	**BWR**
FCR	**Soy**	2.57 ± 0.03	3.93 ± 0.28	3.97 ± 0.09	3.42 ± 0.15	3.76 ± 0.13	3.75 ± 0.82
	VC+	2.58 ± 0.03	4.25 ± 0.28	4.04 ± 0.09	3.23 ± 0.15	3.76 ± 0.13	3.42 ± 0.82
	VC−	2.54 ± 0.03	4.39 ± 0.28	4.01 ± 0.09	3.56 ± 0.15	3.89 ± 0.13	3.53 ± 0.82
DFI	**Soy**	95.2 ± 1.6	86.9 ± 2.3	89.0 ± 2.5	95.6 ± 1.5	86.7 ± 2.6	96.1 ± 1.9
	VC+	95.7 ± 1.6	93.2 ± 2.3	87.2 ± 2.5	94.7 ± 1.6	84.2 ± 2.6	92.6 ± 1.9
	VC−	95.1 ± 1.6	94.2 ± 2.3	87.3 ± 2.5	96.1 ± 1.5	85.4 ± 2.6	93.6 ± 1.9

BG: Bresse Gauloise; VH: Vorwerkhuhn; WR: White Rock; VBG: VH male × BG female; VWR: VH male × WR female; BWR: BG male × WR female; FCR: feed conversion ratio; DFI: daily feed intake, VC+: VC−rich faba bean diet, VC−: VC-poor faba bean diet. Values in the same column with no superscript are not significantly different ($p > 0.05$).

Table 2.5. Least square means (±SE) for feed conversion ratio (FCR. in kg/kg) from week four to ten by genotype*feeding treatment and by genotype.

	Purebreds			Crossbreds		
	BG	**VH**	**WR**	**VBG**	**VWR**	**BWR**
Soy	2.57 ± 0.06^{a}	2.76 ± 0.06^{ab}	2.86 ± 0.06^{b}	2.77 ± 0.06^{ab}	2.90 ± 0.06^{b}	2.79 ± 0.06^{ab}
VC+	2.58 ± 0.06	2.90 ± 0.06	2.86 ± 0.06	2.75 ± 0.06	2.83 ± 0.06	2.70 ± 0.06
VC−	2.54 ± 0.06^{a}	2.79 ± 0.06^{ab}	2.80 ± 0.06^{ab}	2.77 ± 0.06^{ab}	3.00 ± 0.06^{b}	2.74 ± 0.06^{ab}
	2.56 ± 0.04^{a}	2.82 ± 0.04^{bc}	2.84 ± 0.04^{bc}	2.76 ± 0.04^{bc}	2.91 ± 0.04^{c}	2.74 ± 0.04^{b}

BG: Bresse Gauloise; VH: Vorwerkhuhn; WR: White Rock; VBG: VH male x BG female; VWR: VH male x WR female; BWR: BG male x WR female, VC+: VC-rich faba bean diet, VC−: VC-poor faba bean diet. $^{a, b, c}$ Values in the same row with different superscripts are significantly different ($p < 0.05$).

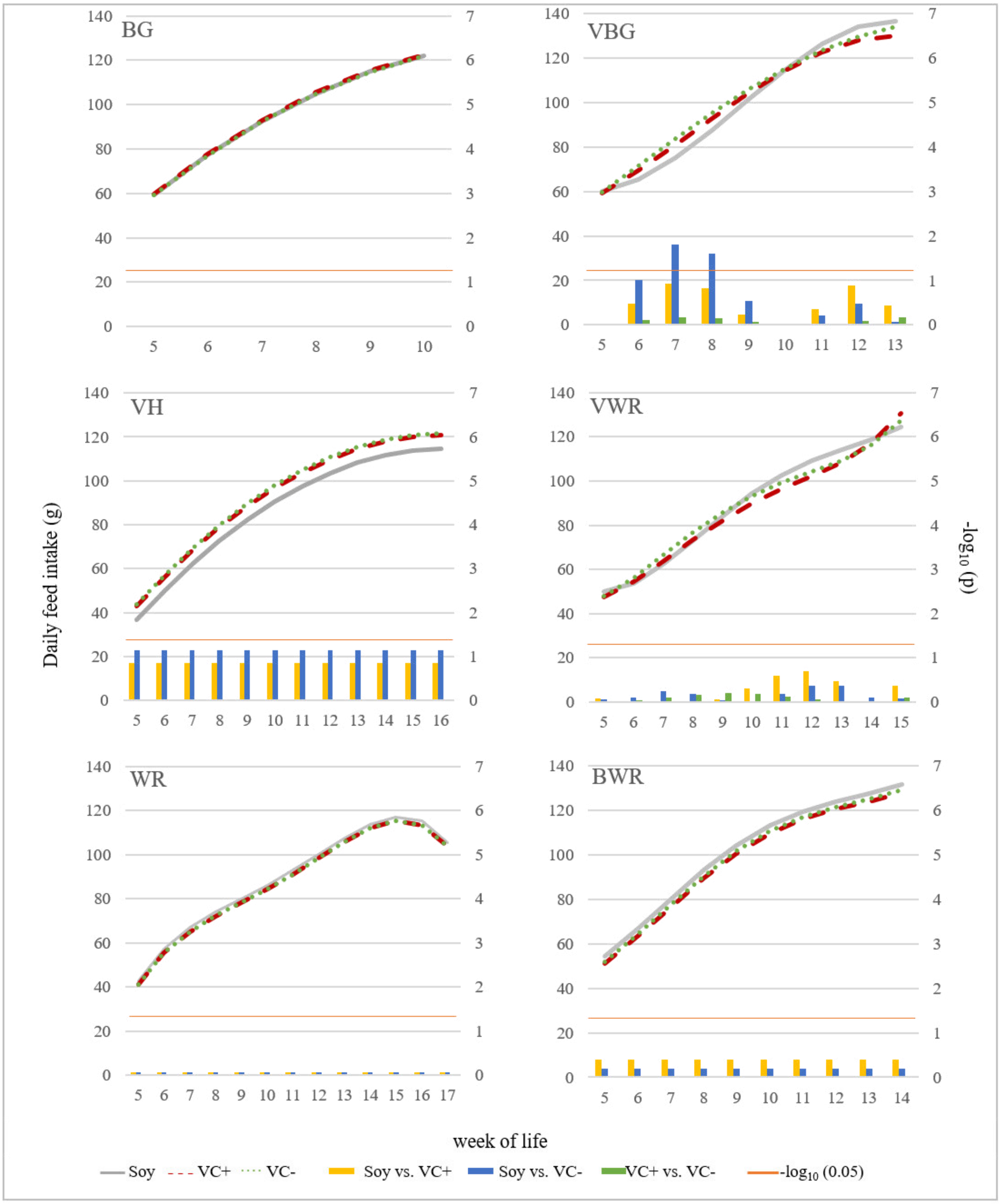

Figure 2.4. Effect of feeding treatment on daily feed intake (DFI) within genotypes. Curves show development of DFI for the respective feeding groups. The bar charts exhibit the differences between LS-means of the different combinations of feeding groups on a weekly base. The orange line represents the significance threshold of $p = 0.05$. Bars that cross this line imply significant differences between feeding groups in the respective week.

Slaughtering Performance

The final weights and the measured parameters of fattening performance are shown in Table 2.6. The yield, which represents the ratio of carcass to live animal weight, varied between 66.3% to 69.9% for the different genotypes. Within the genotypes the differences between diets were in general less than 1%. Only between the VC− and soy group of BG the difference was 1.2%, although not significantly different.

The breast yields were very similar in all six genotypes, too. The BG reached generally the highest values with the maximum of 13.3% in the soy group, while the VH and WR showed the lowest values with less than 11% in all feeding groups. The VWR yielded breast percentages of 11.3% (soy, VC+) and 11.2% (VC−). The breast yields of VBG and BWR have been slightly higher. In VBG the soy group had the highest breast yield with 11.9%, while in BWR the VC+ group performed best (12.0%). However, differences between the respective feeding groups were less than 0.5%.

With regard to leg yield, no fundamental differences were observed between the feeding groups of the six genotypes. The overall range was from 31.5% to 34.9% with maximum differences within genotypes of equal or less than 0.5%.

There were no significant differences in slaughtering parameters for the genotypes BG, VH, WR, VBG and VWR between feeding groups, while in contrast for the genotype BWR significant differences in the absolute weights of carcass, breast and leg were determined. The respective weights of the VC+ group were significantly higher than that of the soy group, which resulted in a difference of 99 g for carcass weight, 17.1 g for breast and 37.8 g for leg weight. The relative parameters carcass, breast and leg yield showed, as for the other genotypes, no significant differences.

The pre-experimental weights of the chicks affected the weights of carcass, breast and leg significantly in all six genotypes.

Table 2.6. Effect of feeding treatment on live weight at slaughter and carcass traits.

	Feed	Live Weight	Carcass Weight	Yield	Breast Weight	Breast Percentage	Leg Weight	Leg Percentage
		(g)	(g)	(%)	(g)	(%)	(g)	(%)
BG	Soy	1883 ± 14	1272 ± 18	67.5 ± 0.5	170 ± 3	13.3 ± 0.1	405 ± 9	31.8 ± 0.3
	VC+	1888 ± 14	1262 ± 18	67.1 ± 0.5	163 ± 3	12.9 ± 0.1	398 ± 9	31.5 ± 0.3
	VC−	1905 ± 14	1269 ± 18	66.3 ± 0.5	165 ± 3	13.0 ± 0.1	406 ± 9	32.0 ± 0.3
VH	Soy	2164 ± 21	1442 ± 32	69.5 ± 0.3	155 ± 5	10.8 ± 0.2	478 ± 12	33.1 ± 0.4
	VC+	2139 ± 21	1407 ± 32	69.5 ± 0.3	152 ± 5	10.8 ± 0.2	465 ± 12	33.0 ± 0.4
	VC−	2196 ± 21	1448 ± 32	69.0 ± 0.3	156 ± 5	10.7 ± 0.2	478 ± 12	32.9 ± 0.4
WR	Soy	2308 ± 21	1571 ± 32	68.7 ± 0.5	169 ± 7	10.7 ± 0.3	538 ± 8	34.3 ± 0.4
	VC+	2279 ± 21	1573 ± 32	69.1 ± 0.5	170 ± 7	10.8 ± 0.3	538 ± 8	34.2 ± 0.4
	VC−	2233 ± 21	1518 ± 32	69.5 ± 0.5	161 ± 7	10.6 ± 0.3	519 ± 8	34.2 ± 0.4
VBG	Soy	2114 ± 25	1431 ± 25	68.7 ± 0.3	170 ± 4	11.9 ± 0.2	479 ± 10	33.5 ± 0.2
	VC+	2124 ± 25	1481 ± 22	68.5 ± 0.2	170 ± 4	11.5 ± 0.2	505 ± 9	33.9 ± 0.2
	VC−	2122 ± 25	1482 ± 25	68.8 ± 0.3	176 ± 4	11.8 ± 0.2	498 ± 10	33.6 ± 0.2
VWR	Soy	2081 ± 22	1431 ± 18	69.4 ± 0.3	162 ± 4	11.3 ± 0.2	492 ± 12	34.4 ± 0.3
	VC+	2042 ± 22	1407 ± 19	69.3 ± 0.3	159 ± 4	11.3 ± 0.2	487 ± 12	34.5 ± 0.3
	VC−	2052 ± 22	1412 ± 18	68.6 ± 0.3	158 ± 4	11.2 ± 0.2	488 ± 12	34.5 ± 0.3
BWR	Soy	2195 ± 20 ^{b}	1505 ± 27 ^{b}	68.9 ± 0.3	175 ± 4 ^{b}	11.6 ± 0.2	521 ± 10 ^{b}	34.6 ± 0.2
	VC+	2299 ± 20 ^{a}	1604 ± 26 ^{a}	69.9 ± 0.3	192 ± 4 ^{a}	12.0 ± 0.2	559 ± 10 ^{a}	34.9 ± 0.2
	VC−	2271 ± 20 ^{a}	1573 ± 26 ^{ab}	69.5 ± 0.3	185 ± 4 ^{ab}	11.7 ± 0.2	547 ± 10 ^{ab}	34.7 ± 0.2

LS-means ± standard error. BG: Bresse Gauloise; VH: Vorwerkhuhn; WR: White Rock; VBG: VH male × BG female; VWR: VH male × WR female; BWR: BG male × WR female, VC+: VC-rich faba bean diet, VC−: VC-poor faba bean diet. $^{a, b}$ Means with different superscripts within one column and genotype show significant differences ($p < 0.05$).

Discussion

The results of the present study suggest that feeding male birds of the six genotypes studied with 20% vicin-rich or vicin-poor faba beans as compared to a soybean-based diet had no negative effects on growth, feed efficiency and fattening performance.

This finding is in agreement with Farrel et al. [36], who recommended 20% faba beans as the maximum inclusion rate in broiler diets. With 24% faba beans, the authors noticed reduced weight gain in finishing broilers compared to the group with only 18% faba beans. Koivunen et al. [37] also observed lower bodyweight and reduced feed consumption with 24% faba beans in the diet compared to a soybean-based control. In contrast, another study found that even the feeding of 50% faba beans in the ration did not affect weight development, feed intake, dressing percentage and the weights of breasts and legs [38].

These aforementioned studies were conducted to assess the effects of faba beans in broiler feeding, but did not provide information on the VC content of the faba beans used.

Dal Bosco et al. [39] fed slow-growing broilers a ration with 16% faba beans and a VC content of 0.3%. This resulted in lower daily weight gain and reduced feed efficiency until day 60 and lower carcass weights in the faba bean group at slaughter. The carcass, breast and thigh yield were not affected by the feeding regime. The growth depression, that was observed during the early stage of development, is probably due to the higher susceptibility of younger animals towards the anti-nutritional factors of the faba bean such as VC or the higher requirements for essential amino acids at this age.

However, Laudadio et al. [22] found no differences in growth, feed intake and carcass traits of broilers fed 31% micronized faba beans with a VC content of 0.12% of dry matter.

In the present study, no influence of faba bean feeding on daily feed intake and feed efficiency was observed. In other studies even better feed efficiencies have been described when feeding 30% faba beans [36,40].

The mortality in the present study was rather low in both experiments A and B and no significant link to any of the feeding treatments was detected. These results are consistent with those from Laudadio et al. [22] and Gous [28], who did not report any influence of the faba bean feeding on the cases of death occurring during their experiments.

In addition to faba beans, which were with their differing VC contents the focus of the present study, the grain legumes blue sweet lupine and field pea were also used in the experimental diets to compose soybean meal free diets. Although the portion of lupines of 28.6% was clearly higher than recommended in the literature [41] and the total legume content was almost 60%, no disadvantage of the two faba bean groups in comparison to the control group was found in the present study. This may be an indication that the combination of different grain legume species allows a higher legume content in total without the anti-nutritional effects of the individual legumes. However, to prove this theory, further research is needed.

Due to the different ages at slaughter, a statistical comparison of the genotypes was only possible for a certain period of the fattening phase, while the carcass traits could only be compared descriptively. There are few studies available in the performance of BG and crosses thereof, and this is to the best of our knowledge the first study providing data regarding the other genotypes. Muth et al. [42] compared the fattening performance of BG males with that of ISA 657 broilers under organic conditions. In that study, BG reached a live bodyweight of 2570 g at an age of 84 days. The carcass yield was 69.1% and breast yield was 18.7%. Another study showed a yield of 66.6% and breast yield of 19% for BG at a slaughter age of

17 weeks [43]. These values are comparable with those reported by Lambertz et al. [44] for BG and their crosses with New Hampshire hens. Siekmann et al. [45] reported a breast yield of 12.7% in the commercial dual-purpose chicken Lohmann Dual after 9 weeks of fattening. However, in the studies referred to above both the superficial and profound breast muscles were taken together for determining the breast weight, while in the present study and the one of Siekmann et al. [45] only the superficial muscle (*M. pectoralis supf.*) was used, which is probably the reason for the lower breast yield.

Differences between the genotypes are visible in the growth curves. As the curves of VH, WR, VBG, VWR and BWR have already started to flatten, the different time points for the slaughter of these genotypes are reasonable. In contrast, the curve of BG still has a linear slope, indicating that higher weights would have been possible, because this breed is still growing fast.

When comparing the genotypes, the BG showed the highest growth rate, while the WR showed the lowest. The VH grew slightly faster than the WR, while the crossbreds each performed between their parental breeds. The difference between the purebreds is caused by the genetic background of these genotypes, which indicates the BG is a meat-type dual purpose breed, while the VH is a layer-type dual purpose breed and the WR is a parental layer genotype.

In the present study, the feed conversion ratio varied from 2.54 (BG, VC−) to 4.39 (VH, VC−) depending on the genotype and feeding group and on the time point of analysis. Due to technical reasons, feed wastage during the experiments could not be avoided completely and so the daily feed intake and FCR may be overestimated. Nevertheless, it has been reported in the literature that the feed efficiency of local breeds and layer males is inferior to that of broilers. The high specialization of broilers went along with a constant increase in feed efficiency, leading to feed conversion ratios (FCR) as low as 1.5 kg/kg under optimum conditions [46]. In contrast, Lichovníkova et al. [47] reported values of 3.1 for Ross Broilers and 3.8 for ISA brown cockerels after a fattening period of 90 days under free-range husbandry conditions. Local breeds as the Belgian Malines and the Schweizerhuhn required 2.55 and 2.73 kg of feed to produce 1 kg life weight [48] and Perella et al. [49] reported a FCR of 3.8 kg/kg for the Italian slow growing genotype Gaina. In the case of layer males, FCRs of up to 10.0 kg/kg have been reported, depending on the husbandry system and length of the fattening period [50].

To gain more information on the impact of faba bean feeding and the role of VC on certain chicken genotypes, further studies investigating the protein digestibility of the diets and the bursa weight and abdominal fat content of the chicken carcass would be of value.

Conclusions

Regardless of their VC content, the feeding of 20% faba beans during the fattening period did not affect the growth and fattening performance of the six examined genotypes, when compared to a standard soy diet. Therefore, the studied faba bean varieties, both VC rich and VC poor, do not appear to have adverse effects on animal mortality and growth performance for these chicken genotypes and could be an alternative to soybeans from this point of view. Because of its high growth rate and good feed efficiency, the meat-type BG is the most suitable genotype for fattening among the tested ones. As a cross-breeding partner, BG shortens the fattening period and improves the feed efficiency of the layer-type genotypes VH and WR. However, because BG, VH and their crosses are dual-purpose chickens, the results of the present study need to be consolidated with performance data of the respective hens, which will be communicated in a separate publication.

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Chapter 3

Egg Production and Bone Stability of Local Chicken Breeds and Their Crosses Fed with Faba Beans

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Simple Summary: Poultry production systems are currently facing important issues like animal welfare, the environmental impact of soy imports from overseas and the decline in genetic diversity. The current study aims at testing an alternative production system that could provide niche markets with regional poultry products. Six different chicken genotypes were tested in this study regarding egg production traits and bone stability. As a regional alternative to soy, two varieties of locally grown faba beans have been used in the animals' diets. A limited adverse effect of the vicin-rich faba bean diet on egg weight was observed. The crossbred chicken of the local breed Bresse Gauloise with the commercial laying hen White Rock seems to be the most promising.

Abstract: Poultry production is raising concerns within the public regarding the practice of culling day-old chicks and the importation of soy from overseas for feedstuff. Therefore, an alternative approach to poultry production was tested. In two consecutive experiments, two traditional chicken breeds, Vorwerkhuhn and Bresse Gauloise, and White Rock as a commercial layer genotype as well as crossbreds thereof were fed diets containing either 20% vicin-rich or vicin-poor faba beans, though addressing both subjects of debate. Hen performance traits and bone stability were recorded. All parameters were considerably influenced by the genotype with White Rock showing the significantly highest ($p < 0.05$) laying performance (99.4% peak production) and mean egg weights (56.6 g) of the purebreds, but the lowest bone breaking strength (tibiotarsus 197.2 N, humerus 230.2 N). Regarding crossbreds, the Bresse Gauloise x White Rock cross performed best (peak production 98.1%, mean egg weight 58.0 g). However, only limited dietary effects were found as only the feeding of 20% vicin-rich faba beans led to a significant reduction of egg weights of at most 1.1 g ($p < 0.05$) and to a significant reduction of the shell stability in the crossbred genotypes. In terms of dual-purpose usage, crossing of Bresse Gauloise with White Rock seems to be the most promising variant studied here.

Keywords: bone stability; crossbreeding; egg weight; faba bean; laying performance; local breeds; vicin

Introduction

Public demands placed on agriculture have changed drastically in recent years. While only a few decades ago, the main goal was to produce sufficient good quality food at a favorable price, today's farmers are encouraged to consider more ethical issues, such as animal welfare and the conservation of natural and climatic resources.

One major ethical concern considers the killing of day-old male chicks of the layer lines due to economic reasons as very critical leading to a ban (of this procedure) in Germany as soon as alternative solutions are ready for practical use [1]. One possible alternative could be the use of dual-purpose chickens, where the hens can be used for egg production while the cocks are fattened for meat production. The main problem of this production system is the negative genetic correlation between laying and fattening performance [2,3], making it difficult to improve both traits in the same line. However, crossbreeding could improve the chickens' overall performance, as shown in local chicken breeds by Götze and von Lengerken [4]. Moreover, the use of local chicken breeds in agricultural production contributes to the conservation of these breeds as genetic resources [5].

