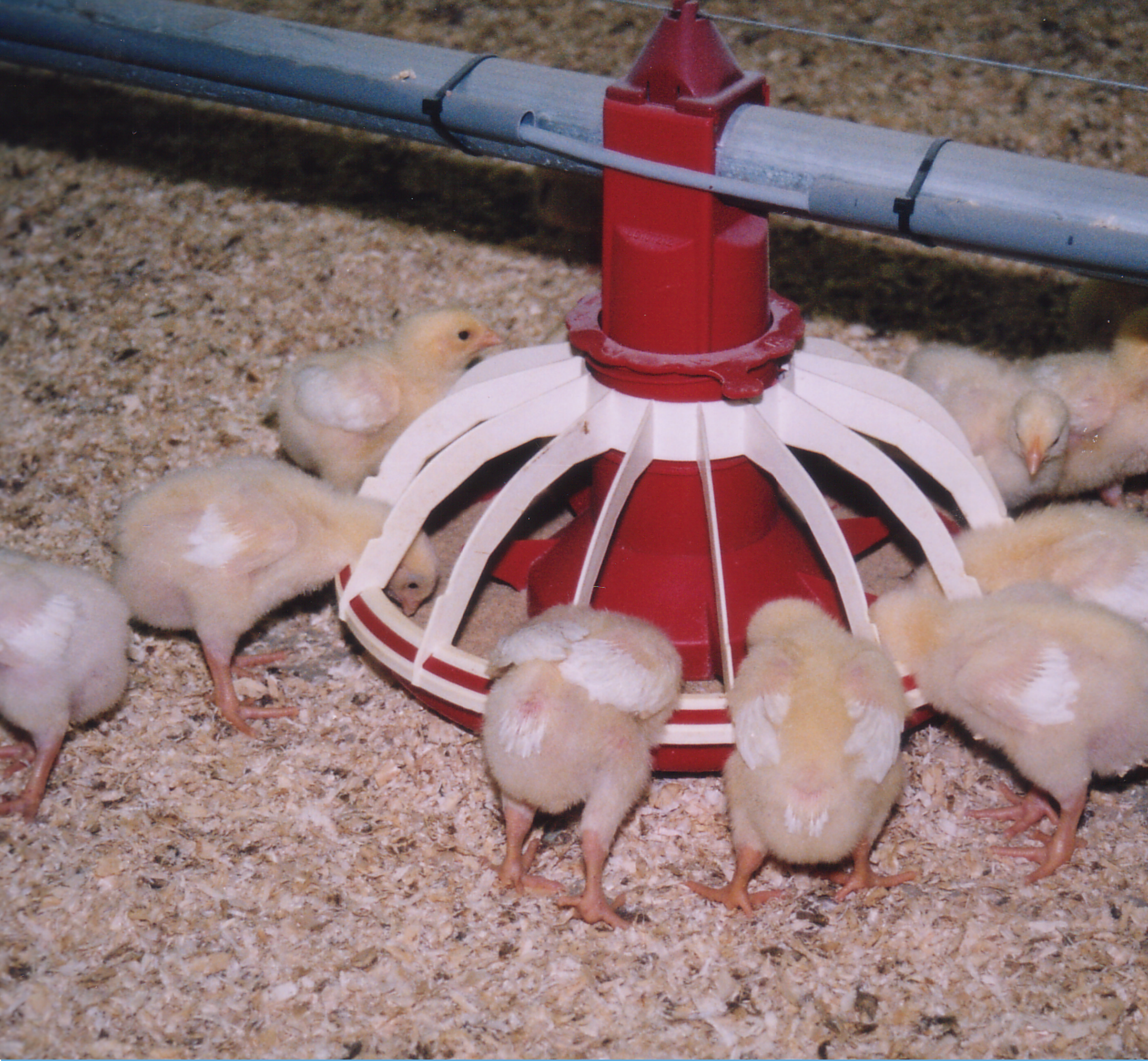

Effects of using regionally (EU) grown, protein-rich ingredients in diets on the growth performance of fast and slow growing broilers

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Effects of using regionally (EU) grown, protein-rich ingredients in diets on the growth performance of fast and slow growing broilers

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Samenvatting
Dit rapport beschrijft de resultaten van een experiment waarin de effecten van gedeeltelijke en volledige vervanging van sojaschroot door regionaal (EU) geteelde eiwithoudende ingrediënten op de productieresultaten van snel en trager groeiende vleeskuikens zijn onderzocht. Gedeeltelijke vervanging van sojaschroot door erwten, veldbonen, zonnebloemzaadschroot of raapzaadschroot is mogelijk zonder dat dit ten koste gaat van de groeiprestaties. Volledige vervanging van sojaschroot door regionaal geteelde eiwithoudende ingrediënten resulteerde echter in verminderde productieresultaten. Snel en trager groeiende vleeskuikens reageren niet hetzelfde op voeders met regionaal geteelde eiwitbronnen. Zo leidde gedeeltelijke vervanging van sojaschroot door raapzaadschroot bij snelgroeiende vleeskuikens tot verminderde productieresultaten, terwijl bij trager groeiende vleeskuikens er een verbetering van de productieresultaten optrad ten opzichte van de referentiegroep. Uit dit onderzoek bleek verder dat het mogelijk is om de CO_2-voetafdruk van voeders te verlagen door regionaal geteelde eiwithoudende ingrediënten te gebruiken.

Summary UK
This report describes the results of an experiment in which the effects of partial and complete replacement of soybean meal with more regionally (EU) grown protein-rich ingredients on the performance results of fast and slow-growing broilers were studied. Partial replacement of soybean meal with peas, field beans, sunflower seed meal or rapeseed meal is possible without compromising performance results. However, complete replacement of soybean meal with more regionally grown ingredients gave reduced performance results. Fast and slow-growing broilers do not respond similar to diets with more regionally grown protein-rich ingredients. In fast-growing broilers, partial replacement of soybean meal with rapeseed meal led to a deterioration in performance results, while in slow-growing broilers an improvement was observed. This study also showed that it is possible to reduce the carbon footprint of diets using more regionally grown protein-rich ingredients, but including or excluding the footprint related to Land Use Change (LUC) from soybean production influences the outcome.

This report can be downloaded for free at https://doi.org/10.18174/684963 or at www.wur.nl/livestock-research (under Wageningen Livestock Research publications).

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Foreword

Feed4Foodure-III - Sustainable animal nutrition in circular agri-food systems” is a public-private partnership between the Dutch Ministry of Agriculture, Nature and Food Quality and a consortium of various stakeholders within the animal production chain and Wageningen Livestock Research. Feed4Foodure-III is a research and innovation program targeted at sustainable animal nutrition, circular agri-food systems, robust livestock and resource use efficiency.

The current report describes the results of an experiment in which the effects of more regionally grown protein-rich ingredients as partly or entire replacement for soybean meal on the growth performance results of fast and slower growing broilers was evaluated. This study was performed within the project subject “Maximizing non-human edible and circular / sustainable feed materials in pig and poultry diets”.

The study was designed with input from scientists of Wageningen Livestock Research and representatives of VDN in the F4F-consortium and performed at the broiler facility of ForFarmers. The authors thank all members of the project team for their worthwhile input. The authors also thank Fam. Nikkels for performing the experiment and for their hospitality.

Jan van Harn, Henrieke Berman, Albert Dijkslag en Alfons Jansman

Summary

It is becoming increasingly important for broiler farming to produce food (meat) in a more circular food system. One aspect of circular food production is the focus on implementing short production chains and the use of more regionally grown or produced feedstuffs. Peas, field beans, sunflower seed meal (SFM) and rapeseed meal (RSM) are examples of regionally (EU) grown feedstuffs that can reduce the dependence on soybean meal from outside EU.
An experiment was conducted with 1,044 fast-growing (Ross 308) and 1,044 slow-growing (Hubbard JA757) male broilers to study the possibility of partial and complete replacement of Brazilian soybean meal (SBM) with peas (P), field beans (FB), sunflower seed meal (SFM) or rapeseed meal (RSM) in broiler diets to improve the sustainability of broiler meat production. The fast-growing and slow-growing broilers were separately housed in floor pens (2.1 m^2) bedded with wood shavings. In total six diet programs were evaluated. Each diet program consisted of four phases: a starter diet was provided from 0 – 9 d, a grower I diet from 9 – 21 d, a grower II diet from 21 – 29 d and a finisher diet from 29 -38 d. Diet program A was the control diet and contained 25.3, 21.7, 20.2 and 18.0% SBM in the starter, grower I, grower II and finisher phases, respectively. In diet program B, compared to reference program A, relatively 11, 47, 61 and 71% of the SBM in the starter, grower I, grower II and finisher diets, respectively, were replaced by peas. In diet program C, 21, 54, 61 and 72% of the SBM in the starter, grower I, grower II and finisher diets, respectively, were replaced by field beans. In diet program D, 25, 71, 85 and 75% of the SBM in the starter, grower I, grower II and finisher diets, respectively, were replaced with SFM. In diet program E, 13, 33, 36 and 40% of the SBM in starter, grower I, grower II and finisher diets, respectively, were replaced with RSM. In diet program F all SBM was replaced with a combination of P, FB, SFM and RSM. Within a feeding phase, diets were iso-caloric and had the same SID EAA (Lys, M+C, Thr, Trp, Ile, Val and Arg) content. Both breeds received the same diet programs and therefore the concentrations of metabolizable energy and SID EAA of the diets were set at values which were intermediate to the recommendations for Ross 308 (fast growing) and Hubbard JA 757 (slow growing) broilers. Each experimental diet was fed ad libitum from 0 to 38 d of age to 12 replicates of 29 birds. Parameters studied were body weight, feed intake, feed efficiency, mortality, visual litter quality, footpad lesions and the effects on the calculated carbon footprint (CFP) of the diets and per kg body weight gain of the birds.

From this study it can be concluded that:

- Partial replacement of SBM in diets with regionally grown protein-rich ingredients (peas, field beans, or rapeseed meal) is possible without compromising the European Production Efficiency Factor (EPEF) of fast and slow-growing broilers.
- Partial replacement of SBM with SFM resulted in impaired performance. This effect was more pronounced in fast-growing broilers.
- Complete replacement of SBM with regionally grown protein-rich ingredients in broiler diets resulted in lowered performance in both fast and slow-growing broilers.
- Partial replacement of SBM with peas resulted in more severe footpad lesions compared to the control diet program. This effect was more pronounced in fast-growing broilers.
- Complete replacement of SBM and partial replacement of SBM with FB or SFM resulted in fewer footpad lesions compared to the control diet program.
- Complete replacement of SBM from Brazilian origin reduced the carbon footprint (incl. LUC) of the diets with approximately 50%. Partial replacement of Brazilian SBM with peas, field beans, SFM or RSM reduced the carbon footprint (incl. LUC) of the feed with 10-40%. Excluding LUC into account, there were no differences in the calculated carbon footprint of the diets.
- Fast-growing broilers had a lower carbon footprint (including and excluding LUC) per kg body weight gain compared to slow-growing broilers.
- Use of regionally grown protein-rich ingredients reduced the carbon footprint (incl. LUC) per kg BWG. Complete replacement of (Brazilian) SBM reduced the carbon footprint (incl. LUC) per kg BWG with almost 50%.