Animal welfare is an issue that is becoming increasingly important in poultry production. One major problem the egg industry facing currently is the high incidence of laying hens with skeletal disorders [6,7], both in intensive and extensive housing systems. It has been shown that not only nutrition and husbandry of the hens, but also genetics play an important role in bone stability [8]. While the majority of studies have investigated bone stability in contemporary laying hybrids, the number of such studies conducted in local chicken breeds is very limited. However, it was found that bone characteristics differ considerably between genotypes [9], which is why findings from high performing lines can probably not fully be transferred to local breeds. So far, there has been little discussion about how purebred local chicken breeds and their crossbreds differ in terms of bone characteristics.

In order to provide the meat producing sector with high-quality protein feed, the EU imports huge amounts of soybeans from overseas, namely 15.1 million metric tons in the season 2019/20 [10]. While the use of genetically modified seeds is common in the main producing countries USA and Brazil, this is seen critically by European consumers [11]. In addition, the negative environmental impact of the soybean production especially in South America [12] leads to a reduction of soybean imports in favor of regional protein crops in Germany [13]. A suitable regional protein plant is the faba bean (*Vicia faba L.*) [14]. However, until now, anti-nutritive substances, like for example, the endogenous glycosides vicin and convicin (together abbreviated as VC) limit the use of faba beans in animal nutrition. Today few VC-poor varieties are available, for example, the variety Tiffany, but the majority of cultivated faba beans is still VC-rich, as less than one-third of the cultivation area used for seed production is planted with VC-poor varieties [15].

The influence of faba bean feeding and of VC on the performance and health of laying hens has been studied for decades. However, the results of the studies and the recommended maximum levels for hen diets vary greatly and are usually evaluated with commercial chicken genotypes. Jeroch et al. [16] recommend maximum levels of 10% for conventional faba bean varieties and 20% of varieties with reduced VC content during the laying period. Described consequences of higher faba bean fractions in the feed are, for example, increased animal mortality [17], reduction of laying performance [17,18], and of egg weight [19–21]. In contrast, Daenner [22] did not find any performance reductions feeding a diet with 30% vicin-rich faba beans.

Addressing alternative ways of poultry production for niche markets, the present study aims at evaluating different egg production and bone stability traits of two local chicken breeds, one commercial line, and of their crossbreds, while feeding regional faba beans with differing VC-contents. By comparing pure and crossbreds, we aim to investigate whether the high performing line will increase the performance of the local breeds to such an extent that it will become economically viable for poultry production.

Materials and Methods

Ethical Note

The current experiments were performed in accordance with the German Animal Welfare Law and approved by the Lower Saxony State Office for Consumer Protection and Food Safety (LAVES) (33.19-42502-04-17/2600).

Stock and Husbandry

The study included three purebred and three crossbred genotypes of domestic chicken (*Gallus gallus domesticus*). The purebred genotypes were Vorwerkhuhn (VH), Bresse Gauloise (BG) and White Rock (WR). VH is a local chicken breed from Germany, which was originally bred for dual-purpose usage. The BG hens originate from the Bresse region in the south of France Burgundy County, where they are marketed as a delicacy with protected designation of origin (PDO) [23]. They achieve an annual laying performance of around 250 eggs [24]. WR is a commercial layer line from Lohmann Tierzucht GmbH (Cuxhaven, Germany), which originates, amongst others, from Plymouth Rock chicken. As high performing line, WR is a founder population of some modern laying hybrids with brown eggshell color. To build up grandparent stocks, birds of the VH and BG breed were provided by fancy breeders of a conservation flock for poultry species. Based on these animals, parent stocks of VH and BG purebreds were generated, which were then used to generate the test animals. The WR hens were provided as day-old chicks by Lohmann Tierzucht GmbH (Cuxhaven, Germany).

Two consecutive experiments were conducted. In experiment A, purebred hens of the VH, BG and WR breeds were tested. Experiment B dealt with crossbreds thereof, which were generated by crossing cocks of VH and BG either with hens of BG or WR, resulting in three crossbreds: VH x BG (VBG), VH x WR (VWR) and BG x WR (BWR).

Hatching and rearing procedures were identical in both experiments. After being wing-tagged for identification, blood samples were taken from VH and BG chicks within the first week of life for sex determination via DNA analysis [25]. Female WR chicks were provided by the breeding company. In the case of experiment B, blood sampling and molecular sexing was only done for BWR chicks, because VWR and VBG chicks could be sexed visually based on different plumage color. Commercially available complete feeding stuffs for chicks (until 6 weeks of age; 11.4 MJ AMEn/kg DM, 180.0 g/kg crude protein, 26.1 g/kg crude fat, 37.5 g/kg crude fiber, 56.0 g/kg crude ash, 7.8 g/kg calcium, 4.7 g/kg phosphorous) and pullets (from 7 to 17 weeks of age; 11.0 MJ AMEn/kg DM, 145.0 g/kg crude protein, 37.0 g/kg crude fat, 65.05 g/kg crude fiber, 59.0 g/kg crude ash, 10.0 g/kg calcium, 6.0 g/kg phosphorous), as well as water were offered ad libitum. A standard lighting program was applied to the birds, where day length increased gradually from 8 h (8th weeks of age) to 14 h (23rd week of age). After hatch at the Institute of Animal Welfare and Animal Husbandry of the Friedrich-Loeffler-Institut (Celle, Germany), the chicks were raised in a floor housing system. At 7 weeks of age, all birds of the respective experiment were transferred to floor pens at the Institute of Farm Animal Genetics of the Friedrich-Loeffler-Institut (Mariensee, Germany), which were later used as experimental sites. The pens of 12.5 m^2 were equipped with wood chips, feeding and drinking troughs, a wooden perch, a dust bath, and nine laying nests.

Experimental Procedure

The experimental setup is shown in Figure 3.1. In both experiments, the testing period lasted from 18th until 52nd week of age. The hens were subjected to three different feeding treatments. While two diets contained faba beans as an alternative source of protein, the third diet was a soybean-based standard feed as control (Soy). In order to examine the effect of anti-nutritive substances on performance and bone characteristics, the experimental diets contained either 20% of the VC-rich faba bean variety Fuego (VC+) or 20% of the VC-poor variety Tiffany

(VC−). To meet the nutritional requirements of the hens without soybean meal, 21% blue sweet lupines (*Lupinus angustifolius* cv. Boruta) were added to all diets. The protein plants were produced GMO-free in Germany. The composition of the diets is specified in Table 3.1. The changeover to the layer diets has been progressively implemented during the 17th week of life. From the beginning of the 18th week, all hens were fed exclusively with the respective layer diet. A total number of 756 hens entered both experiments. In experiment A, 120 purebred hens per genotype, i.e., a total of 360 hens, were allocated to 18 floor pens (2 × 9) of 20 hens each, whereas in experiment B there were 132 crossbred hens per genotype, resulting in 22 hens per pen and 396 in total. Given the three genotypes per experiment and the three different diets, nine groups of genotype x diet combinations were formed, resulting in 40 purebred or 44 crossbred hens for each experimental group (genotype x diet combination). The housing conditions were the same as described above for the rearing period.

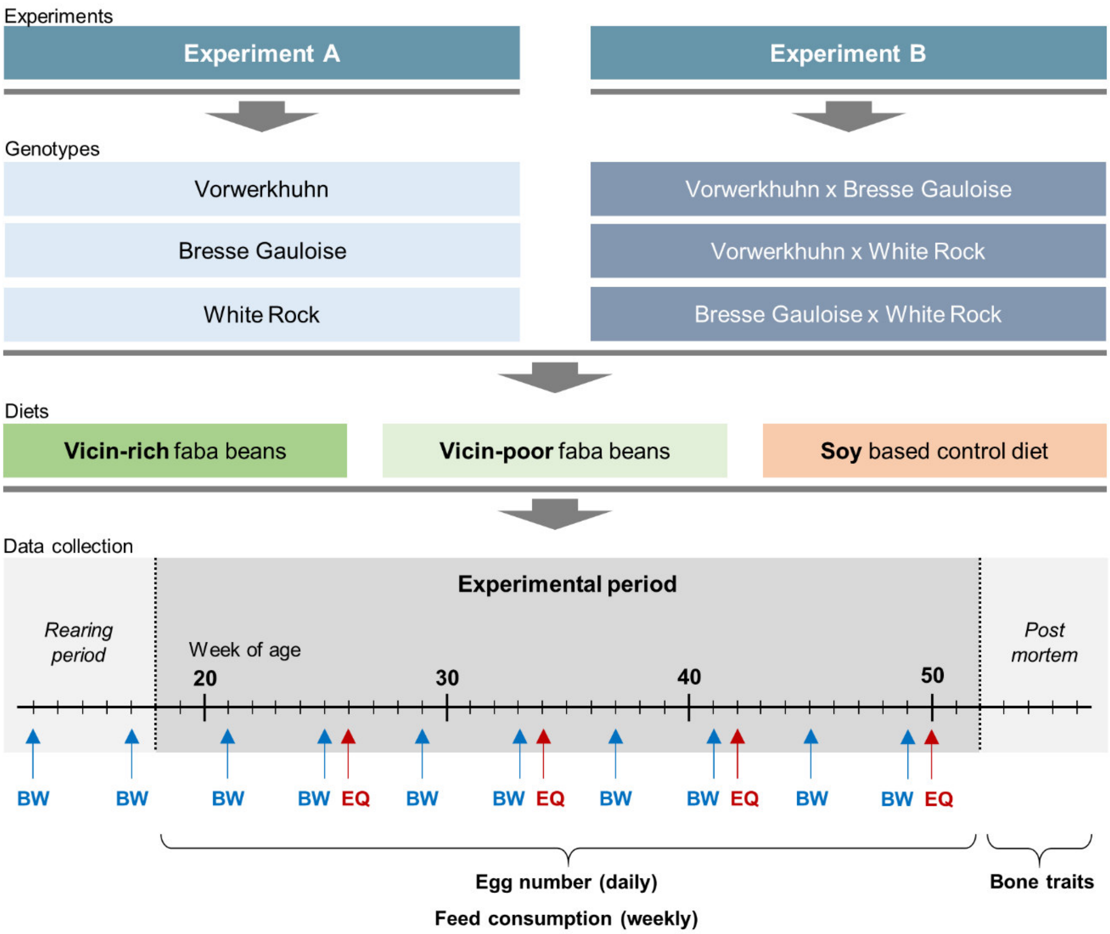

Figure 3.1. Schematic illustration of the experimental setup. In experiment A, three purebred chicken genotypes were allocated to one of three diets containing either faba beans differing in their vicin content or soybean. Experiment B comprised three crossbred genotypes, which were allocated to the same diets. In experiment A (B), 120 (132) hens per genotype were tested with two replicates per genotype x diet combination consisting of 20 (22) hens each. The data collection was identical in both experiments. Data on egg number, egg quality (EQ), feed consumption and body weight (BW) were collected as indicated. Post mortem, bone morphometry, bone mineral density, bone breaking strength and the cortical bone proportion were assessed.

Table 3.1. Composition, analyzed and calculated nutrient composition of the experimental diets.

Item	Experiment A			Experiment B		
	Soy	VC+	VC−	Soy	VC+	VC−
Ingredients (%)						
Wheat	40.39	29.78	29.78	40.39	29.78	29.78
Corn	10.00	10.89	10.89	10.00	10.89	10.89
Soybean meal (39.8% CP)	11.84	-	-	11.84	-	-
Blue sweet lupine cv. Boruta	21.00	21.13	21.13	21.00	21.13	21.13
Faba bean cv. Fuego	-	20.00	-	-	20.00	-
Faba bean cv. Tiffany	-	-	20.00	-	-	20.00
Soybean oil	4.00	5.77	5.77	4.00	5.77	5.77
Dicalcium phosphate	2.76	2.51	2.51	2.76	2.51	2.51
Calcium carbonate	8.39	8.25	8.25	8.39	8.25	8.25
Sodium chloride	0.32	0.25	0.25	0.32	0.25	0.25
DL-Methionine	0.21	0.28	0.28	0.21	0.28	0.28
Lysine	0.09	0.10	0.10	0.09	0.10	0.10
Tryptophan	-	0.04	0.04	-	0.04	0.04
Premix [1]	1.00	1.00	1.00	1.00	1.00	1.00
Chemical composition						
Dry matter abs (%) [2]	89.60	89.50	89.50	90.90	91.20	90.90
Crude ash (g/kg DM) [2]	152.60	149.30	148.70	129.70	139.50	146.70
Crude protein (g/kg DM) [2]	182.40	171.90	185.30	185.20	202.10	184.10
Crude fat (g/kg DM) [2]	95.00	88.80	97.40	91.10	83.70	91.80
Crude fiber (g/kg DM) [2]	54.60	50.30	54.50	61.80	51.30	59.20
Starch (g/kg DM) [2]	362.60	393.10	349.50	365.90	349.5	347.00
Sucrose (g/kg DM) [2]	29.70	25.00	24.00	24.60	26.60	24.60
SFA (g/100g fat) [2]	17.70	17.00	16.10	17.6	16.80	16.40
MUFA (g/100g fat) [2]	22.50	22.60	22.80	22.70	22.80	21.80
PUFA (g/100g fat) [2]	59.80	60.40	61.10	59.6	60.40	61.70
Vicine (%) [2]	0.016	0.079	0.003	0.0	0.095	0.015
Convicine (%) [2]	0.006	0.037	0.002	0.0	0.039	0.004
VC (Vicin + Convicin; %) [3]	0.022	0.116	0.005	0.0	0.134	0.019
Tannin (mg/g) [2]	3.51	3.02	3.33	3.22	3.91	3.67
AMEn (MJ/kg) [3,4]	12.53	12.60	12.36	12.43	12.19	12.12
Methionine (%) [3]	0.42	0.44	0.44	0.42	0.44	0.44
Lysine (%) [3]	0.81	0.83	0.83	0.81	0.83	0.83
Tryptophan (%) [3]	0.16	0.17	0.17	0.16	0.17	0.17
Threonine (%) [3]	0.58	0.55	0.55	0.58	0.55	0.55

CP: crude protein, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, AME_n: nitrogen-corrected apparent metabolizable energy; [1] Premix-hens: feed additives (per kg premix): Vitamin A, 1,000,000 IU; Vitamin D3, 250,000 IU; Vitamin E, 2000 mg; Vitamin B1, 250 mg; Vitamin B2, 700 mg; Vitamin B6, 400 mg; Vitamin B12, 2000 µg; Vitamin K3, 400 mg; Nicotin amide, 4000 mg; Calcium-D-pantothenate, 1000 mg; Folic acid, 60 mg; Biotin, 2500 µg; Choline chloride, 40,000 mg; Fe, 4000 mg; Cu, 1000 mg; Mn, 10,000 mg; Zn, 8000 mg; I, 120 mg; Se, 25 mg; Co, 20.5 mg; Butylated hydroxy toluene (BHT), 12,500 mg; Beta-carotene, 400 mg; Canthaxanthin, 400 mg; [2] Analyzed; [3] Calculated; [4] Apparent metabolizable energy concentrations corrected to zero nitrogen balance (AMEn), calculated according to the energy estimation equation of the World's Poultry Association (Vogt, 1986).

Data collection included performance traits and bone characteristics. Egg production was recorded daily at pen-level, with each observation representing the egg production of 40 (purebreds) or 44 hens (crossbreds). Laying performance was calculated by dividing the total number of eggs by the number of hens present at that day. Feed consumption (g) was recorded weekly at pen-level by weighing the remaining feedstuff. Starting at the 13th week of life, individual body weights (g) of the hens were recorded every four weeks using a digital table scale with a weighing accuracy of 0.1 g (CPA 16001S, Sartorius, Göttingen, Germany). Egg quality was analyzed at four points in time (week 26, 34, 42 and 50). At each point in time, 96 randomly selected eggs from four consecutive days were analyzed per genotype x diet combination. Egg weight (g) was recorded using a digital table scale (CPA 16001S, Sartorius, Goettingen, Germany). Eggshell breaking strength (N) was determined using a texture analyzer (TA.XTplus, Stable Micro Systems, Hamilton, MA, USA) equipped with a 50 N (Newton) load cell showing the maximum load in N that was required to break the eggshell. Eggshell weight (g) was determined after emptying the egg with a spoon and drying the shell for 30 s in a microwave (800 watt). After removing the shell membranes, equatorial eggshell thickness (mm) was measured using a caliper with an accuracy of 0.01 mm.

All hens were sacrificed by carbon dioxide inhalation during the 52nd week of age. The tibiotarsi of both sides and the left humerus were dissected and relieved from muscles and tendons. Bone weight (g), length (mm) and thickness (mm) were recorded and the bones were vacuum-packed and stored frozen (−20 °C) until further examination. Bone mineral density was examined by dual energy X-ray absorptiometry (GE Lunar *i*DXA scanner, GE Healthcare, Solingen, Germany) as described by Jansen et al. [9]. Bone breaking strength (N) of left tibiotarsus and humerus were assessed at the mid-diaphyseal region via three-point bending test (Instron Materials Testing System, Instron Corporation, Canton, MA, USA) using a 5 kN load cell. The span length was 40 mm (humerus) or 80 mm (tibiotarsus). The right tibiotarsus was used to measure diaphyseal cortical bone proportion (%) planimetrically [26].

Statistical Analyses

The data were analyzed separately for experiments A and B using the statistical program SAS (SAS 9.3, SAS Institute Inc., Cary, NC, USA). The separate analysis was chosen, because the two experiments were performed in consecutive years, and an overlap between year- and genotype-effect was not safe to exclude. Therefore, it was also not possible to distinguish between heterosis and year effect.

For the analysis of hen body weight and laying performance, a polynomial growth function was applied according to the model

$$Y_{ijkl} = \mu + G_i + D_j + G_iD_j + \sum_{v=1}^{5} b_{rv}(A_{ij})^v + \sum_{v=1}^{2} b_{sv}G_i(A_{ij})^v + \sum_{v=1}^{2} b_{tv}D_j(A_{ij})^v + \sum_{v=1}^{2} b_{uv}G_iD_j(A_{ij})^v + p_k + e_{ijkl} \quad (3.1)$$

where Y_{ijkl} is the body weight respectively of laying performance, μ is the overall mean, G_i is the fixed effect of the genotype (i = 1 to 3), D_j is the fixed effect of the diet (j = 1 to 3), G_iD_j is the interaction of genotype x diet, b_{rv} are the fixed regression coefficients up to the fourth polynomial degree of age (A_{ij}) for body weight and up to the fifth polynomial degree of age (A_{ij}) for laying performance, b_{sv} are the fixed regression coefficients of the interaction between genotype and age, b_{tv} are the fixed regression coefficients of the interaction between diet and age, b_{uv} are the fixed regression coefficients of the interaction between genotype, diet and age, p_k is the random effect of the pen and e_{ijkl} is the random error. For the polynomial analysis the MIXED procedure of SAS was used. Akaike's information criterion (AIC) was used to determine the model with the best fit according to Koehn et al. [27]. Sample sizes for body weight are listed in the Supplementary Material (Table S3.1).

For the analysis of daily feed consumption, the experiment was split up into 3 periods (Period 1: week 18–30; Period 2: week 31–39; Period 3: week 40–51), because the variability of the daily feed use between the single weeks was rather high. The statistical model was similar to that described below for the egg quality traits (Equation (3.2)). Results are presented in Supplementary Material S3.1 (Figure S3.1).

Data for egg weight, eggshell breaking strength and eggshell thickness were analyzed with a linear mixed model as following:

$$Y_{ijklm} = \mu + G_i + D_j + A_k + G_iD_j + G_iA_k + D_jA_k + G_iD_jA_k + p_l + e_{ijklm} \quad (3.2)$$

where Y_{ijklm} is the respective parameter, μ is the overall mean, G_i is the fixed effect of the genotype (i = 1 to 3), D_j is the fixed effect of the diet (j = 1 to 3), A_k is the fixed effect of the age in weeks, G_iD_j, G_iA_k, D_jA_k and $G_iD_jA_k$ are the interactions of the respective variables, p_l is the random effect of the pen and e_{ijklm} is the random error.

As shell weight and egg weight are highly correlated [28], shell weight was calculated with and without including egg weight as a covariate in the analysis. The applied model was the same as described above for the other egg traits (Equation (2)). To verify the correlation of egg weight and shell weight in the experimental data, Pearson's correlation coefficients (r_p) between egg and shell weight were calculated. They were $r_p = 0.70$ in experiment A and $r_p = 0.74$ in experiment B. The calculation of the least squares means (LS-means) and testing of significant differences was carried out as described in Nolte et al. [29]. The calculation of daily feed intake and egg parameters was performed with the GLIMMIX procedure of SAS.

In the first experiment, an infestation with the northern fowl mite (*Ornithonyssus sylviarum*) took place in the barn and during this period a massive discrepancy in the data compared to the time before and after the infestation was realized. For that reason, the affected data from week 31–39 were excluded from the final analysis. In the case of body weight and laying performance a calculation with stepwise exclusion of data were applied to model the growth and laying curves. The full data, analyzed with a linear mixed model together with the final curves is presented in Supplementary Material Figures S2 (Body weight) and S3 (Laying performance). The bone characteristics were analyzed using the GLIMMIX procedure of SAS according to the following model:

$$Y_{ijkl} = \mu + G_i + D_j + G_iD_j + S_k + e_{ijkl} \quad (3.3)$$

where Y_{ijkl} is the respective bone characteristic, μ is the overall mean, G_i is the genotype (i = 1 to 3), D_j is the diet (j = 1 to 3), G_iD_j is their interaction, S_k is the random effect of the sire and e_{ijkl} is the random error. Since bone weight attributed a relatively large effect on bone strength [12], this factor was considered as a covariate for the analysis of bone breaking strength. Sample sizes for bone characteristics are listed in the Supplementary Material (Table S1).