- The carbon footprint (excl. LUC) per kg BWG was only reduced when SBM was replaced with rapeseed meal. Complete replacement of Brazilian SBM with regionally grown protein-rich ingredients increased even the carbon footprint (excl. LUC) per kg BWG.
- Fast-growing broilers had a higher N and P efficiency compared to slow-growing broilers.
- The highest N-efficiency was observed in the pea diet program, but this was more pronounced in fast-growing broilers than in slow-growing broilers. The lowest N efficiency was observed with the RSM diet program for both fast and slow-growing broilers.
- Partial or complete replacement of SBM with regionally grown protein-rich ingredients reduced the calculated P efficiency in both fast and slow-growing broilers.
- The SFM diet program resulted in the lowest P efficiency.

From this study it can be concluded that by partly replacing Brazilian soybean meal by peas, field beans, sunflower seed meal or rapeseed meal, it is possible to make broiler meat production more sustainable without compromising performance. However, complete replacement of SBM with regionally grown ingredients led to reduced broiler performance. Slow-growing broilers may be better able to handle the regionally grown protein-rich ingredients.

1 Introduction

It is fundamental for broiler production to search for options to keep poultry for food production as part of a circular food production system, in which impact on climate and future availability of resources are taken into consideration. Soybean meal (SBM) is the most used plant protein feed ingredient in poultry diets. Its wide market availability, combined high concentrations of digestible essential amino acids and low concentrations of antinutritive factors (ANF), has made SBM a popular feed ingredient. However, factors such as the EU dependency on non-EU SBM, consumer perceptions regarding the use of genetically modified feedstuffs and environmental concerns (deforestation) are driving the search for alternative protein sources for poultry diets to minimise / reduce the amount of imported soybean products from overseas. To make feed production more sustainable, the concept of circularity has gained increasing attention in the poultry sector and in current research. One aspect of circular food production is the focus on short production chains, including the use of more regionally grown or produced feedstuffs. Peas, field beans, sunflower seed meal (SFM) and rapeseed meal (RSM) are examples of regionally grown feedstuffs that can, at least, partially reduce dependence on overseas (especially South-American) SBM.

Table 1.1 *Nutritional values of the different protein sources (CVB, 2021).*

		Soybean meal	Peas	Field beans	Sunflower seed meal	Rapeseed meal
AMEn broiler	kcal/kg	2192	2644	2455	1473	1490
Starch (am)	g/kg	8	416	336	8	8
Crude fat	g/kg	24	10	10	21	39
Crude fibre	g/kg	36	53	82	176	125
Crude protein	g/kg	489	201	264	368	339
Sum amino acids	g/kg	483 (419)	191 (164)	244 (205)	340 (285)	313 (250)
Lysine	g/kg	30.3 (26.7)	14.3 (12.6)	16.6 (14.3)	12.9 (10.5)	18.6 (14.5)
Methionine	g/kg	6.8 (6.2)	2.0 (1.7)	2.1 (1.8)	8.1 (7.4)	6.8 (6.0)
Methionine + Cysteine	g/kg	14.1 (11.7)	5.0 (3.9)	5.5 (4.3)	14.3 (12.0)	15.3 (12.4)
Threonine	g/kg	19.1 (15.8)	7.4 (6.0)	9.2 (7.2)	13.6 (10.3)	14.9 (10.9)
Tryptophan	g/kg	6.4 (5.7)	1.8 (1.4)	2.4 (1.6)	4.4 (3.7)	4.4 (3.5)
Isoleucine	g/kg	22.5 (19.6)	8.2 (6.8)	10.8 (8.6)	15.1 (12.8)	13.2 (10.3)
Arginine	g/kg	36.7 (33.0)	17.7 (15.9)	24.0 (20.9)	29.8 (27.1)	20.7 (17.6)
Valine	g/kg	23.5 (20.2)	9.3 (7.6)	11.9 (9.4)	18.0 (14.9)	17.3 (13.3)
Glycine + Serine	g/kg	45.9 (39.2)	18.4 (15.3)	23.7 (19.9)	36.7 (27.2)	32.5 (250)

Between brackets the SID AA content is given.

Compared to SBM, peas and field beans are relatively rich in lysine and poor in sulphur-containing amino acids (methionine and cysteine) (Table 1.1). The digestibility of sulphur-containing amino acids in peas and field beans is low compared to SBM. In addition to the relatively high protein content, peas and field beans also are energy rich, due to the high starch content. Sunflower seed meal (SFM) has an average protein content, which is characterized by a high content of sulphur-containing amino acids and a relatively low lysine content. The digestibility of the essential amino acids in SFM is slightly lower than that of SBM. Furthermore, SFM has a low energy content and a high fibre content compared to SBM. Compared to SBM, rapeseed meal (RSM), similar to SFM, has a moderate protein content, characterized by a relatively high content of sulphur-containing amino acids and a low lysine and isoleucine content. The digestibility of the essential amino acids is lower compared to that of SBM. The energy content of RSM, similar to SFM, is limited by the high fibre content.

The experimental approach of the present study was a partial or complete substitution of (Brazilian) SBM with peas, field beans, sunflower seed meal and/or rapeseed meal and aimed at answering the following questions:

1. Can SBM be partially replaced with one of these alternative protein sources or completely replaced with a combination of these alternative protein sources in diets of fast and slow-growing broilers?
2. Furthermore, which effects could be expected of using these alternative protein sources on the litter quality and the occurrence of footpad lesions compared to the use of SBM?
3. What are the effects of (partial) replacement of SBM with these alternative protein sources on the carbon footprint of feed and per kg body weight gain?
4. What are the effects of (partial) replacement of SBM with these alternative protein sources on the N- and P-efficiency?

2 Materials and Methods

2.1 Study objective

The objective of the present study was to evaluate the effects of 1) partial replacement of soybean meal with peas, field beans, sunflower seed meal or rapeseed meal, and 2) complete replacement of soybean meal with a combination of aforementioned ingredients on the performance results, calculated N- and P-efficiency, litter quality, welfare and environmental impact of fast and slow-growing broilers.
Response parameters were growth performance, carbon footprint per kg body weight gain, litter quality and incidence of and severity of footpad lesions.

2.2 Start date and end date of the experiment

Start date: 10 May 2022
End date: 17 June 2022

2.3 Experimental animals

The study was performed with 1044 fast-growing (Ross 308) and 1044 slow-growing (Hubbard JA757) male broiler chickens. The fast and slow-growing broilers were housed separately and allocated to 72 floor pens bedded with wood shavings (29 birds/pen). The broilers were sexed at the hatchery. The experiment started at 1 day of age and the experiment was completed at 38 days of age. The fast-growing broilers originated from one parent stock of 44 weeks of age at the start of the incubation. The slow-growing broilers were from one parent stock aging 48 weeks.

2.4 Experimental design

The experiment was carried out at the experimental broiler house of ForFarmers, Bathmen, The Netherlands. This mechanically ventilated house contained 72 floor pens of 2.1 m^2 (1.46m*1.46m) arranged in four rows of 18 pens (see Appendix 1). A four-phase diet program was applied: 0 – 9 days starter diet, from 9 – 21 days grower I, 21 – 29 days grower II and from 29 – 38 days the experimental finisher diets were provided. A 2*6 factorial block design was used in this experiment, with breed (fast-growing/slow-growing) and diet (n=6, see Table 2.2) as factors. In total there were 6 blocks, and each block consists of 12 pens (see Appendix 1). One breed was used within a block of 6 consecutive pens and the dietary treatments were randomly distributed among these pens. Each treatment (breed * diet) was replicated six times and the experimental unit was a pen with 29 male broilers (Table 2.1). The treatments and a description of the treatments are presented in Table 2.2.

Table 2.1 *Experimental design in summary.*

Item	Number
Total number of broilers	2088 (= 1044 fast-growing and 1044 slow-growing)
Total number of pens	72
Number of treatments	12 (= 2 breeds * 6 diets)
Replicates (pens) per treatment (breed*diet)	6
Replicates (pens) per breed	36
Replicates (pens) per diet	12
Broilers per pen	29
Broilers per treatment	174

Table 2.2 *Experimental treatments.*

Treatment	Description
A	Control diet (SBM as main protein source)
B	Diet program in which SBM was partly replaced with peas (P)
C	Diet program in which SBM was partly replaced with field beans (FB)
D	Diet program in which SBM was partly replaced with sunflower seed meal (SFM)
E	Diet program in which SBM was partly replaced with "double-zero" rapeseed meal (RSM)
F	SBM-free diet program. SBM replaced with P, FB, SFM and RSM

2.5 Experimental diets

The experimental diets were formulated by ForFarmers and produced at the experimental feed plant of ForFarmers, Heijen, The Netherlands. A four-phase diet program was provided. Starter, grower I, grower II and finisher diets were provided from 0 – 9, 9 – 21, 21 – 29 and 29 – 38 days of age, respectively. From start (day 0) till end six experimental diets programs were provided (Table 2.3). Before formulating the diets the main feed ingredients, e.g. maize, wheat, SBM, peas, field beans SFM and RSM, were analysed (Appendix 2). Based on the results of these analyses the experimental diets were formulated that within a feeding period the concentrations of metabolizable energy and SID EAA (lysine, methionine+cysteine, threonine, tryptophan, isoleucine, valine and arginine) were set at values which were in between the recommendations for Ross 308 (fast growing) and Hubbard JA 757 (slow growing) broilers. All diets for each period were prepared with the same batches of ingredients.

Table 2.3 *Inclusion level of the protein-rich ingredients in the experimental diets.*

Treatment	Description
A	**Control**. Diets contained 25.3/ 21.7/20.2/18.0% SBM in starter/grower1/grower2/finisher phase.
B	Diet program with **peas** (P) as partial replacement of SBM. Diets contained 22.5/11.5/7.8/5.3% SBM and 15.0/40.0/40.0/40.0% P in starter/grower1/grower2/finisher phase
C	Diet program with **field beans** (FB) as partial replacement of SBM. Diets contained 19.9/9.9/7.8/5.1% SBM and 15.0/35.0/35.0/35.0% FB in starter/grower1/grower2/finisher phase
D	Diet program with **sunflower seed meal** (SFM) as partial replacement of SBM. Diets contained 19.1/6.2/3.1/4.5% SBM and 10.0/25.0/25.0/20.0% SFM in starter/grower1/grower2/finisher phase
E	Diet program with "double-zero" **rapeseed meal** (RSM) as partial replacement of SBM. Diets contained 22.1/14.5/12.9/10.8% SBM and 8.0/18.0/18.0/18.0% RSM in starter/grower1/grower2/finisher phase
F	**SBM-free** diet program with 20.6/13.4/12.1/11.9% P, 15.0/14.0/11.3/10.0% FB, 8.2/11.8/9.0/8.0 % SFM and 5.0/11/0/9.0/8.0 % RSM in starter/grower1/grower2/finisher phase

Table 2.3 presents the inclusion levels of the protein-rich ingredients per feeding phase per treatment. The ingredient composition and the calculated nutrient contents of all experimental diets are presented in Appendix 3 (Starter + Grower I diets) and Appendix 4 (Grower II + Finisher diets). Appendix 5 lists the relative share of SBM that has been replaced by the more regionally grown protein-rich ingredients.
All diets were pelleted (starter diets as 2.5 mm diameter pellets, and grower I, grower II and finisher diets as 3.2 mm diameter pellets) using a limited amount of steam (pellet temperature < 70 ^{o}C).