Results

Hen Performance

The growth curves of the genotype x diet combinations and of the genotypes in comparison with each other are shown in Figure 3.2. In both experiments, there were no significant differences between the feeding groups within the genotypes (Figure 3.2A). Although the growth curves started to flatten between week 21 and 25, all hens except WR gained weight until the end of the experiment. The BG-crosses VBG and BWR reached final weights of almost 2.5 kg, which was about 300 g less than the BG. The VH and VWR achieved weights of ca. 2.0 kg, while the WR did not exceed 1.9 kg (Figure 3.2A). The comparison of the purebreds showed significant differences during the whole experiment with BG being significantly heavier than

VH and WR. VH and WR differed statistically significantly from each other only in weeks 17–25 and 45–49 (Figure 3.2B), while in the first section WR were heavier than VH, this was opposite at the later section. In Figure 3.2C the equivalent analysis for the crossbreds is shown. In experiment B, the VWR hens have been significantly lighter than the other two genotypes during the whole experiment, while VBG and BWR only differed significantly in weeks 13–21 and 45–49, VBG being slightly heavier. Adjusted by the mean of age, no significant differences between the diets were found in both experiments.

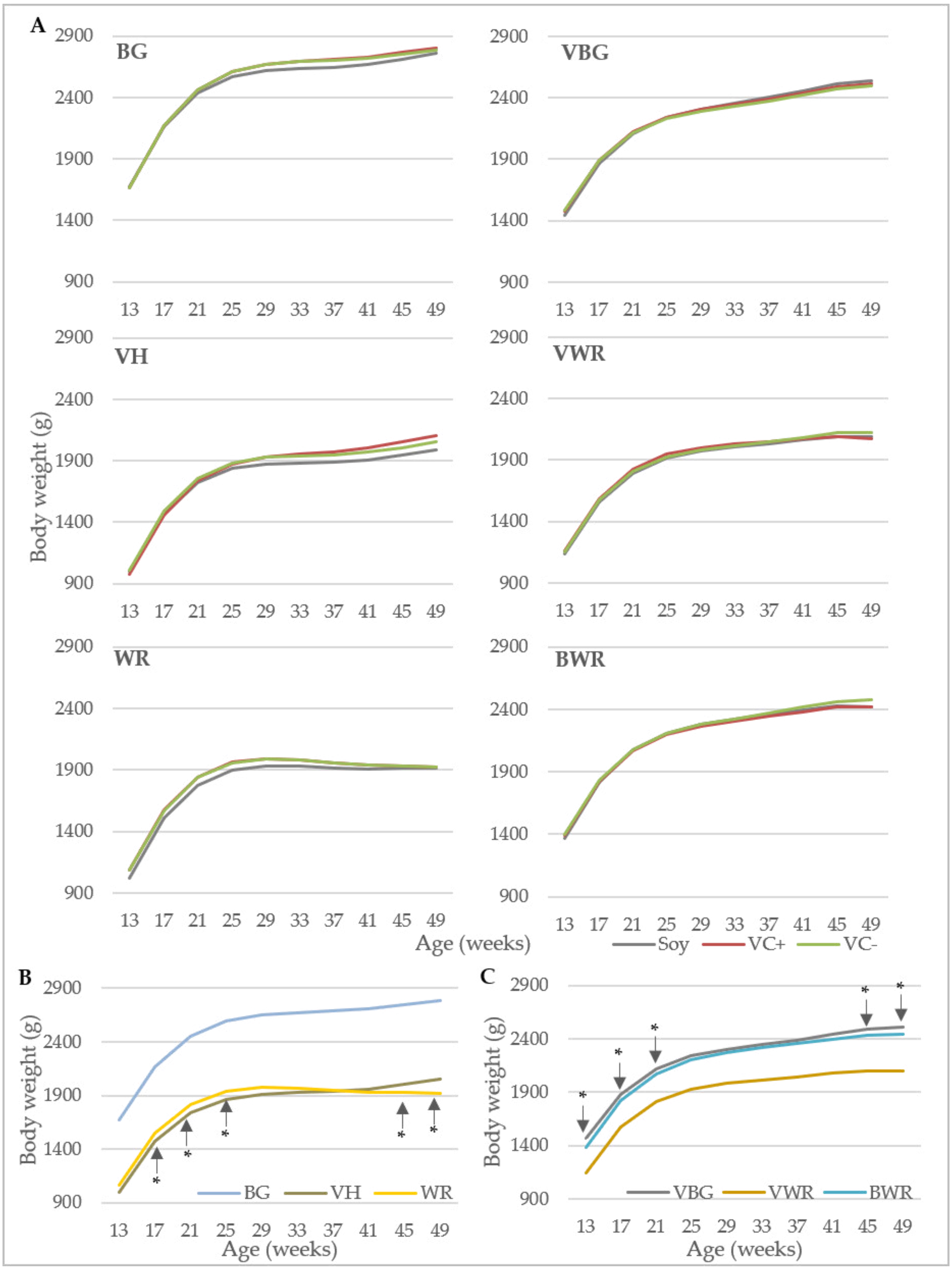

Figure 3.2. Body weight of hens. (**A**) Body weight development of six genotypes under the influence of different diets (BG, VH, WR: *n* = 38; VBG, VWR, BWR: *n* = 43). Within genotype and week, there were no significant differences between the feeding groups. (**B**) Comparison of body weight development of the purebreds (*n* = 113). (**C**) Comparison of body weight development of the crossbreds (*n* = 131). * mark significant differences between VH and WR (**B**) and VBG and BWR genotypes (**C**), respectively; in all other weeks the respective genotypes differ only significantly from

BG (**B**) and VWR (**C**), respectively. BG: Bresse Gauloise, VH: Vorwerkhuhn, WR: White Rock, VBG: VH male x BG female, VWR: VH male x WR female, BWR: BG male x WR female.

Figure 3.3 illustrates the laying performance over the course of the experimental period. In Figure 3.3A, the comparison of the feeding groups within genotypes is shown. In BG and WR no significant differences between the different diets were found, while in VH in the last two weeks of the experiment the difference between the VC− and the soy group became statistically significant with a difference of −5.56% and −12.02% in weeks 50 and 51, respectively. A significant difference between the soy and VC− group was also found in experiment B in VBG at the end of the experiment, but here the soy group showed a significantly higher laying performance in weeks 49–51 compared to the VC− group of 5.41–6.97%. In VWR and BWR no significant differences in the laying curve between the feeding groups could be detected.

The WR and BWR groups showed the highest peak production of about 100% in the respective experiments (Figure 3.3B,C). Among all genotypes, the VH hens showed the lowest laying performance, which is reflected in a later laying maturity and an overall peak production of only about 56%. The laying performance of BG was in between the WR and VH, all purebreds differed significantly from each other at all time points of the experiment (Figure 3.3B). VWR hens performed similar as the BWR but were behind in terms of laying persistency (Figure 3.3C). The laying performance of VBG was lower than that of BWR and VWR, but comparable to that of BG in experiment A regarding laying maturity and peak production. The crossbred genotypes differed significantly from each other from week 18–48, but in weeks 49–51 the difference between VWR and VBG became smaller and was not statistically significant anymore, due to a low persistency of the VWR. Regarding laying maturity, VH hens reached 50% egg production only at week 25, while the other genotypes exceeded this threshold between 20 and 22 weeks. All crossbred genotypes reached peak egg production one week earlier than the purebreds, namely at week 28. No significant differences regarding laying performance could be detected between the feeding groups in both experiments.

Regarding daily feed consumption, significant differences were only detected in experiment B (Figure S3.1). In period 3, in cross VBG a significant difference was found between the soy and VC+ group, with the soy group consuming almost 40 g of feed more per day. Regarding the main factor genotype, a significant difference of 10.8 g existed between the crossbreds VBG and VWR.

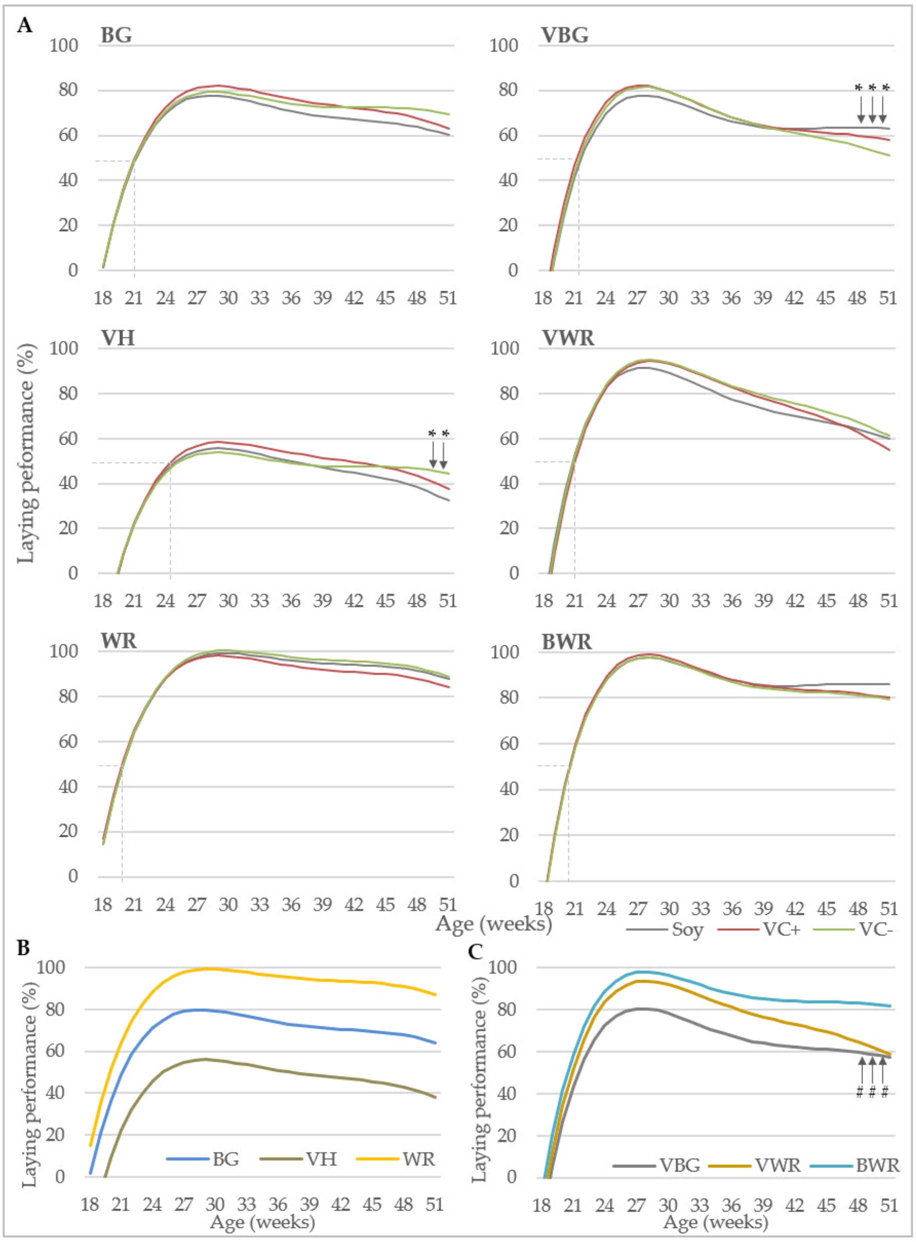

Figure 3.3. Laying performance. (**A**) Laying performance of six genotypes under the influence of different diets ($n = 2$). Dashed lines indicate the age at 50% egg production. * mark significant differences between soy and VC− groups ($p < 0.05$). (**B**) Comparison of laying performance of purebreds ($n = 6$). All genotypes differ significant from each other in every week at $p < 0.05$. (**C**) Comparison of laying performance of crossbreds ($n = 6$). # indicates that there is no significant difference between VBG and VWR in the respective weeks; at all other time points, all genotypes differ significantly at $p < 0.05$. BG: Bresse Gauloise, VH: Vorwerkhuhn, WR: White Rock, VBG: VH male x BG female, VWR: VH male x WR female, BWR: BG male x WR female.

Egg Parameters

The effects of genotype, diet, age and their interactions on egg weight, shell breaking strength, shell weight and thickness are shown in Table 3.2. In both experiments, the egg quality was significantly influenced by genotype and age. While for the purebreds, a dietary effect was only accounted for egg weight and shell weight, all egg quality traits were significantly influenced by the diet in the crossbreds.

Table 3.2. Effect of genotype, diet, age and their interactions on different egg quality parameters.

Parameter	Statistics	Purebreds							Crossbreds						
		Genotype	Diet	Age	Genotype x Diet	Genotype x Age	Diet x Age	Genotype x Diet x Age	Genotype	Diet	Age	Genotype x Diet	Genotype x Age	Diet x Age	Genotype x Diet x Age
Egg weight	F value	357.36	2.32	947.31	1.33	68.39	4.62	2.38	59.95	4.26	834.78	3.18	21.75	6.59	0.77
	p value	<0.0001	0.0018	<0.0001	0.2576	<0.0001	0.0010	0.0149	<0.0001	0.0143	<0.0001	0.0129	<0.0001	<0.0001	0.6820
Shell breaking strength	F value	50.97	2.61	10.44	0.17	5.57	1.94	2.22	52.47	11.53	35.48	0.89	18.57	1.74	1.65
	p value	<0.0001	0.0736	<0.0001	0.9543	0.0002	0.1019	0.0233	<0.0001	<0.0001	<0.0001	0.4711	<0.0001	0.1079	0.0705
Shell weight	F value	251.58	4.42	44.30	0.08	31.12	4.91	3.37	65.96	18.96	291.10	1.93	20.70	2.13	2.18
	p value	<0.0001	0.0121	<0.0001	0.9881	<0.0001	0.0006	0.0008	<0.0001	<0.0001	<0.0001	0.1026	<0.0001	0.0472	0.0103
Shell weight adj.*	F value	105.11	2.26	91.72	0.23	3.36	2.97	2.85	24.75	22.97	04.11	1.52	5.22	2.36	2.56
	p value	<0.0001	0.1044	<0.0001	0.9239	0.0094	0.0185	0.0037	<0.0001	<0.0001	0.0064	0.1926	<0.0001	0.0284	0.0022
Shell thickness	F value	102.07	2.03	260.47	0.05	8.72	0.79	2.20	70.92	10.16	135.59	0.32	27.11	2.55	3.05
	p value	<0.0001	0.1314	<0.0001	0.9950	<0.0001	0.5331	0.0246	<0.0001	<0.0001	<0.0001	0.9750	<0.0001	0.0180	<0.0001

* Shell weight analyzed with egg weight as co-variable, *p* values for egg weight have been $p < 0.0001$ in pure- and crossbreds.

Figure 3.4A shows the LS-mean values for egg weight of the different genotypes at different measurements. Figures B, C illustrate the LS-mean values for the main factors diet and genotype over all measurements. In all genotypes the egg weight increased with aging of the hens, only WR showed 1 g lighter eggs in week 50 than in week 42 (Figure 3.4A). In case of the purebreds the mean egg weight was highest for WR (56.6 g) and lowest for VH (49.5 g) (Figure 3.4B), while among the crossbreds BWR laid the heaviest eggs (58.0 g) and the VBG the lightest (54.0 g). In both experiments, the egg weights of all genotypes differed significantly from each other, while within genotypes and measurements no significant differences between the feeding groups were detected (Figure 3.4A). Over the whole experiment, the VC+ groups had significantly lighter eggs than the soy and VC− groups, which was true in the pure- (Figure 3.4B) and crossbreds (Figure 3.4C). However, these differences were not pronounced and amounted to 1.1 g or even less.

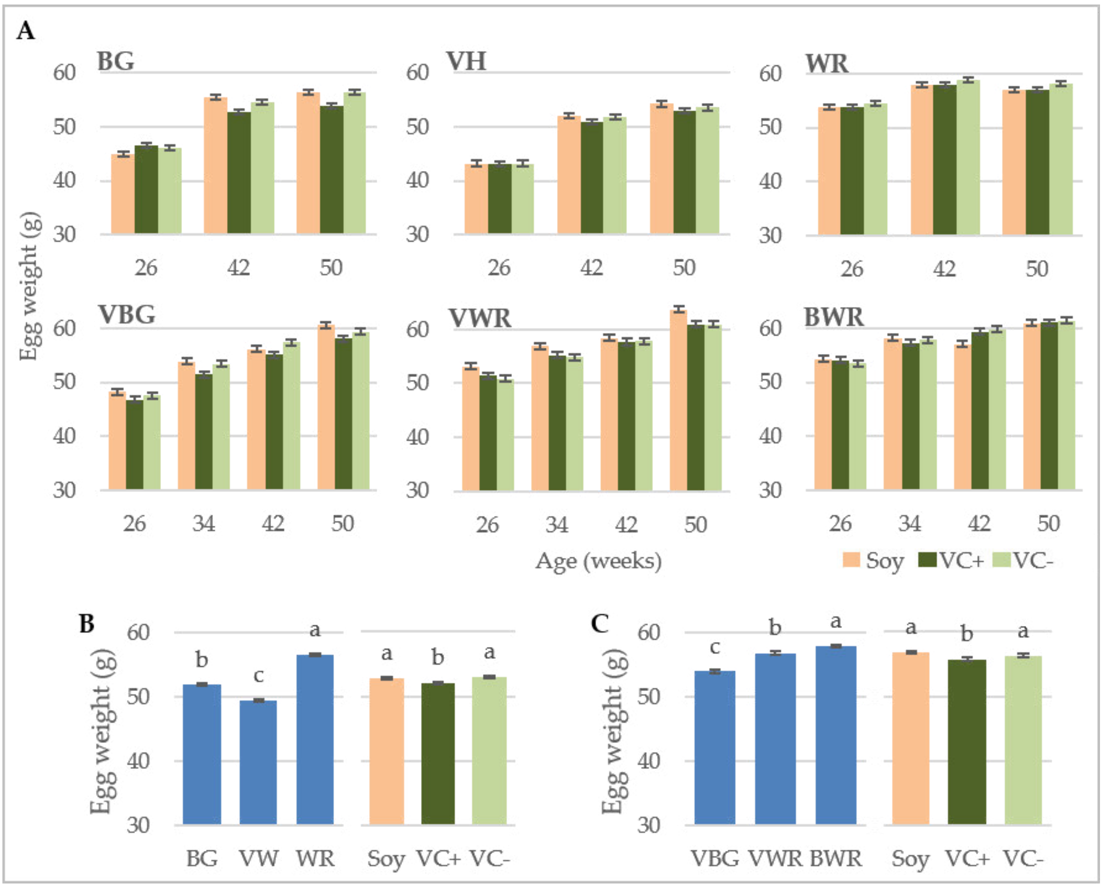

Figure 3.4. Egg weight. LS-means ± SE. (**A**) Effect of diet on egg weight (*n* = 96). (**B**) Purebreds: Mean egg weights of the respective genotypes and diets (*n* = 288). (**C**) Crossbreds: Mean egg weights of the respective genotypes and diets (*n* = 288). BG: Bresse Gauloise, VH: Vorwerkhuhn, WR: White Rock, VBG: VH male x BG female, VWR: VH male x WR female, BWR: BG male x WR female. ^{a,b,c} Bars differ significantly at $p < 0.05$.

Mean values of eggshell breaking strength and eggshell thickness of the genotypes and diets are presented in Tables 3 and 4 for pure- and crossbreds, respectively. The shell strength was significantly highest for WR (57.68 N) and VWR (58.41 N) in experiments A and B, respectively, while purebred BG and VH hens showed values lower than 50 N. Differences between the dietary groups were only found in the crossbreds where the VC+ group showed a significantly reduced eggshell breaking strength. The same holds true for the shell thickness.

Table 3.3. LS-means ± SE for eggshell breaking strength, shell thickness and shell weight in purebreds (experiment A).

Parameter	Genotype			Diet		
	BG	VH	WR	Soy	VC+	VC−
Shell breaking strength (N)	44.71 [b] ± 0.95	47.58 [b] ± 0.96	57.68 [a] ± 0.95	51.22 [a] ± 0.96	48.26 [a] ± 0.95	50.50 [a] ± 0.95
Shell thickness (mm)	0.33 [b] ± 0.003	0.32 [c] ± 0.003	0.38 [a] ± 0.003	0.35 [a] ± 0.003	0.34 [a] ± 0.003	0.34 [a] ± 0.003
Shell weight (g)	5.09 [b] ± 0.05	4.74 [c] ± 0.05	6.13 [a] ± 0.05	5.39 [a] ± 0.05	5.21 [b] ± 0.05	5.37 [a] ± 0.05
Shell weight adj * (g)	5.17 [b] ± 0.04	5.01 [c] ± 0.04	5.85 [a] ± 0.04	5.40 [a] ± 0.04	5.27 [a] ± 0.04	5.36 [a] ± 0.04

* Shell weight analyzed with egg weight as co-variable (n = 288). [a,b,c] Values not sharing a letter within row and category differ significantly at $p < 0.05$.

Table 3.4. LS-means ± SE for eggshell breaking strength, shell thickness and shell weight in crossbreds (experiment B).

Parameter	Genotype			Diet		
	VBG	VWR	BWR	Soy	VC+	VC−
Shell breaking strength (N)	53.61 [b] ± 0.52	58.41 [a] ± 0.52	50.96 [c] ± 0.52	56.06 [a] ± 0.52	52.52 [b] ± 0.52	54.40 [a] ± 0.52
Shell thickness (mm)	0.33 [c] ± 0.002	0.35 [b] ± 0.002	0.36 [a] ± 0.002	0.35 [a] ± 0.002	0.34 [b] ± 0.002	0.35 [a] ± 0.002
Shell weight (g)	5.28 [b] ± 0.03	5.65 [a] ± 0.03	5.70 [a] ± 0.03	5.67 [a] ± 0.03	5.43 [c] ± 0.03	5.53 [b] ± 0.03
Shell weight adj* (g)	5.44 [b] ± 0.02	5.61 [a] ± 0.02	5.57 [a] ± 0.02	5.63 [a] ± 0.02	5.47 [c] ± 0.02	5.53 [b] ± 0.02

* Shell weight analyzed with egg weight as co-variable (n = 288). [a,b,c] Values not sharing a letter within row and category differ significantly at $p < 0.05$.