2.6 Animal and housing conditions

A total number of 1,044 day-old fast-growing (Ross 308) and 1,044 slow-growing (Hubbard JA757) male broilers were obtained from a commercial hatchery (Probroed & Sloot, Groenlo, The Netherlands). The fast-growing and slow-growing broilers were housed separately and allocated to 72 floor pens (floor space: 2.13 m^2; 29 birds/pen) bedded with wood shavings (2 kg/pen) in the mechanically ventilated experimental broiler house of feed producer ForFarmers, Bathmen, The Netherlands (Figure 2.1).

The housing, management, feeding and husbandry conditions were standard and representative for a modern commercial broiler operation in the Netherlands. Water and feed were provided for *ad libitum* intake during the entire experimental period (0 – 38 days of age). Feed was supplied via feeding bins (feeding space/pen 1.36 m). These bins were constructed in a way that feed spillage was limited. Water was supplied by six drinking nipples with drip cups (Val) per pen. One day prior to placement of the broilers, the house was pre-heated to 30°C. The temperature at the time of placement of the broilers was 34.5°C and this temperature was gradually decreased to 20°C at 34 days of age. This temperature was kept until the end of the experiment (Table 2.4). During the first three days the light was nearly continuous on (23L:1D), from 3 – 7 days of age a day/night schedule of 20h light and 4h dark (20L:4D), from 8 – 35 days a day/night schedule of 18h light and 6h dark (18L:6D), from 36 – 37 days a day/night schedule of 20h light and 4h dark (20L:4D) and during the last day 23h light and 1h dark (23L:1D) was applied per 24h. Light intensity was 20 lux during the entire experimental period.
Visual observation of the birds was done twice per day to check animal health. All broilers were vaccinated at the hatchery against Infectious Bronchitis (IB primer, spray) and on day 8 of age against New Castle Disease (Nobilis ND C2, spray vaccination) and Infectious Bronchitis (Nobilis IB Primo QX, spray vaccination) and on day 16 of age against Gumboro (Nobilis Gumboro D78, drinking water) at the experimental facility.

Figure 2.1 *Overview of the experimental house (age broilers 35 days). Pens with the slow-growing broilers were covered with a metal mesh placed on top to prevent birds from jumping outside or in another pen.*

Table 2.4 *Temperature schedule.*

Age	Set temperature (°C)
0	34.5
2	33.5
4	31.5
6	30.5
10	30
14	28
21	25
28	22
34 – slaughter	20

2.7 Observations and measurements

- Diets
  After pelleting, and just before bagging, each diet was sampled (i.e. at intervals of 20 kg diet). These samples were pooled to form a composite, representative sample of each diet. All diets (6 starter, 6 grower I, 6 grower II and 6 finisher diets; n=24) were analysed on contents of dry matter, crude protein, crude fat, crude fibre, ash, starch (Brunt), calcium, phosphorus, sodium, chloride and potassium. In addition to these chemical analyses, the hardness and durability of the pellets were also determined at the ForFarmers feed mill, Delden. All chemical analyses were performed at the laboratory of ForFarmers, Lochem, The Netherlands.

- Performance
  Body weight (BW) of birds per pen was determined at 0, 9, 21, 29 and 38 days of age. Feed intake (FI) per pen was determined at 9, 21, 29 and 38 days of age (provided feed minus remaining feed at 9, 21, 29 and 38 days of age). Body weight gain (BWG), average daily gain (ADG), feed conversion ratio (FCR), feed intake (FI), and average daily feed intake (ADFI) were calculated on a pen basis from these data for the following periods: 0 – 9 d, 9 – 21 d, 21 – 29 d, 29 – 38 d and 0 – 38 d. BWG = BW end period – BW start period; ADG = BWG / length period; FCR = (Total FI / (Total BW end period – total BW start period + total BW of dead or culled birds)); FI = FCR x BWG; ADFI = FI / length period. In all cases of mortality or culling, the birds were weighed, and the date of removal was recorded. EPEF (European Production Efficiency Factor) was calculated per pen at 38 days. EPEF was calculated as (mean daily body weight gain (g)/FCR*10) x (100 - % mortality).

- Litter quality assessment
  Litter quality was visually scored at 21, 29 and 37 days of age by three persons. These persons scored friability and wetness of the litter in each pen on a 1-to-5-point scale. The scores and the description of each score are presented in Table 2.3. The scores are presented as an average of the three independent visual scores.

Table 2.3 *Scores for friability and wetness of the litter and a description of each score.*

Score	Friability description	Wetness description
1	100% friable litter	Dry litter
2	25% of the area is caked	Almost dry litter
3	50% of the area is caked	Moist litter
4	75% of the area is caked	Wet litter
5	Complete caked litter	Very wet litter

- Footpad lesions
  Occurrence of footpad lesions and their severity (scale 0, 1, 2) of ten broilers per pen was determined at 34 days of age by an experienced assessor. Only the right footpad was scored per broiler according to the so-called ‘Swedish’ classification, i.e. score 0: no lesions or very small discolouration; score 1: discoloration but no deep lesion; score 2: deep lesion with ulcers or scabs, bumble foot (Berg, 1998). An overview of scores for footpad lesions is presented in Appendix 6.

The severity of footpad lesions was expressed as footpad score (FPS) per pen. This score is calculated as follows: 100% * ((0.5 * the total number of birds with score 1) + (2 * the total number of birds with score 2)) / the total number of scored birds. The flock FPS ranges from 0 (all birds having no lesions) to 200 (all birds having score 2).

- Nitrogen (N) and phosphorus (P) efficiency

N and P efficiency was calculated on pen level as:

N-efficiency (%) = N-retention (kg) /Total N intake (kg) × 100%
N-retention (kg) = (BW_{end} (kg) × 0.0283 (kg/kg)) – (BW_{start} (kg) × 0.0258 (kg/kg))
Total N-intake (kg) = (($FI_{starter\ period}$ (kg) × $N_{starter\ feed}$ (g/kg)) + ($FI_{grower1\ period}$ (kg) × $N_{grower1\ feed}$ (g/kg)) + ($FI_{grower2\ period}$ (kg) × $N_{grower2\ feed}$ (g/kg)) + ($FI_{finisher\ period}$ (kg) × $N_{finisher\ feed}$ (g/kg))) / 1000

P-efficiency (%) = P-retention (kg) / Total P intake (kg) × 100%
P-retention (kg) = ((BW_{end} (kg) × 0.0101 (kg/kg)) – (BW_{start} (kg) × 0.0058 (kg/kg))/2.29)
Total P intake (kg) = (($FI_{starter\ period}$ × $P_{starter\ feed}$ (g/kg)) + ($FI_{grower1\ period}$ × $P_{grower1\ feed}$ (g/kg)) + ($FI_{grower2\ period}$ × $P_{grower2\ feed}$ (g/kg)) + ($FI_{finisher\ period}$ × $P_{finisher\ feed}$ (g/kg))) / 1000

in which N-content in the body of broiler chickens at start and finish is 0.0258 and 0.0283 kg/kg BW and the P_2O_5 content is 0.0058 and 0.0101 kg/kg BW ("Stikstof en fosfaat in dieren"; RVO (2022). P conversion factor from P_2O_5 to P: 2.29. BW = Body weight (kg); FI = Feed Intake (kg)

- Carbon footprint (CFP) of the feed and per kg body weight gain
Carbon footprint of the feed (g CO_2 eq./kg) was based on the list version 5 of Nevedi (Nevedi, 2022) including and excluding Land Use Change (LUC). The values of feed materials used in this study are included in Appendix 7. In addition, the CFP per kg of body weight gain (BWG) was calculated.
Carbon footprint per kg BWG was calculated on pen level as follows:
CFP per kg BW gain (g CO_2 eq./kg) = (total FI (kg)[1] × CFP per kg feed) / (BW_{end} (kg) – BW_{start} (kg))

[1] Total FI (kg) = $FI_{starter\ period}$ (kg) + $FI_{grower1\ period}$ (kg) + $FI_{grower2\ period}$ (kg) + $FI_{finisher\ period}$ (kg)

- Microbiome composition
At 7 and 21 days of age, cloacal swabs from 2 birds per pen (24 birds per treatment) were collected and analysed according to the Cargill Galleon© protocol. Appendix 8 describes the Galleon© procedure and shows the effects of the dietary treatments on the microbiome composition of faeces from fast- and slower-growing broilers.

2.8 Statistical analysis

Raw data were analysed for statistical outliers. An outlier was defined as an observation which is deviating more than 2.5 times the standard deviations from the mean. If the deviation of an observation was more than 2.5 times the standard deviation from the mean and there was a plausible reason for this deviation, the observation was excluded from the dataset. The experimental data were analysed using GenStat statistical software (GenStat™ Release 21.1).
The P-value of the treatment effect and the SEM (Standard Errors of Means) were provided per response parameter. Differences between treatments were analysed using the Fisher's unprotected least significant difference test. Treatment effects with a *P-value* <0.05 were considered to be statistically significant.

Response parameters were analysed using ANOVA (analysis of variance) according to the following model:

$Y_{ij} = \mu + Block_i + Breed_j + Diet_k + Breed*Diet_{jk} + Error_{ijk}$

Where:

Y	Response parameter
μ	General mean
Block	Effect of block (i = 1…6)
Breed	Effect of breed (j = 1,2; slow-growing vs. fast-growing)
Diet	Effect of diet program (k = 1…6)
Breed*Diet	Interaction effect between breed and diet program
Error	Error term

3 Results

3.1 General

No adverse events occurred during the trial. Average body weight of the fast-growing and slow-growing broilers at arrival was 48 and 42 grams, respectively. Overall mortality during the experiment for the fast-growing broilers was 3.2% and 1.3% for the slow-growing broilers. These mortality rates can be considered as low (Dutch standard for regular broilers = 3.5% and for slow-growing broilers = 2.5%, KWIN 2022/2023). At day 38 the average body weight of the fast-growing and slow-growing male broilers was respectively 184 gram (2958 vs. 2774 gram) and 193 gram (1906 vs. 1713 gram) higher than the breed male broiler performance objectives (Figure 3.1). The feed conversion ratio (FCR) of the fast-growing male broilers exceeded from day 9 onwards the breed performance objectives. The FCR of the slow-growing male broilers was lower compared to the breed performance objectives. The higher growth of the fast-growing broilers can most likely be attributed to the lower animal density and the better microclimate at bird-level, while the higher growth of the slow-growing broilers is probably due to the fed luxurious diet and a better bird-level microclimate compared to field conditions on which the breeder's performance objectives are based. The higher FCR of the fast-growing birds was most likely due to the diet provided, as the nutritional values of the diets were in between the requirements of the Ross 308 and Hubbard JA757 broilers. This means that the fast-growing broilers were fed below the CVB recommendation for maximum growth, while the slow-growing broilers were fed above the breeder's recommendation.
On day 38, the weight of the birds in pen 26 (Fast-growing, diet E), pen 40 (Fast-growing, diet E) and pen 57 (Slow-growing, diet H) has not been properly determined probably due to failure of the weighing scale or false registration. As a result, the performance results over the finisher phase and the total experimental period could not be calculated properly for these pens and were therefore marked as missing values. Other than these, the database showed no outliers and therefore all other data were used in the analysis.