The results of the statistical analysis of the shell weight with and without consideration of the egg weight as a covariate in the model are shown in Tables 3.3 and 3.4. In the purebreds, there was no significant interaction between diet and egg weight and consequently the values changed due to the correction but not the significances. Nevertheless, the shell weight of the local breeds was increased as a result of the correction factor, but in both applied models the WR group exhibited a significantly higher shell weight than the local breeds. A similar situation was found in experiment B, where the cross of the two local breeds, VBG, showed significantly lower shell weights than the other genotypes, even after the covariate was considered in the statistical model. With regard to the effect of the diet, the correction eliminated the significant differences in favor of the VC+ group in Experiment A, which was present without the consideration of the egg weight as a correction factor in the model. In the second experiment (B) there were significant differences between all feeding groups with the soy group having the highest shell weights and the VC+ group the lowest and this was the case with and without the covariate egg weight.

Bone Characteristics

The effects of genotype, diet and their interaction on the bone characteristics within the purebreds and crossbreds are shown in Table 5. In both experiments, the genotype had a highly significant effect on all bone traits. In contrast, the diet influenced only the bone mineral density of the tibiotarsus and the keel bone in the purebreds. The genotype by diet interaction was not significant at all. In both bone types, the breaking strength was significantly influenced by considering bone weight in the statistical model.

Table 3.5. Effect of genotype, diet and their interaction on bone characteristics of tibiotarsus, humerus and keel bone.

Bone Type	Parameter	Statistics	Purebreds Genotype	Purebreds Diet	Purebreds Genotype x Diet	Purebreds Bone Weight	Crossbreds Genotype	Crossbreds Diet	Crossbreds Genotype x Diet	Crossbreds Bone Weight
Tibiotarsus	Breaking strength	F value	333.01	0.64	1.44	297.77	219.91	1.60	1.25	312.87
		p value	<0.0001	0.5274	0.2213	<0.0001	<0.0001	0.2038	0.2905	<0.0001
	Mineral density	F value	152.82	5.24	0.42	---	44.14	0.38	0.50	---
		p value	<0.0001	0.0058	0.7967	---	<0.0001	0.6869	0.7388	---
	Cortical area	F value	102.62	1.62	0.67	---	46.19	1.25	1.54	---
		p value	<0.0001	0.2002	0.6154	---	<0.0001	0.2863	0.1912	---
	Weight	F value	109.36	2.87	0.13	---	27.50	0.40	0.79	---
		p value	<0.0001	0.0580	0.9725	---	<0.0001	0.6730	0.5292	---
	Length	F value	144.58	1.15	0.92	---	20.11	0.08	0.29	---
		p value	<0.0001	0.3193	0.4534	---	<0.0001	0.9250	0.8863	---
	Thickness	F value	15.58	1.84	1.01	---	3.57	0.16	0.72	---
		p value	<0.0001	0.1604	0.3998	---	0.0292	0.8502	0.5788	---
Humerus	Breaking strength	F value	255.24	0.37	1.52	223.51	23.45	0.53	0.13	332.89
		p value	<0.0001	0.6880	0.1972	<0.0001	<0.0001	0.5893	0.9731	<0.0001
	Mineral density	F value	110.86	0.50	0.03	---	58.36	1.49	1.36	---
		p value	<0.0001	0.6043	0.9977	---	<0.0001	0.2273	0.2468	---
	Weight	F value	61.14	0.30	0.04	---	29.63	1.26	0.68	---
		p value	<0.0001	0.7431	0.9966	---	<0.0001	0.2848	0.6093	---
	Length	F value	50.95	0.38	0.80	---	7.83	0.31	1.00	---
		p value	<0.0001	0.6872	0.5248	---	0.0005	0.7313	0.4092	---
	Thickness	F value	15.81	2.36	0.68	---	3.26	0.12	0.13	---
		p value	<0.0001	0.0962	0.6082	---	0.0394	0.8839	0.9715	---
Keel bone	Mineral density	F value	61.52	3.05	0.31	---	16.33	1.19	0.96	---
		p value	<0.0001	0.0489	0.8710	---	<0.0001	0.3058	0.4282	---

The corresponding LS-means of the purebreds are listed in Table 3.6. Group VH showed the highest tibiotarsus breaking strength, followed by group BG and WR. In the case of the humerus, group BG exhibited a higher breaking strength compared to the VH group. The WR group showed the lowest breaking strength for this bone type as well. The significantly highest bone mineral density in all three bones was observed in BG hens, followed by VH and WR. The BG group had the significantly heaviest and longest bones. For tibiotarsus thickness, the highest values were found in VH hens, while in humerus the WR group was inferior to the other genotypes. With exception of the humerus weight, the WR hens showed the lowest values in all bone characteristics among the purebred genotypes. Regarding the effect of the diet, the VC+ group significantly differed from the controls (Soy) showing a higher bone mineral density for both the tibiotarsus and keel bone, whereas the VC− group was intermediate.

Table 3.6. LS-means ± SE for characteristics of tibiotarsus, humerus and keel bone under the effect of genotype and diet for experiment A (purebreds) (*n* = 113).

Bone Type	Parameter	Genotype			Diet		
		BG	VH	WR	Soy	VC+	VC−
Tibiotarsus	Breaking strength (N)	274.87 ^{b} ± 4.13	321.81 ^{a} ± 3.36	197.23 ^{c} ± 3.67	261.61 ± 3.41	265.38 ± 3.32	266.93 ± 3.41
	Mineral density (g/cm^2)	0.382 ^{a} ± 0.004	0.347 ^{b} ± 0.004	0.279 ^{c} ± 0.004	0.326 ^{b} ± 0.004	0.345 ^{a} ± 0.004	0.338 ^{a,b} ± 0.004
	Cortical area (%)	47.63 ^{a} ± 0.67	46.30 ^{a} ± 0.64	35.78 ^{b} ± 0.63	44.18 ± 0.65	42.70 ± 0.63	42.83 ± 0.65
	Weight (g)	14.52 ^{a} ± 0.14	12.54 ^{b} ± 0.14	11.68 ^{c} ± 0.13	12.67 ± 0.14	13.14 ± 0.13	12.93 ± 0.14
	Length (mm)	127.69 ^{a} ± 0.40	123.03 ^{b} ± 0.38	118.44 ^{c} ± 0.37	122.58 ± 0.39	123.27 ± 0.37	123.32 ± 0.39
	Thickness (mm)	6.34 ^{b} ± 0.03	6.57 ^{a} ± 0.03	6.39 ^{b} ± 0.03	6.39 ± 0.03	6.48 ± 0.03	6.43 ± 0.03
Humerus	Breaking strength (N)	359.06 ^{a} ± 4.68	327.82 ^{b} ± 4.21	230.15 ^{c} ± 4.03	308.56 ± 4.10	304.40 ± 3.98	304.06 ± 4.11
	Mineral density (g/cm^2)	0.272 ^{a} ± 0.003	0.229 ^{b} ± 0.003	0.218 ^{c} ± 0.003	0.237 ± 0.003	0.241 ± 0.003	0.240 ± 0.003
	Weight (g)	6.96 ^{a} ± 0.09	5.63 ^{b} ± 0.09	5.89 ^{b} ± 0.09	6.11 ± 0.09	6.20 ± 0.09	6.18 ± 0.09
	Length (mm)	81.73 ^{a} ± 0.23	79.85 ^{b} ± 0.22	78.47 ^{c} ± 0.22	79.86 ± 0.23	80.07 ± 0.22	80.12 ± 0.23
	Thickness (mm)	5.86 ^{a} ± 0.03	5.88 ^{a} ± 0.03	5.68 ^{b} ± 0.03	5.78 ± 0.03	5.84 ± 0.03	5.82 ± 0.03
Keel bone	Mineral density (g/cm^2)	0.222 ^{a} ± 0.002	0.195 ^{b} ± 0.002	0.187 ^{c} ± 0.002	0.197 ^{b} ± 0.002	0.205 ^{a} ± 0.002	0.202 ^{a,b} ± 0.002

^{a,b,c} Values not sharing a letter within bone trait and category differ significantly at $p < 0.05$.

Table 3.7 shows the results of bone characteristics of the crossbreds. The significantly highest breaking strength and bone mineral density in all bone types was observed in VBG hens. Group VWR had the significantly lowest keel bone mineral density, while the other two groups did not differ from each other. The groups VWR and BWR both had a significantly higher cortical area than group VBG. The BG crosses had significantly higher values for tibiotarsus weight and length, while in the humerus it was more differentiated. The latter also applies to the bone thickness in both bone types. The diet had no effect on the crossbreds.

Table 3.7. LS-means ± SE for characteristics of tibiotarsus, humerus and keel bone under the effect of genotype and diet for experiment B (crossbreds) (n = 131).

Bone Type	Parameter	Genotype			Diet		
		VBG	VWR	BWR	Soy	VC+	VC−
Tibiotarsus	Breaking strength (N)	297.65 [a] ± 2.92	244.91 [b] ± 3.01	227.16 [c] ± 2.92	255.55 ± 2.89	254.65 ± 2.89	259.52 ± 2.88
	Mineral density (g/cm^2)	0.379 [a] ± 0.004	0.328 [b] ± 0.004	0.339 [b] ± 0.004	0.346 ± 0.004	0.350 ± 0.004	0.349 ± 0.004
	Cortical area (%)	55.42 [b] ± 0.43	60.09 [a] ± 0.43	60.75 [a] ± 0.43	58.42 ± 0.43	59.30 ± 0.43	58.54 ± 0.43
	Weight (g)	14.12 [a] ± 0.14	12.85 [b] ± 0.14	14.06 [a] ± 0.14	13.58 ± 0.14	13.72 ± 0.14	13.73 ± 0.14
	Length (mm)	126.37 [a] ± 0.37	123.95 [b] ± 0.37	127.15 [a] ± 0.37	125.89 ± 0.37	125.70 ± 0.37	125.87 ± 0.37
	Thickness (mm)	6.42 [b] ± 0.03	6.48 [a,b] ± 0.03	6.54 [a] ± 0.03	6.47 ± 0.03	6.50 ± 0.03	6.48 ± 0.03
Humerus	Breaking strength (N)	350.65 [a] ± 4.47	305.89 [c] ± 4.60	335.58 [b] ± 4.38	333.65 ± 4.43	327.30 ± 4.36	331.16 ± 4.36
	Mineral density (g/cm^2)	0.263 [a] ± 0.003	0.223 [c] ± 0.003	0.243 [b] ± 0.003	0.246 ± 0.003	0.239 ± 0.003	0.244 ± 0.003
	Weight (g)	7.15 [a] ± 0.10	6.12 [c] ± 0.10	6.82 [b] ± 0.10	6.77 ± 0.10	6.57 ± 0.10	6.75 ± 0.10
	Length (mm)	81.64 [a,b] ± 0.22	81.00 [b] ± 0.22	82.23 [a] ± 0.22	81.53 ± 0.22	81.76 ± 0.22	81.57 ± 0.22
	Thickness (mm)	5.99 [a] ± 0.03	5.89 [b] ± 0.03	5.94 [a,b] ± 0.03	5.94 ± 0.03	5.95 ± 0.03	5.93 ± 0.03
Keel bone	Mineral density (g/cm^2)	0.221 [a] ± 0.002	0.204[b] ± 0.002	0.214 [a] ± 0.002	0.210 ± 0.002	0.214 ± 0.002	0.214 ± 0.002

[a,b,c] Values not sharing a letter within bone trait and category differ significantly at $p < 0.05$.

Discussion

In the two experiments of this study, hens of different genotypes were fed diets with 20% VC-rich and VC-poor faba beans. In general, the chicken genotype had more impact on the parameters measured than the different diets.

Comparison of Genotypes

The body weight is a breed characteristic defined in the breed standard. For both Vorwerkhuhn and Bresse Gauloise hens, it is 2000–2500 g [30,31], which matches with the final weight of the VH hens, while the BG in the present study have been clearly heavier with more than 2700 g. Lambertz et al. [24] recorded weights of 2957 g for BG hens slaughtered at 75 weeks of age. The weight of the WR hens is in the range indicated for commercial brown laying hybrids [32,33]. Despite the differences in body weight, all purebreds had a similar feed consumption.

In the case of BG and WR, this is assumed to be due the high performance either regarding growth (BG) or laying (WR). For VH possible explanations are an unfavorably high metabolic rate or feed wastage by foraging. The latter probably also applies to the VWR group in experiment B.

The laying performance of the local breeds and especially of the VH has been considerably lower than that of the WR. Similar differences between local and commercial genotypes have also been described by Lange [34] and Götze and von Lengerken [4], who investigated both the laying performance of several German local breeds. The described difference is due to the different breeding history of these breeds. While commercial laying hybrids have been intensively selected for high number of saleable eggs for many generations as part of the breeding program [35], the local breeds were typically presented on exhibitions and therefore the type was the most important trait in the last decades. The performance divergence is also reflected by the laying maturity and age at 50% egg production. The persistency of all three genotypes was similar in relation to their difference in total laying performance, as indicated by the almost parallel course of the laying curves.

Furthermore, the egg weights of the local breeds have been lower than of WR, which is in agreement with Sirri et al. [36] and Moula et al. [28], who compared the performance of commercial laying hybrids with Italian and Belgian local breeds, respectively. Lambertz et al. [24] described a high amount of small eggs (<53 g) at the beginning of the laying period of BG, whereas at the end of the laying period small eggs amounted only 3%.

With regard to the eggshell quality, i.e., breaking strength, thickness and weight, there was also a clear difference between the commercial WR line and the local breeds BG and VH in the present study, whereas Moula et al. [28] did not find this difference in breaking strength, but also in shell weight and thickness. Tixier-Boichard et al. [37] and Götze and von Lengerken [4] also found no differences in the breaking strength of eggshells of local versus commercial genotypes.

The laying performance of the crossbreds was in the first half of the laying period more similar to the performance level of the maternal genotype than to the paternal, while in the second half, there was a decrease in direction of the paternal performance, although BWR showed a much better persistency than VWR in their laying performance. The mean egg weights of the crossbreds have been higher than the mean of the parental pure lines for all genotypes, which, however cannot be construed directly as heterosis, since the difference might be confounded with a year effect. Concerning the eggshell parameters, the crossbreds’ values have been in between that of the parental lines.

Consistent with the literature [38], this research revealed considerable phenotypic differences in terms of bone traits between the genotypes. Our findings support the hypothesis that bone morphometry has only limited influence on bone breaking strength [9]. The results suggest the crossbreds being heterotic, as they showed enhanced values in comparison with the respective purebred parents [39]. This is especially true for the BG crosses. However, this must be interpreted with caution, as the experiments were conducted separately and a direct comparison is not possible. Another source of uncertainty is that the results are somewhat contradictory. In the case of the breaking strength, possible hybrid vigor occurred in the humerus but not in the tibiotarsus. In terms of bone mineral density, it was the opposite. However, this result cannot be conclusively clarified based on the available data but suggests more specifically designed follow-up studies.

Comparison of Diets

Regarding the hens' body weight, no influence of faba bean feeding on the weight development was observed, which is in accordance with previous reports [21,40], where laying hens were fed 25% or 24% faba beans and no effect on the laying performance was reported. In contrast, however, Halle [17] observed performance reductions already at a faba bean level of 10% and Fru-Nji et al. [18] from a level of 16% faba beans and higher. These differences might be explained by the faba beans that have been used in the respective studies, because the VC content differs between varieties [41] and the percentage of faba beans in the diet gives no direct information about the VC content.

The reduction of egg-weight with a faba-bean diet was shown in several studies [17,21,42] with an extent of 2–4 g per egg, therefore vicin has also been known as 'egg-weight-depressing factor' since its identification in faba beans forty years ago [19]. Egg-weight reduction was also evident in the VC+ groups in the present study but the difference amounted to less than 1 g compared to control and VC− groups.

Regarding eggshell stability, no influence of faba beans on breaking strength or shell thickness was observed by other authors [40,43], which is in accordance with the results of experiment A, whereas both parameters have been reduced in the VC+ groups of experiment B.

To our knowledge, this is the first trial studying the effects of VC on bone characteristics in laying hens. Although the VC+ group showed a significantly higher bone mineral density than the soy group, this difference is still small. This especially applies to the keel bone, where the p-value was only slightly below the critical threshold. Furthermore, no effects on the humerus were observed, although the bones of the purebreds otherwise showed a very consistent pattern.

Thus, a distinct influence of VC on the bones cannot be confirmed. A negative effect of tannin on bone development, as demonstrated in broilers [44,45], is also considered unlikely, as the tannin content fluctuated only marginally between diets and the VC+ diet even had the lowest value.

Conclusions

Taken together the dietary effect, only little negative impact of VC was observed. However, as it concerned mainly the economically important parameter egg weight, the VC− diet should be preferred when replacing soy with regional faba beans.
With regard to the genotype, the commercial WR line was superior in performance parameters but characterized by inferior bone stability compared to the local breeds. Although no direct comparison of the two experiments is possible, the findings suggest that the crossbreeding with the meat-type BG improved the bone characteristics of WR with almost equal laying performance.
Because this study is dealing with dual-purpose genotypes, the male performance also has to be considered for a final conclusion. The performance test of the males showed BG to improve the fattening performance of the layer-type VH and WR chickens [29]. Therefore, considering both sexes, the BWR hybrid seems to be the most promising cross. However, further research is needed to characterize crossbreeding as a possibility for agricultural use of local chicken breeds. Moreover, the inner egg quality is a topic to be discussed, which is underway in a follow-up study.

Supplementary Materials: The following are available online at https://www.mdpi.com/2076-2615/10/9/1480/s1, Table S3.1: Sample sizes of the analysis separated by genotype x diet combination; Figure S3.1: Daily feed consumption; Figure S3.2: Modeling body weight of hens; Figure S3.3: Modeling of laying performance.

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Supplementary Materials

Table S3.1. Sample sizes of the analysis separated by genotype x diet combination.

	BG			VH			WR			VBG			VWR			BWR		
Variable	VC+	VC-	Soy	VC+	VC-	Soy	VC+	VC-	Soy	VC+	VC-	Soy	VC+	VC-	Soy	VC+	VC-	Soy
Bone breaking strength Tibiotarsus	39	35	32	39	39	37	39	38	40	43	44	44	44	44	43	44	44	44
Bone mineral density Tibiotarsus	39	35	32	39	39	37	39	38	40	44	44	44	44	44	43	44	44	44
Weight Tibiotarsus	39	35	32	39	39	37	39	38	40	44	44	44	44	44	43	44	44	44
Length Tibiotarsus	39	35	32	39	39	37	39	38	40	44	44	44	44	44	43	44	44	44
Thickness Tibiotarsus	39	35	32	39	39	37	39	38	40	44	44	44	44	44	43	44	44	44
Cortical area Tibiotarsus	39	35	32	39	39	37	39	38	40	44	44	44	44	44	43	44	44	44
Bone breaking strength Humerus	39	35	32	39	39	37	39	38	40	44	44	44	44	44	43	44	44	41
Bone mineral density Humerus	39	35	32	39	39	37	39	38	40	44	44	44	44	44	43	44	44	43
Weight Humerus	39	35	32	39	39	37	39	38	40	44	44	44	44	44	43	44	44	43
Length Humerus	39	35	32	39	39	37	39	38	40	44	44	44	44	44	43	44	44	43
Thickness Humerus	39	35	32	39	39	37	39	38	40	44	44	44	44	44	43	44	44	43
Bone mineral density Keel bone	39	35	32	39	39	37	39	38	40	44	44	44	44	43	43	44	44	43
Body weight	39	35	32	39	39	37	39	38	40	44	44	44	44	43	43	44	44	44

BG: Bresse Gauloise, VH: Vorwerkhuhn, WR: White Rock, VBG: VH male x BG female, VWR: VH male x WR female, BWR: BG male x WR female

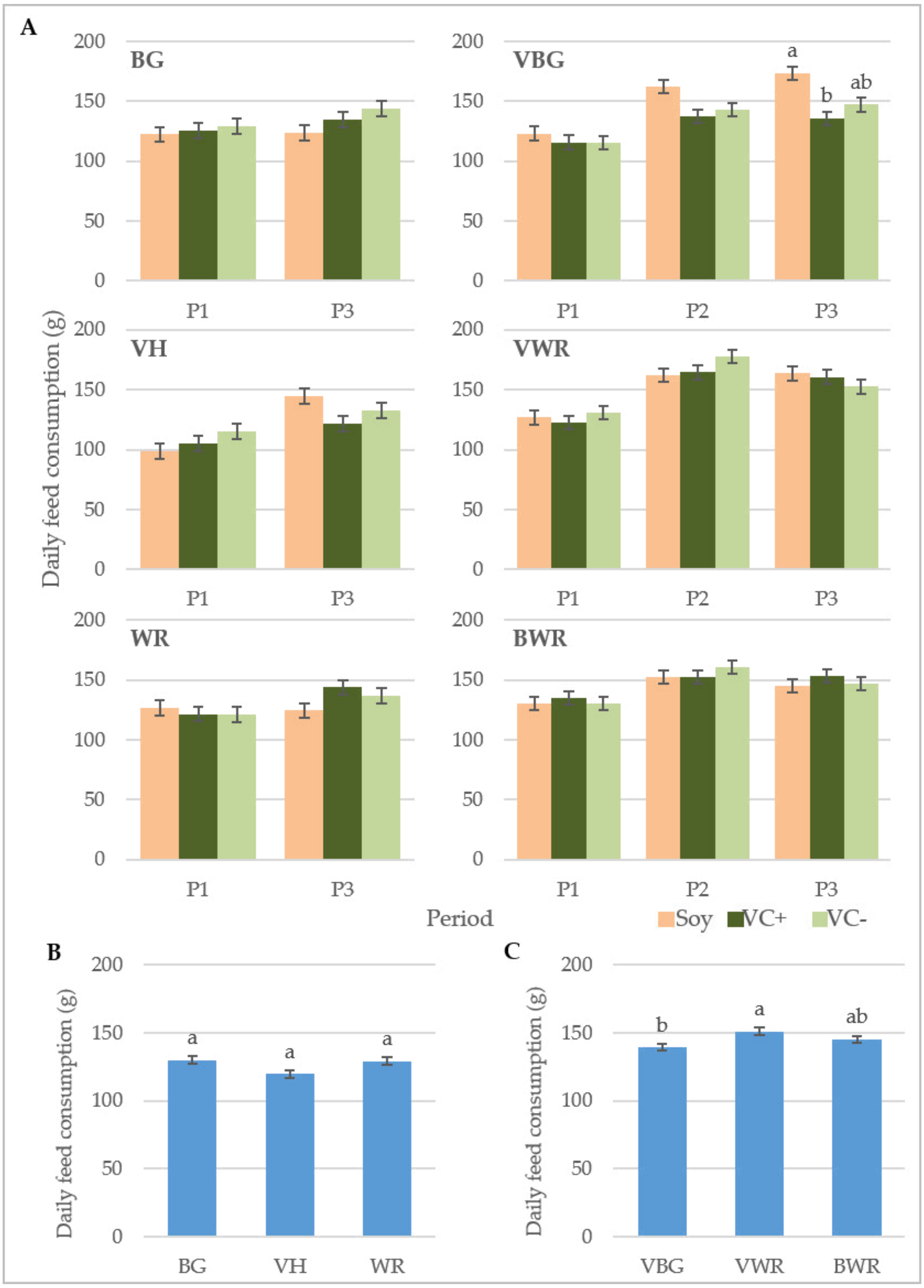

Figure S3.1. Daily feed consumption, LS-means ± SE. (A) Daily feed consumption of six genotypes under the influence of different diets during three periods. Period 1 from week 18-30, period 2 from week 31- 39, period 3 from week 40-51. (B) Daily feed consumption of the respective purebreds. (C) Daily feed consumption of the respective crossbreds. BG: Bresse Gauloise, VH: Vorwerkhuhn, WR: White Rock, VBG: VH male x BG female, VWR: VH male x WR female, BWR: BG male x WR female. a,b Bars not sharing a letter differ at $p < 0.05$. In (A) significant differences were only shown within genotype and period. Groupings without superscripts shown no significant differences.