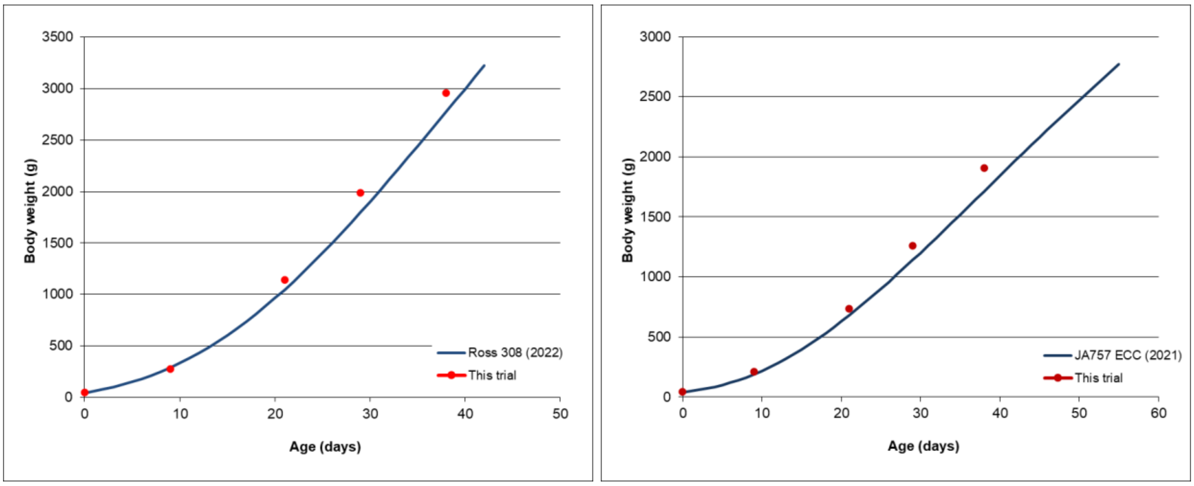

Figure 3.1 *Average body weight of the Ross 308 and Hubbard JA757 male broilers achieved in the present study compared to the Ross 308 broiler male performance objectives (Aviagen, 2022) Hubbard JA757 ECC performance objectives (Hubbard, 2021).*

3.2 Diet

The calculated and analysed nutrient composition of the starter and grower I diets, and the grower II and finisher diets are given in Tables 3.1 and 3.2, respectively. In Table 3.3 the results of the pellet hardness and durability measurements of all experimental diets are given. Table 3.4 shows the carbon footprint values (CFP) including Land Use Change (LUC) of the diets. In Appendix 6 the CFP values of the ingredients and diets including and excluding LUC are given.

Table 3.1 *Calculated and analysed nutrients in the starter and grower I diets.*

Phase		**Starter diets**						**Grower I diets**					
Feed Code[1]		**1A**	**1B**	**1C**	**1D**	**1E**	**1F**	**2A**	**2B**	**2C**	**2D**	**2E**	**2F**
Calculated													
Crude protein	g/kg	196	197	198	197	200	194	184	182	193	189	193	191
Crude fat	g/kg	56	54	56	71	70	70	60	54	61	96	90	95
Crude fibre	g/kg	27	32	35	42	35	57	27	39	45	63	44	65
Crude ash	g/kg	61	62	61	62	62	60	53	54	52	56	56	56
Starch Brunt	g/kg	400	398	400	371	368	377	419	420	411	345	347	341
Calcium	g/kg	8.8	8.8	8.8	8.8	8.8	8.8	6.8	6.8	6.8	6.8	6.8	6.8
Phosphorus	g/kg	4.9	4.9	4.9	5.4	5.2	5.4	3.8	3.7	3.9	5.1	4.5	4.8
Sodium	g/kg	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4
Chloride	g/kg	2.2	2.2	2.2	2.2	2.2	2.2	2.0	2.0	2.0	2.0	2.0	2.0
Potassium	g/kg	8.4	8.7	8.4	8.3	8.4	7.3	7.7	8.2	8.0	7.5	7.7	7.6
Analysed													
Crude protein	g/kg	198	198	197	193	203	195	182	185	186	181	196	192
Crude fat	g/kg	55	54	56	67	66	67	59	52	59	93	88	93
Crude fibre	g/kg	24	31	33	41	34	54	25	37	49	59	41	63
Crude ash	g/kg	56	59	58	60	60	58	50	54	52	53	53	55
Starch Brunt	g/kg	406	395	404	370	369	376	421	401	404	342	338	325
Calcium	g/kg	8.4	9.3	9.1	9.3	8.8	9.3	7.0	7.7	7.3	6.9	6.5	7.0
Phosphorus	g/kg	3.9	6.8	4.6	5.5	5.3	5.6	3.9	3.8	4.0	5.0	4.6	4.9
Sodium	g/kg	1.3	1.3	1.4	1.4	1.4	1.4	1.4	1.3	1.4	1.3	1.5	1.4
Chloride	g/kg	2.1	2.0	2.1	2.1	2.0	2.0	1.9	1.8	2.0	1.8	1.8	1.9
Potassium	g/kg	8.7	9.0	8.5	8.2	8.5	7.2	7.8	8.2	7.7	7.2	7.6	7.4

[1] the number indicates the feeding phase (1 = starter phase; 2 = Grower I phase), the letter indicates the feeding program (A: Control diet; B: Diet with peas; C: Diet with field beans; D: Diet with sunflower seed meal; E: Diet with rapeseed meal; F: SBM-free diet).