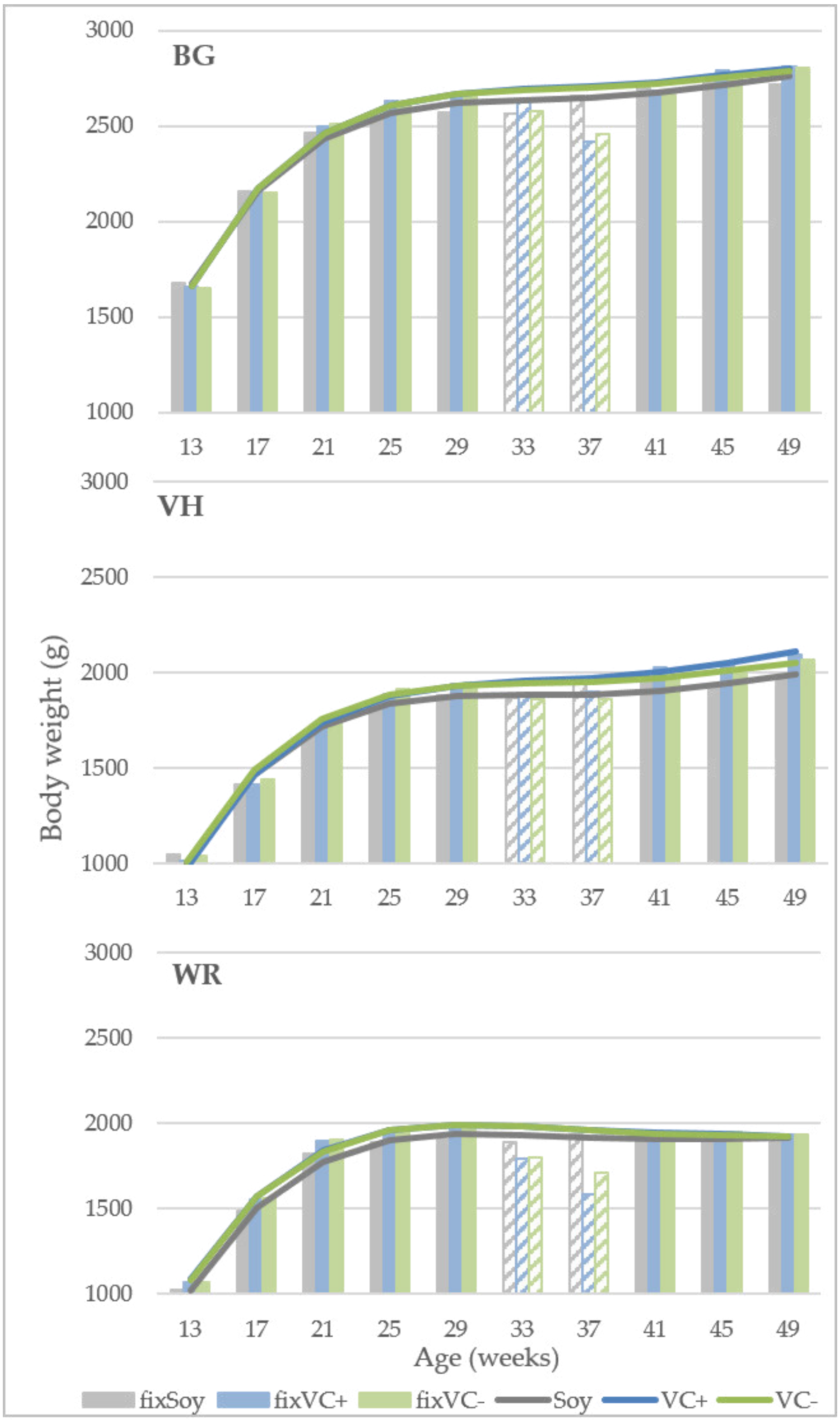

Figure S3.2. Modeling body weight of hens. Bar diagrams represent the complete data set. Data of striped bars was excluded from the final model, because of massive discrepancy between expected and measured values during a mite infestation in the chicken population. The exclusion of data took place iterative. The final growth curves (lines) were calculated via polynomial regression.

Figure S3.3. Modeling of laying performance. Bar diagrams represent the complete data set modeled with a linear mixed model. Data of striped bars was excluded from the final model, because of massive discrepancy between expected and measured values during a mite infestation in the chicken population. The exclusion of data took place iterative. The final curves (lines) were calculated via polynomial regression.

CHAPTER 4

Genotypic and Dietary Effects on Egg Quality of Local Chicken Breeds and Their Crosses Fed with Faba Beans

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Simple Summary: The quality of chicken eggs is important for reasons of food safety and the consumers' choice at the point of sale. Faba beans are a regionally produced alternative to soybeans, but they contain substances that could influence the egg quality. The aim of the present study was to test the influence of feeding faba beans on the egg quality of six different chicken genotypes including traditional breeds. The tested chicken genotypes were two local breeds, the Vorwerkhuhn and the Bresse Gauloise, as well as the commercial line White Rock and crossbreds thereof. The genotype had an influence on yolk weight, Haugh units, yolk and shell color, the frequency of inclusions in the eggs and the composition of the eggs. The feeding of faba beans influenced the yolk and shell color as well as Haugh units and shell portion. Egg traits were significantly influenced by the genotype.

Abstract: The quality of chicken eggs is an important criterion for food safety and the consumers' choice at the point of sale. Several studies have shown that egg quality can be influenced by the chickens' genotype and by the composition of the diet. The present study aimed to evaluate the effect of faba beans as a substitute for soybeans in the diet of chickens originating from traditional low-performance breeds in comparison with high-performing laying type hens and their crosses on egg quality parameters. Chickens of six different genotypes were fed either with a feed mix containing 20% faba beans with high or low vicin contents or, as a control, a feed mix containing soybeans. The genotypes studied were the local breeds Vorwerkhuhn and Bresse Gauloise, as well as commercial White Rock parent hens and their crosses. Yolk weight, Haugh units, yolk and shell color, the frequency of blood and meat spots and the composition of the eggs were significantly influenced by the genotype. The feeding of faba beans had an effect on yolk and shell color, Haugh units and shell portion, while there was no significant influence on the frequency of blood and meat spots.

Keywords: egg quality; faba bean; local breeds; vicin

Introduction

Chicken eggs are an important component of human nutrition, because they have a high nutritional value, are cheap to produce and are not subjected to religious restrictions [1]. Since the middle of the last century, poultry production systems have undergone a massive transformation from backyard farming to a highly specialized sector [2,3], which promoted the development and use of genotypes with high laying performance and high egg quality. The utilization of these high-performing lines in commercial poultry production led to the displacement of local chicken breeds due to their comparatively low performance level. Local breeds were since then mainly kept by hobby breeders who ensured their survival but did not systematically select for performance parameters. In the course of the discussion about the killing of day-old male chicks of layer lines, old local breeds came back into the focus of wider interest. Although it is clear that local breeds cannot match the specialized lines regarding performance parameters, economic value and resource efficiency, it is worth evaluating their potential as dual-purpose breeds to supply niche markets and to study how they perform as partners in cross-breeding. However, it is not clear whether the egg quality of local chicken breeds can keep up with that of commercial laying hens.

From the European consumer's point of view, the most important quality characteristics of eggs are shell strength, albumen consistency and yolk color [4]. The preference for specific yolk colors varies around the world [5], with a darker yellow yolk preferred in Europe [4,6]. The color of the shell is also important to consumers, with different regional preferences. For example, in Europe, brown-shelled eggs dominate, while in the U.S., white-shelled eggs make up the largest part [2]. Furthermore, the albumen consistency, measured as albumen height and converted to Haugh units, serves as an indicator of perceived freshness of eggs [2,4]. Inclusions in the egg, namely blood and meat spots, which develop through the rupture of small blood vessels or displacement of tissue in the oviduct, are generally considered undesirable [7].

It has been shown that these quality parameters are influenced by both the genetics and the composition of the diet. Well known is the effect of different feed components on yolk color, for example, alfalfa, marigold or yellow lupines [8,9]. The egg industry takes advantage of this fact to achieve the right yolk color for certain consumer segments by supplementing feeding stuff additives [10]. Haugh units decrease as the egg ages, but this parameter is also influenced by many other factors, such as genetic background and hen nutrition [5]. In the case of blood spots, Sauter et al. [11] described an influence of nutrition and genetics, but also of the season. The proportions of yolk and albumen are strongly influenced by genetic components. For example, eggs of commercial chicken lines have a higher amount of albumen and less yolk than eggs of local chicken breeds [1,12,13], which is likely due to the selection of commercial lines for higher egg weights and the negative correlation between yolk proportion and egg weight [2]. However, the diet also can influence the shares of yolk and albumen [14,15]. Regarding shell color, Hocking et al. [13] described a high genetic variation within and between commercial lines and traditional breeds. Wilson [16] also described an influence of genetics on shell color variation within lines but pointed out that the diet plays a role, too.

Among the ingredients used in chicken feedstuff, the faba bean (*Vicia faba* L.) is known to affect egg quality. Faba beans contain antinutritional factors, for example, the endogenous glycosides vicin and convicin (together abbreviated as VC). These substances were shown to be responsible for lowered egg and yolk weights, an increasing frequency of blood spots [17], as well as higher values in Haugh units [18].

The objective of the current study was to investigate the influence of feeding faba beans with two different concentrations of VC compared to soybean meal on internal egg quality traits and shell color of two local and one commercial chicken genotype and their crosses. Our focus was to assess whether local breeds with lower egg production levels are better able to compensate for the antinutritive substances contained in the faba bean in terms of egg quality than high-performing genotypes, and whether this makes a difference in their crosses as well.

Material and Methods

The current experiments were performed in accordance with the German Animal Welfare Law and approved by the Lower Saxony State Office for Consumer Protection and Food Safety (LAVES) (33.19-42502-04-17/2600).

Experimental Design

Two experiments were conducted to evaluate different parameters of egg quality. In experiment A (purebreds), two local chicken breeds and one commercial layer genotype were tested (Table 4.1). The two local ones were an old German chicken breed, the Vorwerkhuhn (VH), and the French breed Bresse Gauloise (BG). Both breeds have been kept by fancy breeders and were selected according to phenotypic breed standards. While the VH is a layer-type dual-purpose breed from northern Germany, the BG is mainly used for label-meat production in France. The commercial layer hens originated from parent stocks of White Rock (WR) of Lohmann Breeders GmbH (Cuxhaven, Germany). Experiment B (crossbreds) was carried out one year later with the following crosses of the purebreds used in experiment A: Vorwerkhuhn cock × Bresse Gauloise hen (VBG), Vorwerkhuhn cock × White Rock hen (VWR) and Bresse Gauloise cock × White Rock hen (BWR).

Table 4.1. Experimental design.

	Experiment A	Experiment B
genotypes	Bresse Gauloise (BG) Vorwerkhuhn (VH) White Rock (WR)	BG cock × WR hen (BWR) VH cock × BG hen (VBG) VH cock × WR hen (VWR)
diets	Control diet based on soybean meal (Soy) 20% vicin-rich faba bean (Fuego; VC+) 20% vicin-poor faba bean (Tiffany; VC−)	
number of birds	120 per genotype	
replicates	2	

Hens were fed three different diets to evaluate the effect on the internal egg quality. The experimental diets contained 20% faba beans (*Vicia faba L.*), either of the vicin-rich variety *Fuego* (VC+) or the vicin-poor variety *Tiffany* (VC−). The VC contents of the diets in experiment A were 0.12% (VC+) and 0.01% (VC−) and 0.13% and 0.02% in experiment B, respectively. The control diet was based on soybean meal (39.8% crude protein; Soy). As further protein source, all diets contained 21% blue sweet lupine (*Lupinus angustifolius cv. Boruta*). The diets were formulated according to GfE (German Society for Nutritional Physiology) recommendations to be isoenergetic and isonitrogenous [19]. A detailed table of ingredients was published before [20] (Supplementary Material Table S4.1).

The experiments lasted from the 18th until the 52nd week of age. In total, 120 hens per genotype were allocated to six pens of 20 hens each. In combination with the three different diets, this resulted in two replicates of each experimental group (genotype × diet combination). The hens were housed in floor pens equipped with wood chips, perch, dust bath and nine laying nests and had ad libitum access to feed and water. The experimental design and the husbandry conditions were previously described by Nolte et al. [20].

Data Collection

The assessment of internal egg quality was carried out three times for the pure breeds and four times for the crosses during the experiments, at weeks of age 26 (crossbreds only), 34, 42 and 50. Due to an unplanned infestation with the northern fowl mite (*Ornithonyssus sylviarum*) in experiment A, the data obtained in week of age 34 were considered not reliable and therefore excluded from the analysis. As a consequence, only data of week 42 and 50 were used for the purebreds.

On the day before laboratory analysis, 20 eggs of each experimental group (i.e., 10 eggs per pen) were collected randomly. Laboratory analyses started with measuring shell color at two points on the blunt end of the egg with a CM-600d spectrophotometer (Konica Minolta, Munich, Germany). Recorded values were the lightness L*, the redness a* and the yellowness b* of the shell. The blunt end was chosen because it was shown to be representative for the whole egg [21]. Once the eggs were weighed, they were carefully broken on a mirror table. The height of the albumen was measured one centimeter distant from the yolk with the Futura 2a system (Broering information technology, Lohne, Germany), which consists of an albumen height gauge connected to a computer with the appropriate software for data recording. Haugh units were calculated for each egg automatically by the software with the formula $HU = 100 * log\ (h - 1.7w^{0.37} + 7.6)$, where h is the albumen height and w is the egg weight. Placed on the mirror table, the broken eggs were visually examined for blood and meat spots. Blood spots were defined as located at the yolk, while meat spots were found in the albumen [7]. Yolk and albumen were separated from each other and the remains of the albumen on the yolk were removed by rolling the yolk carefully on a paper tissue. The yolk was weighed and the color determined with the Roche color fan (DSM nutritional products GmbH, Grenzach, Germany).

Albumen weight was calculated by subtracting the yolk and shell weight from the egg weight, whereas shell weights of all eggs analyzed in this study were available from the parallel analysis of external egg quality parameters [20]. Relative proportions of the various egg components such as yolk, albumen and shell were calculated from the quotient between the respective weight and egg weight.

Statistical Analysis

The data were analyzed with linear mixed models using the 'GLIMMIX' procedure of the statistical program SAS (SAS 9.3, SAS institute Inc., Cary, NC, USA). The two experiments (purebreds, crossbreds) were analyzed separately. The statistical model for the analysis of yolk weight, yolk color, Haugh units, yolk, albumen and shell percentage was as follows:

$$\mathrm{Y_{ijklm} = \mu + G_i + D_j + A_k + G_iD_j + G_iA_k + D_jA_k + G_iD_jA_k + p_l + e_{ijklm}} \qquad (4.1)$$

where Y_{ijklm} is the respective trait variable, μ is the overall mean, G_i is the fixed effect of genotype, D_j is the fixed effect of diet, A_k is the fixed effect of age, G_iD_j, G_iA_k, D_jA_k, $G_iD_jA_k$ are the interactions of the respective factors, p_l is the random effect of the pen and e_{ijklm} is the random error. The values of yolk, albumen and shell percentage were subjected to an arcsine transformation before analysis. The presented least squares means (LS-means) were then back-transformed to percentages. A similar statistical model was used for the analysis of shell color (L*, a* and b* values), whereby the repeated measurements on the individual egg (I) were considered as random effect in the model as follows:

$$Y_{ijklm} = \mu + G_i + D_j + A_k + G_iD_j + G_iA_k + D_jA_k + G_iD_jA_k + p(I)_l + e_{ijklm} \quad (4.2)$$

The number of blood and meat spots was analyzed by applying a linear logistic model as follows:

$$\log\left(\frac{\pi_{ijk}}{1-\pi_{ijk}}\right) = \varphi + G_i + D_j + A_k + G_iD_j + G_iA_k + D_jA_k + G_iD_jA_k \quad (4.3)$$

where π_{ijk} is the probability for the occurrence of blood or meat spots, φ is the overall mean, G_i is the fixed effect of genotype, D_j is the fixed effect of diet and A_k is the fixed effect of age, G_iD_j, G_iA_k, D_jA_k, $G_iD_jA_k$ are the interactions of the respective factors. LS-means were estimated on the logit scale and then back-transformed to the original scale (probability) by using the inverse link function [22].

For all parameters, significant differences between least squares means were tested using a *t*-test procedure by inclusion of the PDIFF option in the LSMEANS statement (SAS, 2018).

Results

The effects of genotype, diet, age and their interactions on the different egg quality parameters of the purebred hens are presented in Table 4.2.

Table 4.2. The effect of genotype, diet, age and their interactions on parameters of internal egg quality in purebred chicken.

Parameter	Genotype	Diet	Age	Genotype × Diet	Genotype × Age	Diet × Age	Genotype × Diet × Age
Yolk weight	**<0.0001**	0.0844	**0.0001**	0.6404	**<0.0001**	0.7032	0.7622
Yolk color	0.2420	0.0525	**<0.0001**	0.5153	0.5724	**<0.0001**	**0.0221**
Yolk percentage	**<0.0001**	0.3713	0.2232	0.9889	**0.0191**	0.6482	0.7768
Shell percentage	**<0.0001**	0.0789	0.0540	0.4343	0.6163	0.9988	0.3795
Albumen percentage	**<0.0001**	0.0739	0.6854	0.9959	**0.0081**	0.6494	0.7859
Shell color L*	**<0.0001**	**0.0110**	**<0.0001**	**0.0451**	0.9935	0.2120	0.7545
Shell color a*	**<0.0001**	0.0561	**<0.0001**	0.1267	0.1554	0.7685	0.5547
Shell color b*	**<0.0001**	**0.0457**	**0.0031**	0.4040	0.0571	0.1707	0.8498
Haugh units	**<0.0001**	**<0.0001**	0.5972	0.2412	0.5441	0.8835	0.5985
Blood spots	**0.0009**	0.3186	0.8136	0.9479	0.5911	0.5676	0.1443
Meat spots	0.7576	0.9002	0.9534	0.8437	0.1812	0.9006	0.5985

p-values, significant results ($p < 0.05$) are accentuated in bold numbers.

Yolk weight was influenced by genotype, age and their interaction. The BG showed that the highest yolk weight (17.96 g), the weights of VH (16.66 g) and WR (15.29 g) were significantly lower (Figure 4.1). Regarding the effect of age, the yolk weight significantly increased from week 42 to 50 by 0.55 g. In contrast to the local breeds, the yolk weight of WR decreased with increasing age.

Yolk color was not influenced by the main factors genotype and diet, but by the age, the interaction of diet and age and the threefold interaction of all factors. There was an increase in yolk color score from week 42 to 50 by one tint of the Roche color fan (Figure 4.1). This effect could actually be seen in the VC+ and VC− groups of all genotypes, although not statistically significant for VC−. On the contrary, in the soy groups a brightening of yolk color with aging was observed. Comparing the feeding groups between both measurements, the changes were statistically significant in all diets.

All egg components were significantly influenced by the main factor genotype. For yolk and albumen percentages as well interactions of genotype × age were observed (Table 4.2). The yolk percentage was highest in the local breeds BG (32.49%) and VH (31.26%), whereas the WR yolk amounted to 26.28%. All genotypes differed significantly from each other (Figure 4.2).

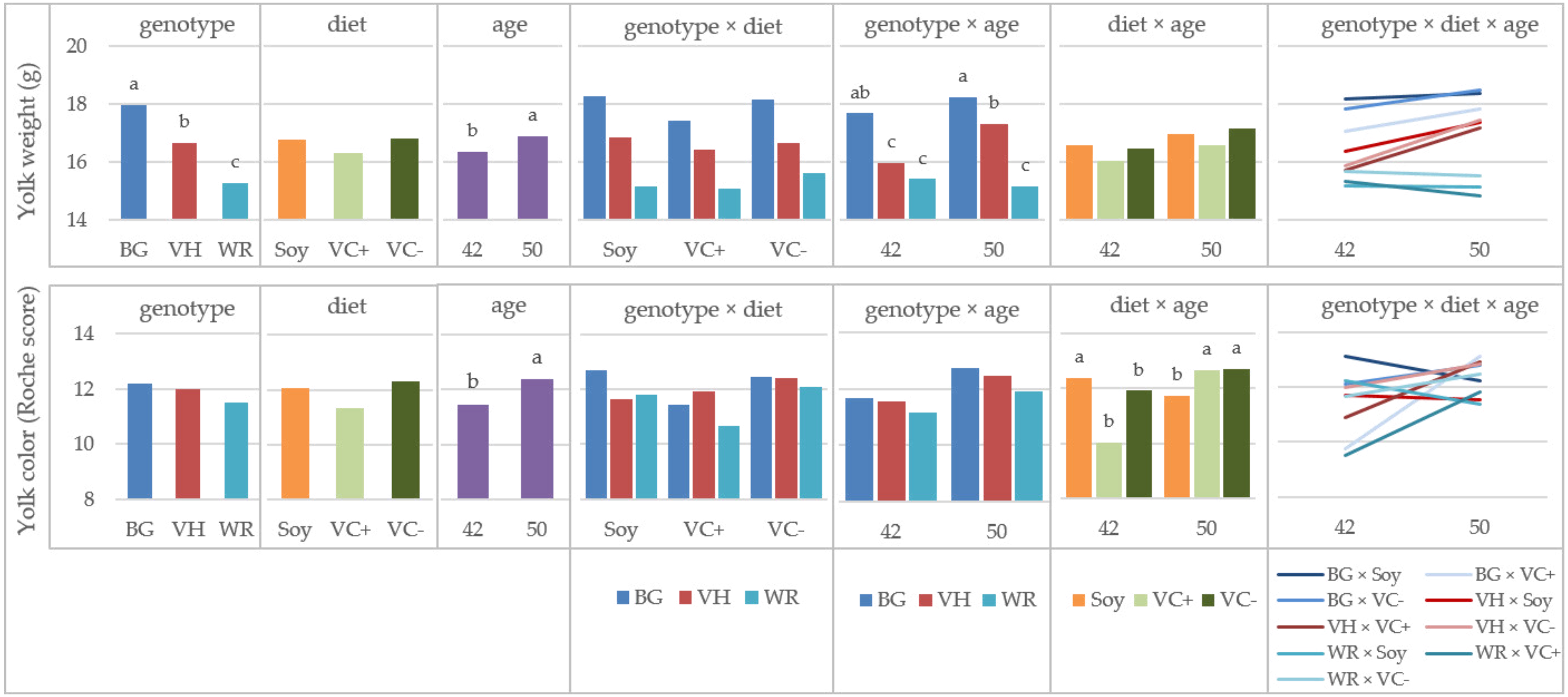

Figure 4.1. Yolk weight and color in purebred chicken, LS-means for the effects of genotype, diet, age and their interactions; BG: Bresse Gauloise, VH: Vorwerkhuhn, WR: White Rock; [a,b,c] Bars in one diagram not sharing a letter differ at $p < 0.05$, letter codes only shown for significant eff ects.

Figure 4.2. Egg components in purebred chicken, LS-means for the effects of genotype, diet, age and their interactions; BG: Bresse Gauloise, VH: Vorwerkhuhn, WR: White Rock; [a,b,c] Bars in one diagram not sharing a letter differ at $p < 0.05$, letter codes only shown for significant effects.

While the portion of yolk in the local breeds increased with aging of the hens, the WR showed a decrease. However, these changes were only small and not statistically significant. The albumen percentage was highest in the WR (63.30%) and lowest in BG (58.08%). The interaction of genotype and age corresponded to a decrease of albumen percentage from week 42 to 50 in BG and VH, while it increased in WR, as well only minimal and not statistically significant. The shell portion was highest in WR (10.41%) and significantly lower in BG (9.40%) and VH (9.12%).

Eggshell lightness (L*) was influenced by the main factors genotype and diet and their interaction as well as by the age (Table 4.2). Only in WR chicken was a significant difference between feeding groups observed (Table 4.3), meaning that the VC− groups produced eggs with a slightly lower shell lightness than the Soy and VC+ groups. With regard to the difference between genotypes, the shell color of BG and VH was cream, while the WR laid dark brown eggs. Therefore, the BG and VH showed significantly higher L* values than the genotype WR. With aging of the hens, the lightness of the eggshell increased significantly from 74.42 to 76.31. The redness (shell a*) was influenced by the genotype and age of the hens. All genotypes differed significantly, with WR having the highest a* value and VH the lowest. A significant decrease in a* value was recorded with increasing age. The yellowness, expressed as b* value, behaved similar to the redness. A significant influence of the diet was observed. This is, however, not reflected in the LS-means, as the feeding groups do not differ significantly from each other.

Table 4.3. Least-squares means ± SE for the effect of genotype, diet, age and the genotype × diet interaction in purebred groups on shell color (L*, a* and b* values).

Effect	Shell L*	Shell a*	Shell b*
Genotype			
BG	84.55 ± 0.32 [a]	3.26 ± 0.17 [b]	13.78 ± 0.31 [b]
VH	85.05 ± 0.32 [a]	2.60 ± 0.17 [c]	12.50± 0.31 [c]
WR	56.50 ± 0.32 [b]	19.82 ± 0.17 [a]	29.25 ± 0.31 [a]
Diet			
Soy	76.01 ± 0.32 [a]	8.46 ± 0.17	18.80 ± 0.31
VC+	75.44 ± 0.32 [ab]	8.33 ± 0.17	17.88 ± 0.31
VC−	74.66 ± 0.32 [b]	8.89 ± 0.17	18.86 ± 0.31
Age (weeks)			
42	74.42 ± 0.26 [b]	8.97 ± 0.14 [a]	19.05 ± 0.25 [a]
50	76.31 ± 0.26 [a]	8.15 ± 0.14 [b]	17.97 ± 0.25 [b]
Genotype × Diet			
BG × Soy	84.69 ± 0.55 [a]	3.32 ± 0.30	13.75 ± 0.54
BG × VC+	84.14 ± 0.55 [a]	3.32 ± 0.30	13.64 ± 0.54
BG × VC−	84.81 ± 0.55 [a]	3.14 ± 0.30	13.95 ± 0.54
VH × Soy	85.47 ± 0.55 [a]	2.64 ± 0.30	12.76 ± 0.54
VH × VC+	85.80 ± 0.55 [a]	2.01 ± 0.30	11.37 ± 0.54
VH × VC−	83.89 ± 0.55 [a]	3.16 ± 0.30	13.39 ± 0.54
WR × Soy	57.86 ± 0.55 [b]	19.44 ± 0.30	29.89 ± 0.54
WR × VC+	56.37 ± 0.55 [bc]	19.64 ± 0.30	28.62 ± 0.54
WR × VC−	55.27 ± 0.55 [c]	20.37 ± 0.30	29.24 ± 0.54

BG: Bresse Gauloise, VH: Vorwerkhuhn, WR: White Rock; [a,b,c] Values in one column and effect not sharing a letter differ significantly at $p < 0.05$.

For Haugh units, two main factors, i.e., genotype and diet, were identified by ANOVA. All genotypes differed significantly from each other, with WR showing the highest values and VH the lowest values (Table 4.4). With regard to the feed treatment, in the VC+ groups significantly higher Haugh units have been measured than in the Soy and VC− groups.

Table 4.4. Least-squares means ± SE for the effect of genotype and diet in purebred groups on Haugh units, blood and meat spots.

Effect	Haugh Units	Blood Spots (%)	Meat Spots (%)
Genotype			
BG	74.71 ± 0.66 [b]	15.28 ± 3.85 [a]	13.15 ± 3.51
VH	67.56 ± 0.66 [c]	7.69 ± 2.71 [a]	16.94 ± 3.74
WR	87.37 ± 0.66 [a]	52.59 ± 5.21 [b]	3.21 ± 168.30
Diet			
Soy	75.04 ± 0.66 [b]	23.51 ± 5.18	2.10 ± 111.57
VC+	78.97 ± 0.66 [a]	24.62 ± 5.12	16.68 ± 3.73
VC−	75.61 ± 0.66 [b]	14.24 ± 4.22	19.23 ± 4.15

BG: Bresse Gauloise, VH: Vorwerkhuhn, WR: White Rock; [a,b,c] Values in one column and effect not sharing a letter differ significantly at $p < 0.05$.

Only the genotype had a significant influence on the frequency of blood spots (Table 4.2). The WR showed blood spots in more than half of the eggs examined. The frequency in VH and BG was significantly lower. The incidence of meat spots was neither influenced by genotype, age or diet, nor were there any significant interactions between factors.
Regarding crossbreed chickens in experiment 2, the effect of genotype, diet, age and their interactions on egg quality parameters is displayed in Table 4.5. Yolk weight was influenced by the main factor genotype, with BWR showing significantly heavier yolks than VWR (16.51 g vs. 15.97 g; Figure 4.3). As well, age had a significant influence on yolk weight, which was expressed in increasing yolk weights with aging. Between age and the main factors, interactions existed. Figure 4.3 shows that the increase of yolk weight is different between genotypes: VBG showed the highest gain of 6.89 g during the experiment, whereas the weight gain in BWRs yolks was only 5.54 g. Furthermore, in week 26, there was a significant difference between these two genotypes. The interaction of diet and age shows differences in the increase of yolk weight between feeding groups. The highest increase was observed for VC+ (6.38 g) and the lowest for VC− (5.56 g).

Table 4.5. The effect of genotype, diet, age and their interactions on parameters of internal egg quality in crossbred chicken.

Parameter	Genotype	Diet	Age	Genotype × Diet	Genotype × Age	Diet × Age	Genotype × Diet × Age
Yolk weight	**0.0088**	0.0723	**<0.0001**	0.1216	**<0.0001**	**0.0168**	**0.0099**
Yolk color	**0.0135**	**0.0128**	**<0.0001**	0.6096	0.0984	0.4923	**0.0465**
Yolk percentage	**<0.0001**	0.4763	**<0.0001**	0.7766	0.1651	**0.0002**	**<0.0001**
Albumen percentage	**<0.0001**	0.4129	**<0.0001**	0.7064	0.1161	**0.0004**	**<0.0001**
Shell percentage	**0.0056**	**0.0139**	**<0.0001**	0.5859	0.9079	0.1994	0.5292
Shell color L*	**<0.0001**	**0.0109**	**0.0085**	0.2232	0.1173	0.7424	0.2797
Shell color a*	**<0.0001**	**0.0212**	**0.0058**	0.2614	0.2981	0.2900	0.1993
Shell color b*	**<0.0001**	**0.0099**	**<0.0001**	0.5107	0.8808	0.7680	0.2584
Haugh units	**<0.0001**	**0.0040**	**<0.0001**	**0.0089**	0.2369	0.2822	0.5560
Blood spots	**<0.0001**	0.7914	0.4928	0.8743	0.3978	0.8752	0.6308
Meat spots	0.9996	1.0000	1.0000	1.0000	0.9621	0.7880	0.9684

p-values, significant results ($p<0.05$) are accentuated in bold numbers.

Yolk color of the crossbreds was influenced by the effects of genotype, diet, age and their three-way interaction. During the experiment, a brightening of yolk color took place in almost all experimental groups with increasing age (Figure 4.3). The highest difference was shown of the BWR VC− group of 1.2 nuances of the Roche color score. In contrast, the difference in the BWR Soy group was only −0.3 and in the VC+ group +0.1. Looking at the single effects of the main factors the VBG showed significantly darker yolks than BWR and VWR, but it must be mentioned that this difference was less than 0.2 Roche tones. The same is true for the effect of diet, where the soy groups show statistically significant brighter yolks than the faba bean groups. In Figure 4.4, the egg components of the crossbreds are shown. Yolk and albumen percentage were significantly influenced by the genotype, the age, the interaction of diet × age and the three-way interaction of all three factors. The VBG had the significantly highest portion of yolk of 29.80%, whereas the WR crosses achieved 28.78% (BWR) and 28.03% (VWR), respectively. A statistically significant increase of yolk percentage with increasing age amounting in total to 5.71% could be observed. The general trend of increasing yolk percentage was not true for all experimental groups, indicated by significant interactions between the three factors genotype, diet and age. In the BWR Soy group from week 42 to 50, a decrease of almost 1% took place. Furthermore, in the BWR VC+ group, a decrease from 29.43% to 28.11% from week 34 to week 42 was observed, which was compensated by an increase of up to 31.01% measured in week 50. In the case of albumen percentage, the effects of genotype and age behaved exactly the other way round. The VWR and BWR showed significantly higher portions than the VBG (61.91% and 61.80% vs. 60.34%). With aging, the albumen percentage was lowered by 5.35% over the experimental period. As well, in this parameter, the BWR groups behaved differently than the general trend. The BWR Soy group was characterized by an increase of the albumen percentage from week 42 to 50, whereas the increase in the BWR VC+ group took place from week 34 to 42, followed again from a decrease of albumen percentage towards week 50. The shell percentage was relevantly influenced by genotype, diet and age. The VWR revealed a significantly higher portion of shell than the VBG (10.03% vs. 9.82%), the BWR being intermediate (9.89%). Of the feeding groups, the Soy group showed a 0.19% higher portion of shell than the VC+ group. With respect to the age, there was a statistically significant decrease of shell portion observed from week 34 to week 42.

Figure 4.3. Yolk weight and color in crossbred chicken, LS-means for the effects of genotype, diet, age and their interactions; VBG: VH male × BG female, VWR: VH male × WR female, BWR: BG male × WR female; [a,b,c,d,e,f,g] Bars in one diagram not sharing a letter differ at $p < 0.05$, letter codes only shown for significant effects.

Figure 4.4. Egg components in crossbred chicken, LS-means for the effects of genotype, diet, age and their interactions; VBG: VH male × BG female, VWR: VH male × WR female, BWR: BG male × WR female; [a,b,c,d,e] Bars in one diagram not sharing a letter differ at $p < 0.05$, letter codes only shown for significant effects.

All parameters of eggshell color (lightness L*, redness a* and yellowness b*) were significantly affected by genotype, diet and age (Table 4.5). The VBG showed significantly higher lightness and significantly lower a* and b* values than VWR and BWR, which did not differ significantly from each other (Table 4.6). Regarding the dietary effect, the VC+ group revealed significantly higher L* and significantly lower a* and b* values than the VC− group. The Soy group behaved intermediately. The effect of age was different between the parameters. In case of the L* value, in week 34 and 50, the value was significantly lower than in week 26. The redness was significantly higher in week 34 than in week 50. The b* was significantly lower in week 26 than in weeks 34 and 42, which did not differ. In week 50, the b* value was significantly lower than at all other time points.

Table 4.6. Least-square means ± SE for the effect of genotype, diet and age in crossbred groups on shell color (L*, a* and b* values).

Effect	Shell L*	Shell a*	Shell b*
Genotype			
VBG	81.85 ± 0.27 ^{a}	5.05 ± 0.16 ^{b}	16.78 ± 0.21 ^{b}
VWR	68.67 ± 0.27 ^{b}	13.69 ± 0.16 ^{a}	25.40 ± 0.21 ^{a}
BWR	67.98 ± 0.27 ^{b}	13.82 ± 0.16 ^{a}	25.90 ± 0.21 ^{a}
Diet			
Soy	72.79 ± 0.27 ^{ab}	10.89 ± 0.16 ^{ab}	22.77 ± 0.21 ^{ab}
VC+	73.42 ± 0.27 ^{a}	10.51 ± 0.16 ^{b}	22.21 ± 0.21 ^{b}
VC−	72.29 ± 0.27 ^{b}	11.15 ± 0.16 ^{a}	23.09 ± 0.21 ^{a}
Age (weeks)			
26	73.70 ± 0.31 ^{a}	10.61 ± 0.19 ^{ab}	22.44 ± 0.24 ^{b}
34	72.30 ± 0.31 ^{b}	11.26 ± 0.19 ^{a}	23.48 ± 0.24 ^{a}
42	72.80 ± 0.31 ^{ab}	11.09 ± 0.19 ^{ab}	23.65 ± 0.24 ^{a}
50	72.54 ± 0.31 ^{b}	10.45 ± 0.19 ^{b}	21.20 ± 0.24 ^{c}

VBG: Vorwerkhuhn male × Bresse Gauloise female, VWR: Vorwerkhuhn male × White Rock female, BWR: Bresse Gauloise male × White Rock female; ^{a,b,c} Values within one column and effect not sharing a letter differ significantly at $p < 0.05$.

The Haugh units for the crossbred chickens are displayed in Table 4.7. The main factors, genotype, diet and age, significantly influenced the Haugh units (HU) as well as the interaction of genotype × diet. In VBG, the VC+ group showed significantly higher HU than the VC− group, while in BWR the VC+ group achieved significantly higher HU than the Soy group. In the main effect of genotype, BWR showed the highest HU followed by VWR and VBG, all differing significantly from each other. With respect to the diet, the VC+ groups had significantly higher HU than Soy and VC−. Aging of hens led to a decrease of HU from week 26 to 34 and to 42, with weeks 42 and 50 not differing statistically significantly.

Table 4.7. Least-square means ± SE for the effect of genotype and diet in crossbred groups on Haugh units and egg inclusions.

Effect	Haugh Units	Blood Spots (%)	Meat Spots (%)
Genotype			
VBG	77.23 ± 0.60 [c]	3.72 ± 97.13	11.70 ± 2.23
VWR	81.01 ± 0.60 [b]	24.06 ± 2.76 [a]	1.00 ± 103.16
BWR	85.12 ± 0.60 [a]	44.46 ± 3.53 [b]	3.67 ± 260.50
Diet			
Soy	80.42 ± 0.60 [b]	24.31 ± 3.06	3.16 ± 225.71
VC+	82.75 ± 0.60 [a]	21.24 ± 3.28	3.44 ± 244.65
VC−	80.19 ± 0.60 [b]	10.16 ± 247.56	4.20 ± 296.63
Age (weeks)			
26	85.56 ± 0.69 [a]	20.15 ± 3.82	3.54 ± 335.59
34	81.84 ± 0.69 [b]	27.82 ± 3.97	1.94 ± 186.71
42	78.76 ± 0.69 [c]	7.40 ± 247.70	7.88 ± 1.88
50	78.32 ± 0.70 [c]	21.24 ± 3.48	2.95 ± 281.82
Genotype × Diet			
VBG × Soy	76.63 ± 1.04 [de]	11.44 ± 3.84	10.41 ± 3.56
VBG × VC+	79.84 ± 1.04 [cd]	8.56 ± 3.41	11.99 ± 4.02
VBG × VC−	75.22 ± 1.04 [e]	0.47 ± 38.41	12.82 ± 4.12
VWR × Soy	81.92 ± 1.04 [bc]	26.95 ± 5.00	0.32 ± 70.30
VWR × VC+	80.40 ± 1.04 [bcd]	21.24 ± 4.79	0.28 ± 62.66
VWR × VC−	80.70 ± 1.04 [bcd]	24.21 ± 4.82	10.14 ± 3.50
BWR × Soy	82.71 ± 1.04 [bc]	41.01 ± 5.64	8.56 ± 3.54
BWR × VC+	88.01 ± 1.04 [a]	43.71 ± 5.89	10.41 ± 3.59
BWR × VC−	84.64 ± 1.04 [ab]	48.72 ±5.83	0.50 ± 111.10

VBG: Vorwerkhuhn male × Bresse Gauloise female, VWR: Vorwerkhuhn male × White Rock female, BWR: Bresse Gauloise male × White Rock female; [a,b,c,d,e] Values within one column and effect not sharing a letter differ significantly at $p < 0.05$.

Bloodspots differed between genotypes, with BWR showing the highest frequency and VBG the lowest. The frequency of meat spots was not significantly influenced by any of the tested effects.

Discussion

In purebred chicken, the yolk weight of the local breeds BG and VH was higher than that of the commercial line WR. A similar difference between local and commercial chickens was also described in several studies comparing different commercial lines and local breeds [1,12,13]. Moreover, Rizzi and Chiericato [23] observed that increasing age of hens led to an increase in yolk weight of Italian local breeds but not in commercial hybrids. The same was shown in the present study. Regarding the diet, some authors described VC leading to lowered yolk weights [17,24]. This cannot be confirmed by the present study.

Concerning the yolk color, a remarkable increase in color score was observed from week 42 to 50 in the VC+ groups of all genotypes. As noted above, there was an infestation of the Northern Fowl mite in the stock around week 34 that led to severe performance losses mainly in the VC+ groups. Both the feeding of faba beans and an infection with fowl mites challenge the immune system [25–27] and influence therefore metabolic processes in the liver. Given that yolk pigments are partly built in the liver, a causal connection between the previous exposure to metabolic stress and the relatively bright yolk colors of the VC+ groups in week 42 might be possible. However, this observation was found by chance, and a more detailed investigation of such a relationship requires further research with a specific experimental design. The increase in color score of the VC− groups was much weaker with less than 1 Roche nuance from weeks 42 to 50, while on the other hand, the Soy groups showed a light brightening. In literature, both darker and brighter yolk color under the feeding of faba beans was described [28,29], as well as no effect [18].

Egg components showed genotypic differences as expected with the local breeds' eggs having a higher portion of yolk and less albumen and shell percentages than the commercial line WR. The breeding for higher egg weights led to a relative increase of albumen, and the breeding for high shell stability led to a higher portion of shell. The genotype × age interaction demonstrated that the genotype differences in yolk and albumen portion become even more clear with aging [23].

Shell color is determined genetically and therefore differs between genotypes. The WR is a brown layer line, while the egg shell colors of BG and VH are described as white or yellowish [30,31]. Although the effects of diet, genotype × diet and age as well have been statistically significant in the analysis, these caused only tiny changes that were scarcely visible nuances to the human eye. However, an influence of feed on eggshell lightness (L*) was recently reported by Mori et al. [32], comparing mixed and fermented feed.

The dependence of Haugh units on chicken genotype is controversially discussed in the literature. Haugh units of local chickens have been higher [6], lower [13] or in between [1] that of commercial lines. The genotype differences were confirmed in the present study, with the commercial genotype showing the highest values. The effect of faba beans on Haugh units is also not distinct. In our study, the highest Haugh units were observed in the groups fed with the VC+ diet. While in some studies an increase of Haugh units along with increased faba bean levels was observed [28,33], Lessire et al. [18] ascribed this effect to VC, leading to higher viscosity of the albumen. In contrast, Daenner [34] did not observe a change in Haugh units while feeding different levels of vicin-rich and vicin-poor faba beans.

In the present study, WR showed a much higher frequency of blood spots than the local genotypes, while in the case of meat spots, the differences between the genotypes were not significant. Hocking et al. [13] found no difference between the frequency of blood spots in traditional breeds compared to commercial lines and, similarly, Sauter et al. [11] negated an influence of laying performance on the amount of blood spots. Brade et al. [7] stated that brown-shelled eggs in general have more blood and meat spots compared to white-shelled eggs. No influence of faba bean feeding on the frequency of blood and meat spots was detected in the present study. This is in accordance with the results of Lessire et al. [18] but contradictory to Muduuli et al. [17], who described four times more blood spots in eggs of hens that were fed 1% vicin in the diet compared to the control group. Robblee et al. [33] also observed a slight increase in the number of blood spots.

Yolk weight of the crossbreds was influenced by the three-way interaction of all factors. There was a trend towards increased yolk weights as hens aged, although its magnitude differed between genotypes and feeding groups. No clear direction of the interaction is visible.

Although statistically significant differences in yolk color were detected, these are of minor relevance, as they were less than one nuance on the Roche color fan.

In general, there is a trend of increasing yolk and decreasing albumen portion with aging of the hens, which was also observed in the local breeds in Experiment A. For the effect of crossbreeding a local with a commercial genotype, this could be a favorable effect, as yolk is the part of the egg containing the valuable ingredients [35].

The shell color tones of the crossbreds' eggs were mixtures of the colors of the parental lines, i.e., light brown in the case of VWR and BWR and white to tinted in VBG. Li et al. [21] observed a similar effect with the crosses of white and brown layers and suggested additive effects of the genes responsible for eggshell color resulting in a mixture of color. Similar as in experiment A, the significant differences in L*, a* and b* values between feeding groups and measurements are negligible, because they were not visible with the human eye at all.

The genotype × diet interaction in Haugh units showed different responses of the crossbreds towards the diets. As described above, the information from other studies regarding the effect of faba beans on Haugh units was not the same between experiments. This is possibly due to the different commercial genotypes used in the respective experiments.

Our results suggest that the genetic predisposition to blood spots was transferred from WR hens to their crossbred offspring. While the frequency in BWR was slightly lower compared to the parental WR, the crossbreeding of WR with VH reduced the frequency of blood spots by half. This reduction was also observed when the local breeds were crossed with each other (VBG).

Conclusions

All crossbred genotypes, especially the two crosses with WR hens, revealed an internal egg quality that is comparable to that of commercial layers.
The apparent susceptibility of WR hens to blood spots is significantly reduced in the progeny of these birds when crossed with the local breeds.
In our companion publication addressing the egg production traits and bone stability of these hens, we concluded BWR to be the most promising genotype of the evaluated crossbreds regarding dual-purpose use [20]. This is still true, although the BWR genotype has the disadvantage of a high frequency of blood spots in the eggs, even though it is lower than WR. Assembling the present study with our previous publications regarding the egg production traits and bone stability of the hens [20] and the fattening performance of the male counterparts [36], it becomes again apparent that faba beans at the portion of 20% are a suitable alternative to soybeans at least for the investigated genotypes.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/ani11071947/s1, Table S4.1: Composition, analyzed and calculated nutrient composition of the experimental diets.

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Data Availability Statement: The data presented in this study are available on reasonable request from the corresponding author.

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Supplementary Material

Table S4.1. Composition, analyzed and calculated nutrient composition of the experimental diets.

Item	Experiment A			Experiment B		
	Soy	**VC+**	**VC−**	**Soy**	**VC+**	**VC−**
	Ingredients (%)					
Wheat	40.39	29.78	29.78	40.39	29.78	29.78
Corn	10.00	10.89	10.89	10.00	10.89	10.89
Soybean meal (39.8% CP)	11.84	-	-	11.84	-	-
Blue sweet lupine cv. Boruta	21.00	21.13	21.13	21.00	21.13	21.13
Faba bean cv. Fuego	-	20.00	-	-	20.00	-
Faba bean cv. Tiffany	-	-	20.00	-	-	20.00
Soybean oil	4.00	5.77	5.77	4.00	5.77	5.77
Dicalcium phosphate	2.76	2.51	2.51	2.76	2.51	2.51
Calcium carbonate	8.39	8.25	8.25	8.39	8.25	8.25
Sodium chloride	0.32	0.25	0.25	0.32	0.25	0.25
DL-Methionine	0.21	0.28	0.28	0.21	0.28	0.28
Lysine	0.09	0.10	0.10	0.09	0.10	0.10
Tryptophan	-	0.04	0.04	-	0.04	0.04
Premix [1]	1.00	1.00	1.00	1.00	1.00	1.00
	Chemical composition					
Dry matter abs (%) [2]	89.60	89.50	89.50	90.90	91.20	90.90
Crude ash (g/kg DM) [2]	152.60	149.30	148.70	129.70	139.50	146.70
Crude protein (g/kg DM) [2]	182.40	171.90	185.30	185.20	202.10	184.10
Crude fat (g/kg DM) [2]	95.00	88.80	97.40	91.10	83.70	91.80
Crude fiber (g/kg DM) [2]	54.60	50.30	54.50	61.80	51.30	59.20
Starch (g/kg DM) [2]	362.60	393.10	349.50	365.90	349.5	347.00
Sucrose (g/kg DM) [2]	29.70	25.00	24.00	24.60	26.60	24.60
SFA (g/100g fat) [2]	17.70	17.00	16.10	17.6	16.80	16.40
MUFA (g/100g fat) [2]	22.50	22.60	22.80	22.70	22.80	21.80
PUFA (g/100g fat) [2]	59.80	60.40	61.10	59.6	60.40	61.70
Vicine (%) [2]	0.016	0.079	0.003	0.0	0.095	0.015
Convicine (%) [2]	0.006	0.037	0.002	0.0	0.039	0.004
VC (Vicin + Convicin; %) [3]	0.022	0.116	0.005	0.0	0.134	0.019
Tannin (mg/g) [2]	3.51	3.02	3.33	3.22	3.91	3.67
AMEn (MJ/kg) [3,4]	12.53	12.60	12.36	12.43	12.19	12.12
Methionine (%) [3]	0.42	0.44	0.44	0.42	0.44	0.44
Lysine (%) [3]	0.81	0.83	0.83	0.81	0.83	0.83
Tryptophan (%) [3]	0.16	0.17	0.17	0.16	0.17	0.17
Threonine (%) [3]	0.58	0.55	0.55	0.58	0.55	0.55

CP: crude protein, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, AME_n: nitrogen-corrected apparent metabolizable energy; [1] Premix-hens: feed additives (per kg premix): Vitamin A, 1,000,000 IU; Vitamin D3, 250,000 IU; Vitamin E, 2000 mg; Vitamin B1, 250 mg; Vitamin B2, 700 mg; Vitamin B6, 400 mg; Vitamin B12, 2000 µg; Vitamin K3, 400 mg; Nicotin amide, 4000 mg; Calcium-D-pantothenate, 1000 mg; Folic acid, 60 mg; Biotin, 2500 µg; Choline chloride, 40,000 mg; Fe, 4000 mg; Cu, 1000 mg; Mn, 10,000 mg; Zn, 8000 mg; I, 120 mg; Se, 25 mg; Co, 20.5 mg; Butylated hydroxy toluene (BHT), 12,500 mg; Beta-carotene, 400 mg; Canthaxanthin, 400 mg; [2] Analyzed; [3] Calculated; [4] Apparent metabolizable energy concentrations corrected to zero nitrogen balance (AMEn), calculated according to the energy estimation equation of the World's Poultry Association (Vogt, 1986).

Cited from: Nolte, T.; Jansen, S.; Halle, I.; Scholz, A. M.; Simianer, H.; Sharifi, A. R.; Weigend, S. Egg Production and Bone Stability of Local Chicken Breeds and Their Crosses Fed with Faba Beans. Animals 2020, 10. doi: 10.3390/ani10091480.

CHAPTER 5

General Discussion

General Discussion

Dual-purpose chickens are currently much-debated in terms of future poultry production. Since the culling of day-old male chicks will be forbidden from 2022 on, alternative practices must be available soon. The topic of the present thesis was to investigate performance levels of two local breeds and crossbreds thereof regarding dual-purpose use.

While the previous chapters dealt with the performance of the male chickens (**Chapter 2**) or the female chickens alone (**Chapters 3, 4**), the following chapter will bring both sexes together as this is crucial for the evaluation of a dual-purpose genotype. A field study to validate the performance of the crossbreds under practical conditions was conducted and the results will be summed up and brought in line with the experiments. Furthermore, the suitability of regional faba beans as protein source in the feed of these genotypes will be discussed. Finally, recommendations for practice will be made and a general conclusion will be drawn.

Comparison of the different genotypes regarding dual-purpose use

In a first experiment, the performance levels of the purebred chickens have been investigated. As expected, the WR showed a low fattening performance and a high laying performance with a peak production of 100%. The BG showed not only a daily weight gain comparable to slow-growing broilers [1], but also a satisfying laying performance (**Table 5.1**). Although the VH is described as dual-purpose chicken [2], both the fattening and even the laying performance have been quite low in the present study. The egg size was small with less than 50 g on average, what is even lower than described by Weigend et al. for this breed [3].

Table 5.1. Overview on dual-purpose performance. Compilation of results from Chapters 2 and 3.

	Cockerels			Hens	
	Slaughter age (weeks)	Live weight [1,2] (g)	Daily Gain [1,3] (g)	Laying performance [4,5] (%)	Egg weight [4,6] (g)
BG	10	1883-1905 ± 14	34.9-35.7 ± 0.6	63.7 ^{b} ± 1.1	51.9 ^{b} ± 0.2
VH	16	2139-2196 ± 21	21.4-22.8 ± 0.6	38.8 ^{c} ± 1.1	49.5 ^{c} ± 0.2
WR	17	2233-2308 ± 21	21.2-22.1 ± 0.6	83.7 ^{a} ± 1.1	56.6 ^{a}± 0.2
BWR	14	2195-2299 ± 20	27.2-27.8 ± 0.6	80.4 ^{a} ± 1.0	58.0 ^{a} ± 0.3
VBG	13	2114-2122 ± 25	27.7-27.8 ± 0.6	60.9 ^{c} ± 1.0	54.0 ^{c} ± 0.3
VWR	15	2042-2081 ± 22	22.5-23.2 ± 0.6	71.1 ^{b} ± 1.0	56.8 ^{b} ± 0.3

LS-means ± SE. BG: Bresse Gauloise, VH: Vorwerkhuhn, WR: White Rock, BWR: BG cock × WR hen, VBG: VH cock × BG hen, VWR: VH cock × WR hen, [1] Parameters analyzed separately by genotype, therefore no statistical comparison of significant differences between genotypes possible, [2] data obtained from table 2.6., [3] data obtained from table 2.3., [4] Parameters analyzed groupwise for purebreds and for crossbreds, [5] mean laying performance over the whole experiment, [6] data obtained from figure 3.4., [a,b,c] Results with different superscripts within one column and experiment differ statistically significant at $p < 0.05$.

In case of the crossbreds, the BG crosses, i.e., BWR and VBG, showed similar fattening performances and reached the 2 kg mark at 12 weeks of age. Though, regarding laying performance, the VBG had a significantly lower egg production and egg weights than the BWR (**Table 5.1**). The VWR showed the slowest growth speed of the crosses, reaching 2 kg at 15 weeks of age. However, the laying performance of VWR was in between that of BWR and VBG (**Figure 3.3**).

It should be mentioned that both experiments (pure- vs. crossbreds) are only comparable to a limited extend, as they have been conducted in two consecutive years and genotypic and year effects cannot be separated from each other without the risk of data confounding.

The suitability of Bresse Gauloise and crosses for dual-purpose use was previously tested by Lambertz et al. [4] and Baldinger and Busemas [5,6]. According to Lambertz et al. [4], male and female purebred BG chickens are superior to Bresse Gauloise × New Hampshire crosses in terms of dual-purpose performance. Baldinger and Busemas [5] evaluated, amongst others, BG × WR crosses and the results of the cockerels were very close to that of the present study with a slaughter weight of 2393 g in week 15 (here 2195 - 2299 g in week 14 according to diet), FCR of 3.6 (3.42 - 3.75) and dressing percentage of 66% (68.9% - 69.5%). The laying performance of the hens was higher in the present study (80.4% vs. 68%), but the mean egg weight was lower (58 g vs. 65 g). The cross of Vorwerkhuhn cock × White Rock hen is also called Kollbecksmoor Huhn and was developed by a conservation breeding program to produce hybrids with a higher performance level for financing conservation breeding activities [7]. The laying performance of the Kollbecksmoor Huhn is said to be 250 eggs/year, which is 68.5%, with an average egg weight of 61 g. In the present study the VWR cross showed a performance level of 71.1% with egg weights of 56.8 g on average. The expectation of increased fattening performance of the VWR compared to purebred VH could not be confirmed in the present study.

The value of crossbreeding

The performances of the BWR and VBG crosses clearly show the advantage of crossing a meat-type with an egg-type genotype to increase the dual-purpose potential of local chicken breeds in the F1 generation. Even if the performance levels are still lower compared to commercial lines, they are higher in comparison with many local dual-purpose breeds [8]. The value of crossbreeding of local breeds was also demonstrated by Vogt-Kaute et al. [9], who crossed cockerels of the endangered meat-type breed Mechelner with Lohmann Brown hens. The laying performance was comparable to that of commercial dual-purpose chickens and the fattening performance between that of layer line cockerels and commercial dual-purpose chickens in his study.

This model of crossing divergent genotypes is basically what the Lohmann Breeders GmbH did, when they bred the Lohmann Dual chicken, which is a cross of a broiler sire with a layer line dam. The performance level of Lohmann Dual lays in between that of specialized commercial lines and local breeds [10–12]. The Lohmann Dual benefits thereby from two breeding schemes, that are crossbreeding and the selection over generations within the specialized lines. For this reason, it can be questioned, if setting up selection programs for local breeds, in parallel to the crossbreeding to improve their performance immediately, could be an option for long-term performance enhancement. Since local chickens are generally kept by private persons, as a hobby or for extra income, establishing such a system might be difficult. A central institution would have to take over data recording and processing as basis for selection decisions, similar to that formerly done in Merbitz (Germany) or Neu-Ulrichstein (Germany) [13,14]. Therefore, a breeding organization would have to take over the collection of a sufficient number of animals to build up a breeding stock or the private fanciers would have to be very engaged in taking part in such a system with data acquisition in the field. Advantageous in improving performance by means of selection within certain breeds would be the possibility to use the breed itself for production instead of crossbred hybrids. A threat to breeders might be that the selection process could modify the genetic characteristics of a breed in an unwanted way, when the animals are selected more intensively for performance traits, i.e. the selection for specific performance traits could lead to simultaneous unplanned selection for undesirable traits due to genetic correlations [15]. Nevertheless, as well for establishing a crossbreeding program for production purposes, an appropriate breeding stock would be necessary to provide hybrids of similar quality constantly to producers, as in most fancy breeds, a lack of breeding program led to inhomogeneity between animals from different breeders. The existence of a nucleus herd would also have facilitated the accomplishment of the present study. Due to the low laying performance of VH it was challenging to produce the needed purebred VH chicks for the first trial which is reflected in the lower number of male VH compared to BG and WR in the fattening experiment (**Chapter 2**). However, the purebred experimental animals had to be the parents for the crossbreds at the same time.

In the egg quality parameters presented in **Chapters 3** and **4**, interesting characteristics of the local breeds were observed, which they may have inherited to the crossbred generation. It should be highlighted that both the yolk weight and the yolk percentage increased with aging of the hens in the eggs of local breeds and crossbreds (**Figures 4.1-4.4**). As the yolk is a valuable source of almost all vitamins and minerals for human nutrition [16,17], the higher proportion of yolk per egg is clearly an advantage of the local breeds and crossbreds compared to commercial hybrids regarding table egg production. Tixier-Boichard et al. [18] demonstrated, that the high proportion of yolk in eggs of the local Fayoumi breed was transferred to their offspring with commercial laying hens. Contradictory to the present work, in commercial laying hens an increase of yolk percentages was observed by some authors [19,20]. However, the total proportion of yolk weight was generally higher in local compared to conventional hens [20–22].

Blood spots in the eggs do not impair the edibility of eggs, but are undesirable from the perspective of marketing anyway. WR showed frequencies of more than 50% of eggs with blood spots and did pass this predisposition to the offspring BWR. In contrast, the VWR crosses clearly showed lower frequencies than the maternal WR. As selection against blood spots proves to be difficult [23], local breeds with low frequencies of blood spots by nature, as for example the VH, could be interesting partners for crossbreeding programs beyond conservation breeding. These two examples of yolk percentage and blood spot frequencies demonstrate the importance of local breeds as reservoir of useful traits that must be preserved.

The impact of the Northern Fowl Mite on the first layer experiment

Local breeds are said to be more robust against environmental stress and diseases than highly specialized lines. However, this common knowledge needs to be verified with scientific facts and with regard to defined targets.

In the experiment with the purebred hens, an infestation with the Northern fowl mite (*Ornithonyssus sylviarum*) in the barn was present around weeks 31 - 39 of hens' age. Different symptoms were observed at that time, including increased mortality, weight loss (**Figure S3.2**), decreased laying performance (**Figure S3.3**) and lowered egg weights (**Figure 5.1**). Interestingly the impact of the infestation was higher in WR chicken than in the local breeds and in the faba bean fed groups compared to soy fed groups. These observations cannot be explained by the localization of the different groups in the barn, but the results suggest an effect of genotype and diet or rather a genotype × diet-interaction. Somehow the impact of the mite infestation and the feeding of faba beans seemed to sum up and impair the chickens' homeostasis as the infestation alone (Soy groups) or the faba bean diets alone (measurements at other time points) did not lead to performance reduction. The performance reduction was most severe in WR chicken.

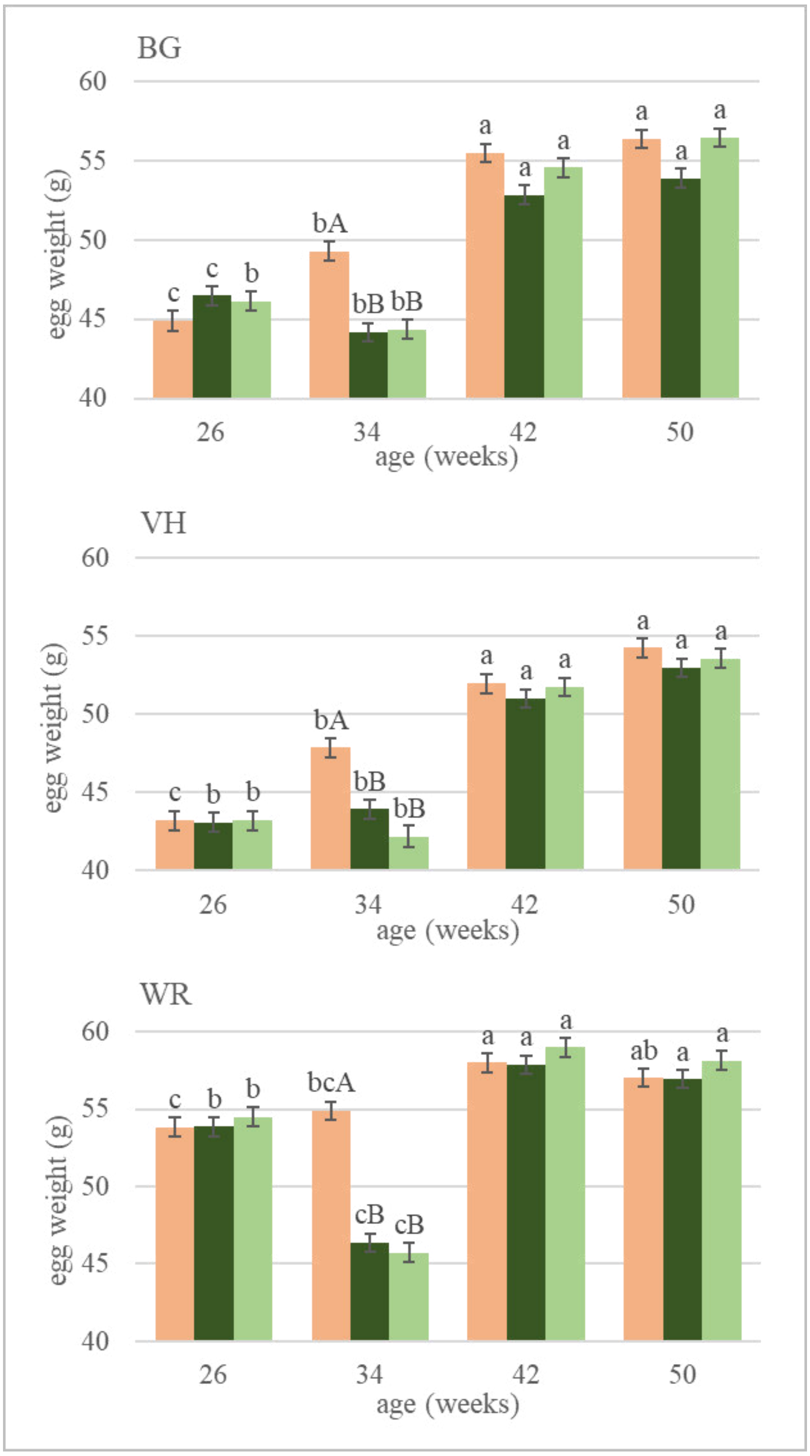

Figure 5.1. Egg weight of the purebreds. LSmeans ± SE, [a,b,c] Small letters mark significant differences between measurements for the respective diets over all measurement for $p < 0.05$, [A,B] Capital letters mark significant differences between diets at one measurement for $p < 0.05$, BG: Bresse Gauloise, VH: Vorwerkhuhn, WR: White Rock.

Northern fowl mites (*Ornithonyssus sylviarum*) are blood feeding parasites that live on the chicken, more precisely on feathers of the vent region [24]. The mites are shown to cause irritation, anemia, reduced feed efficiency, performance reduction and paler yolk color [24,25]. Moreover, the blood feeding induces a severe immune response [24,25]. Sakai et al. [26] reviewed the effect of Soy isoflavones, especially Genistein, in immune responses of humans. Genistein has an anti-inflammatory activity, most likely because of its similar structure to 17ß-estradiol, binding on receptors of different immune cells. Possibly isoflavones from the soybean meal in the Soy groups maintained the immune response to the mites and therefore attenuated the impact on the host. As this was only an accidental finding, further research should be done, to understand the relationship between genotype, diet and the reaction to a mite infestation.

The smaller impact of the mite infestation on the local breeds compared to WR could indicate their higher robustness regarding different stressing factors. Robustness of chicken genotypes against parasites and as well for example inclement climate conditions is of particular importance as extensive production systems with free range or mobile houses currently increase [27]. The indication seen here for the comparably lower sensitivity of the local breeds against mites should be investigated further from this perspective.

Which genotype to choose?

It becomes clear, that especially the BWR cross showed potential as dual-purpose genotype, since it performed well in both categories. For farmers that have no preferences on a certain breed, but want to work with alternative genotypes, the BWR might be an interesting option. Furthermore, it is already available, bred by the Ökologische Tierzucht GmbH [28], and sold together with crosses of BG × New Hampshire as "Coffee and Cream", named so due to the feather coloring. The above mentioned experiments of Baldinger and Busemas [5,6] were actually undertaken with animals of this company. From farmers' view, the advantage of a breeding company as distributor is the availability of chicks in a sufficient number.

Although their overall performance was lower than that of BWR, the results of the VBG are of particular importance with respect to conservation breeding programs. As shown in **Table**

5.1, the VBG reached a comparable weight as VH about three weeks earlier, and the laying performance was clearly improved compared to VH. Thus, the VBG would still be advantageous for breeders to increase the economic viability of this breed.

For other local breeds these results can be considered as a model. The crossbreeding of the meat-type BG with the layer-type WR and VH led to satisfying performance levels in both, laying and fattening performance. Therefore, when the priority is to maintain a particular breed, crossbreeding provides a higher level of performance in a very short time compared to pure-breeding strategies. Choosing the perfect partner for crossbreeding might be difficult, as the number of local breeds is very high, especially in Europe [29], anyway, in the present study as well as in the literature [4–6], Bresse Gauloise proved to be a valuable crossbreeding partner for different genotypes. Further research could investigate if BG is also suitable as crossing partner for heavy local breeds or if in that case a layer-type, of local or commercial origin, would be a better choice.

Results of the field study

The experiments under controlled conditions at research facilities were supplemented by an on-farm study with six breeders from a conservation breeding program. At least two crossbred genotypes with 25 animals each (mixed-sexed groups) were reared on each farm and data regarding bodyweight of both sexes and later on laying performance of the hens were documented. The cockerels have been slaughtered at an age of 12 weeks at Goettingen University (Goettingen, Germany) to evaluate the fattening performance whereas the hens have been observed from week 18 to 52. The number of eggs was counted daily while the egg weight was measured in weeks 30, 40 and 50. Only during the laying period the hens received a faba bean containing feed, more precisely the VC- diet used also in **Chapters 3** and **4**.

Live weights of the cockerels at slaughter have been 1910 g (BWR), 1875 g (VBG) and 1544 g (VWR) respectively, which was lower than the weights in **Chapter 2** at that age. Just like in **Chapter 2**, the BWR cross achieved the best growth performance with the VBG being slightly lighter.

The laying performance of the hens is displayed in **Figure 5.2A**. During the peak production period, the VWR showed a higher laying performance than the BWR, but the crossing of the laying curves in week 41 demonstrates the higher persistency of the BWR. The mean laying performance over the whole laying period was 71.0% for BWR and 69.7% for VWR and did not differ statistically significant. The laying curve of VBG ran underneath that of the VH crosses with an overall mean of 57.5%. The egg weights of BWR have been significantly higher than that of VWR and VBG in week 30 and 40 with 59.0 g and 62.0 g (**Figure 5.2B**), however in week 50 there was no difference between the WR crosses (both 61.7 g). All genotypes showed a lower laying performance in practice than on the experimental sides, with the biggest difference of 10.6% in BWR. Egg weights of the experiments and the field study have been close together. Although the sample size was small and distinct differences between the farms existed, the field study basically confirmed the results of the experiments.

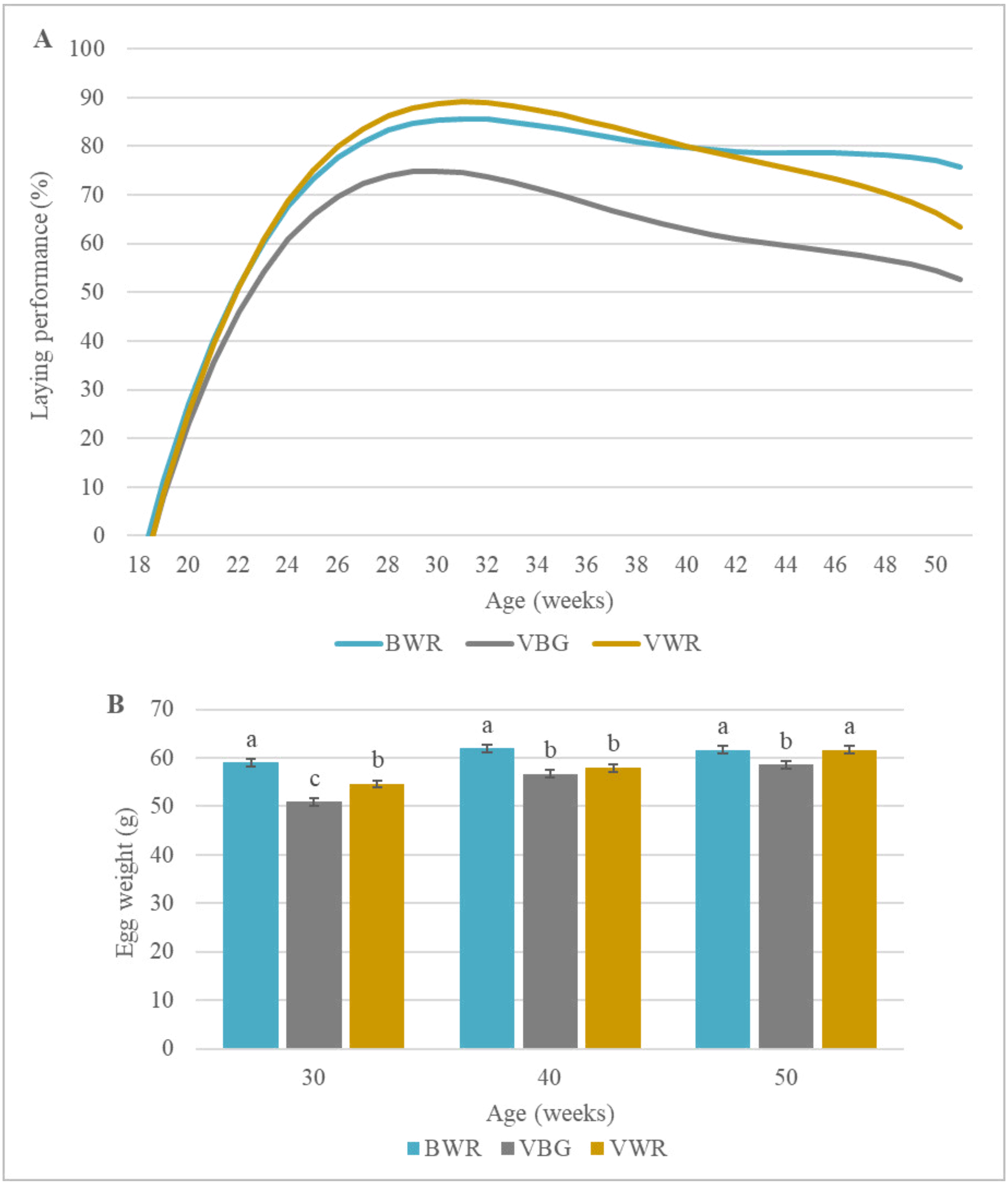

Figure 5.2. Field study. (A) Laying performance. (B) Egg weight; LSmeans ± SE; Bars with different letters at one time point differ significantly at $p < 0.05$. BWR: Bresse Gauloise cock × White Rock hen, VBG: Vorwerkhuhn cock × Bresse Gauloise hen, VWR: Vorwerkhuhn cock × White Rock hen.

Feed efficiency in dual-purpose genotypes

Despite the above discussed advantages of dual-purpose genotypes, it is also clear that the performance levels regarding laying and fattening are still inferior compared to specialized laying hens and broilers. In addition, the feed efficiency was much lower compared to the commercial genotypes. The daily feed intake of the purebred hens was between 119.8 g (VH) and 129.8 g (BG), what was in the range of commercial brown layers (115 – 125 g) [30], but the commercial hybrids achieve higher egg output from the same amount of feed. The average daily feed intake of the crossbred hens was even higher (**Figure S3.1C**). On the male side the daily feed intake as well as the FCR have been calculated (**Figure 2.4**). In this case the feed conversion ratio of all evaluated genotypes was clearly higher than that of commercial broilers which need only 1.5 kg of feed to produce one kg of body mass under optimum conditions. According to Damme et al. [31], the low feed efficiency causes an "ethical dilemma" as the use of less productive genotypes to protect poultry genetic resources and to enhance animal welfare leads to the stressing of environmental resources and sustainability goals on the other side, because these genotypes need more feed, which includes more area, more energy, more water. Therefore, concepts to diminish the environmental impact of dual-purpose chickens should be formed and it seems self-evident to tackle the feed components. This is accredited by the results of Leinonen et al. [32], investigating the environmental impact of poultry production in the UK. He calculated in live-cycle assessment that the use of regional protein sources could reduce the global warming potential of poultry production systems and that the effect is higher with higher inclusion rates of regional beans or peas in the feed.

The effect of faba bean-feeding on the different genotypes

To increase the sustainability of the production system investigated in this research, faba beans were chosen as alternative protein source to soybean meal. In the cockerels of all genotypes, no adverse effects of the feeding of faba beans could be observed. The BWR achieved higher body weights and weights of carcass, breast and legs when fed with the faba bean containing diets. Interactions of genotype × diet could not be interpreted in the fattening trials as the statistical analysis was done separately for each genotype due to the different slaughter ages. However, during week five to ten, where a conjoint analysis was done, no genotype × diet interaction was present.

Similar to previous studies [33–36], a reduction of egg weight through feeding of VC-rich faba beans was observed in the present study, even though it was to a minor degree. Furthermore, a statistically significant genotype × diet interaction was observed in the crossbreds (**Table 3.2; Figure 5.3**). Although differences between diets within genotypes were not statistically significant, differential dietary responses are evident. In BWR, all feeding groups were close together. In contrast, in VBG, egg weights of the VC+ group were almost 2 g lighter than those of the Soy and VC- groups, and in VWR, both faba bean groups had almost 2 g lighter eggs. These differences may indicate that BWR is more robust to faba bean feeding than VBG and VWR. At this point, it should be noted that in cockerels, the faba bean groups of BWR showed significantly higher weights than the Soy group (**Chapter 2**).

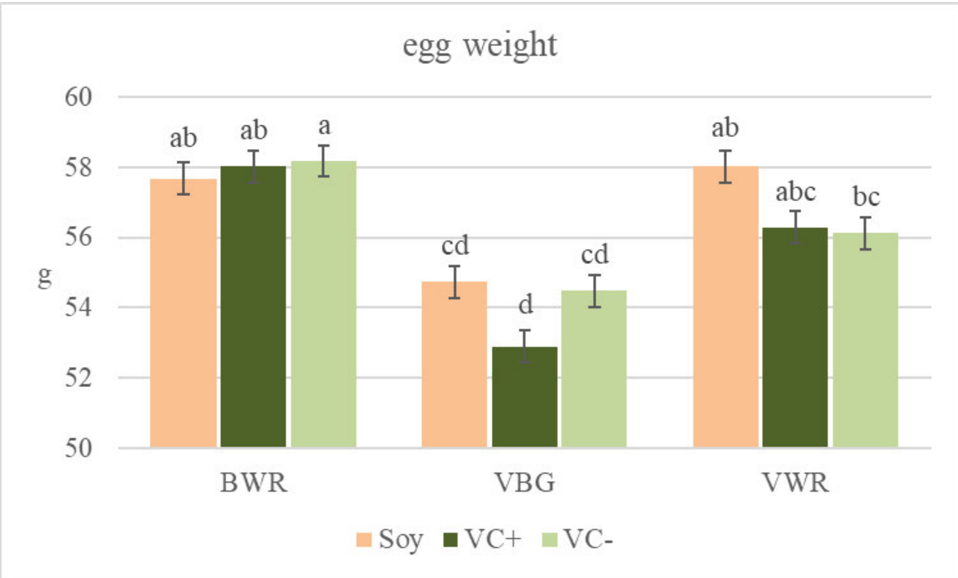

Figure 5.3. Genotype × diet interaction on egg weight of crossbred hens. LSmeans ± SE. Bars with different letters differ significantly at $p < 0.05$. BWR: Bresse Gauloise cock × White Rock hen, VBG: Vorwerkhuhn cock × Bresse Gauloise hen, VWR: Vorwerkhuhn cock × White Rock hen.

Furthermore, there was a dietary effect on the shell quality of the crossbreds, with VC+ leading to lower breaking strength and less shell percentage. The genotype × diet interaction on Shell color lightness (L*) of the purebreds and Haugh units of the crossbreds was discussed in **Chapter 4**.

Interestingly the feed had no influence on the yolk weight and the frequency of blood spots as these parameters have been described to be influenced by VC in the diet with lower yolk weights being causative for reduced egg weights [36,37]. Indeed, these authors fed crude VC to chickens in concentrations of 0.5% and 1% which was much higher than the amounts used in the present studies which are described in **Chapters 2-4** where the analyzed VC-concentrations in the diets did not exceed 0.1% in the VC+ groups.

In both experiments, the faba bean was not the only legume in the diets. To meet nutritional requirements under the abandonment of soybean meal, the experimental diets of the cockerels were formulated including 28.6% Blue sweet lupine (*Boruta*) and 10.5% Field pea (*Astronaute*). Regarding the hens' experiments, all diets including the control diet were composited with 21% Blue sweet lupines (*Boruta*). Due to this, the total legume content in the experimental fattening feeds was 56.1% and in the layer diets 41%. Whereas the pea proportion was clearly below the recommended maximum levels for broilers of 30% [38,39], the lupine portion was quite high in both experiments. Jeroch et al [38] recommend a maximum of 15% sweet lupines for broilers and Farrel et al. [39] indicated to use not more than 10% lupines. In contrast, Roth-Maier et al. [40] observed no adverse effects on broiler chicks at a level of 20% blue sweet lupines whereas at 30% level the FCR was increased. For laying hens the recommended maxima vary between 15 to 25% [38,41–43]. However, Kowalska et al. [19] fed laying hens a diet including 11% peas and 47% lupines, i.e. 25% yellow lupines and 22% blue sweet lupines, without negative effects on egg quality.

As the dietary effect on performance parameters was low in the present studies, the combination of different legumes seems to be a possibility to fulfill the nutritional demand of poultry under the abandonment of soybean meal. Future research should be done with focus on combinations of different legumes. Smaller amounts of the individual legume species could reduce the impact of anti-nutritional factors whereby the total amount of homegrown legumes could be increased. This could not only be of value regarding sustainability of the production system but also to make the individual farmer more independent from the availability of certain protein plants.

Recommendations for practice – development of a niche

The production system of local chicken breeds and regional protein feed is only plausible for niche markets. Because of the low production performance, the output of the system is insufficient for bigger markets. Moreover, higher product prices must be set to compensate for lower production and the increased demand of feed compared to conventionally produced eggs and meat.

With respect to higher product prices, Gangnat et al. [44] found Swiss consumers to have a higher willingness to pay for eggs than for meat in general. The willingness to pay could be increased if the products originate from organic farming. However, the knowledge of consumers about poultry husbandry and dual-purpose chickens was low. According to Heise and Theuvsen [45], the willingness to pay of German consumers was slightly higher for meat than for eggs with a general upper limit around 40% of markup on the usual price. Anyway, it should be mentioned that a bias between the attitude of people and their buying decision at the point of sale exists. Escobedo del Bosque et al. [46,47] identified a segment of consumers, that is interested in the regional origin of the products they purchase. These consumers, although buying meet seldom, could be a target group for the described production system, as they could be convinced by additional information about the local origin of feed and the value of the chicken genotypes used for production. This is in agreement with Kohlschütter et al. [48], who pointed out that conservation of traditional breeds and locality should be addressed in marketing strategies for products of local poultry.

In general, the breast fillet is preferred by consumers [49] and is therefore the most valuable part of a broiler. Unfortunately, the breast fillets of the cockerels in the present study have been too small for separate selling (**Chapter 2**) and other ways of marketing the meat have to be found, possibilities are whole carcasses or convenience products [50].

According to Escobedo del Bosque et al. [51] consumers are often overwhelmed with whole carcasses due to the time required for cooking or the amount of meat, which is why Schuetz et al. [52] encouraged producers to collaborate with butchers to produce different types of sausages or high-quality salami. Direct marketing with the possibility to inform consumers about the peculiarity of the products should be preferred generally, as in supermarkets the competition with cheaper conventional products is too high and consumer expectations are different [52].

Main Conclusions

In the present study, the performance levels of the local breeds Bresse Gauloise and Vorwerkhuhn, the commercial line White Rock and crossbreds thereof were investigated regarding dual-purpose use to enhance the protection of poultry genetic resources. Regional faba beans replaced imported soybean meal for better sustainability of the production system. Of the investigated chicken genotypes, the cross of Bresse Gauloise × White Rock revealed to be the most promising one regarding dual-purpose production. Due to the lower performance level compared to conventional hybrids, the farmer still has to balance the protection of poultry genetic resources against that of environmental resources. At this point, the use of regional legumes can increase the sustainability as the use of 20% faba beans in the present study was without adverse effects on chicken performance. Even the high total amount of legumes in the diets did not cause any problems regarding chicken performance or animal health. With respect to egg weight, however, faba bean varieties with reduced vicin contents should be preferred.

It was also demonstrated that not all local breeds are suitable for dual-purpose production in the same way. The Vorwerkhuhn crosses were either inferior regarding fattening performance (VWR) or laying performance (VBG), but still improvement compared to the pure breed existed that could be used immediately, from one generation to the next, to increase the income from this breed. Likewise, the crossbreeding of local breeds of differing type (meat or egg) is likely transferable to chicken breeds other than VH and BG.

For better comparability of pure- and crossbreds and evaluation of heterosis effects, one experiment with all six genotypes at the same time would have been ideal.

Besides the investigation of performance levels, qualities of the local breeds were shown, that could be of interest for marketing of the products and for poultry breeding in general, e.g., the high proportion of yolk or the ability to lower the frequency of blood spots as crossing partner for commercial hens.

To conclude, the formula ‘local chicken genotype combined with regionally (on-farm) grown protein feed’ is an interesting concept, especially for niche production, where consumers appreciate the kind of husbandry and the special quality of the products and are willing to pay more for it, e.g., via direct marketing.

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