Table 3.2 *Calculated and analysed nutrients in the grower II and finisher diets.*

Phase		**Grower II diets**						**Finisher diets**					
Feed Code[1]		**3A**	**3B**	**3C**	**3D**	**3E**	**3F**	**4A**	**4B**	**4C**	**4D**	**4E**	**4F**
Calculated													
Crude protein	g/kg	178	168	185	179	187	173	169	158	174	169	178	165
Crude fat	g/kg	62	51	61	96	92	83	67	54	66	95	97	85
Crude fibre	g/kg	27	39	45	62	44	57	27	38	44	55	44	54
Crude ash	g/kg	52	52	51	54	55	53	49	49	48	52	52	50
Starch Brunt	g/kg	428	445	424	362	356	388	439	460	438	385	366	402
Calcium	g/kg	6.6	6.6	6.6	6.6	6.6	6.6	5.9	5.9	5.9	5.9	5.9	5.9
Phosphorus	g/kg	3.7	3.6	3.7	4.9	4.3	4.4	3.6	3.5	3.7	4.6	4.3	4.3
Sodium	g/kg	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.4	1.3	1.3
Chloride	g/kg	2.0	2.0	2.0	2.0	2.0	1.7	1.8	1.9	1.9	1.6	1.8	1.4
Potassium	g/kg	7.4	7.5	7.6	6.9	7.4	6.8	7.0	7.0	7.1	6.6	7.0	6.5
Analysed													
Crude protein	g/kg	175	170	176	171	190	172	168	160	168	163	182	164
Crude fat	g/kg	60	50	59	92	89	83	63	51	62	92	93	84
Crude fibre	g/kg	24	36	47	64	40	59	25	37	46	52	41	49
Crude ash	g/kg	50	51	51	52	53	53	48	48	48	49	50	48
Starch Brunt	g/kg	417	427	407	362	354	378	414	416	414	352	325	401
Calcium	g/kg	6.9	7.2	7.1	6.9	6.5	7.3	6.4	6.6	6.5	6.3	6.1	6.4
Phosphorus	g/kg	3.8	3.7	3.9	4.9	4.6	4.6	3.8	3.7	3.8	4.7	4.4	4.3
Sodium	g/kg	1.3	1.2	1.3	1.3	1.4	1.3	1.2	1.2	1.3	1.3	1.4	1.3
Chloride	g/kg	2.0	1.8	2.1	1.7	1.9	1.6	1.7	1.6	1.9	1.6	1.7	1.3
Potassium	g/kg	7.4	7.5	7.3	6.7	7.5	6.7	7.0	7.1	6.9	6.3	7.0	6.4

[1] the number indicates the feeding phase (3 = Grower II phase; 4 = Finisher phase), the letter indicates the feeding program (A: Control diet; B: Diet with peas; C: Diet with field beans; D: Diet with sunflower seed meal; E: Diet with rapeseed meal; F: SBM-free diet).

The analysed nutrient composition of the experimental starter, grower I, grower II and finisher diets were largely in accordance with the calculated values.
The phosphorus (P) content of diet 1A (Control) was 1.0 g/kg (= 20%) lower, while the P content of diet 1B (in which part of the SBM was replaced with peas) was 1.9 g/kg (= 39%) higher than calculated. These differences are, considering the dosing reports prepared upon diet production, not likely to be related to mixing/dosing errors, nor due to the differences between assumed and actual nutrient composition of individual ingredients, as the feed formulation was based on the actual analysed nutrient concentrations in the ingredients. It is not expected that the differences in dietary P concentration had a large impact on the results of the study.

Table 3.3 *Pellet quality of the experimental diets.*

Parameter	**Pellet hardness (kg)** [1]				**Pellet durability (%)** [2]			
Feeding phase	Starter	Grower I	Grower II	Finisher	Starter	Grower I	Grower II	Finisher
Diet								
Control diet program	2.15	2.12	2.42	2.90	85.9	79.9	87.1	84.9
Diet program with peas	1.98	2.73	3.27	2.65	90.4	91.9	93.9	93.9
Diet program with field beans	2.18	2.38	3.10	2.43	89.3	87.2	90.7	90.8
Diet program with sunflower seed meal	1.75	1.58	2.05	1.82	82.8	72.8	78.7	78.4
Diet program with rapeseed meal	1.38	1.52	1.57	1.70	78.7	67.7	66.8	67.8
SBM-free diet program	2.15	1.62	2.07	2.75	89.0	81.7	78.5	82.7

[1] The OK range for hardness is between 2.5 and 3.5 kg. Feed will be disapproved when values are <2.2 kg and >4,0 kg. Corrective measurements will be made when in between 2.2 to 2.5 and 3,5 to 4.0 kg.

[2] The OK range for durability is between 90 and 95%. Feed will be disapproved when values are <87% and >97%. Corrective measurements will be made when in between 87 to 90 and 95 to 97%.

The rapeseed meal diets had the lowest pellet hardness and pellet durability of all diets, closely followed by the sunflower seed meal diets (Table 3.3). The diet programs with peas and field beans had on average the highest pellet hardness. In addition to the highest pellet hardness, the pea and field bean diets had the highest pellet durability. It should be noticed that the pellet quality of various diets does not meet the standards for pellet hardness and durability. However, the stated standards are standards for bulk diets, not for experimental diets. The experimental diets were produced in a specialized feed factory with a small installation and hardly any feed transportation. Immediately after production, the feed was bagged and transported to the experimental trial facility. Bulk production feed undergoes much more transportation, e.g. after pelleting transportation to storage silo in feed factory, transportation from storage silo to the bulk truck, transportation bulk truck to farm silo, transportation from farm silo to feeding hopper in the barn, transportation from feeding hopper to feed pan. The feed produced and fed to the animals appeared visually normal and there were no visual indications of differences in the meal fraction (proportion of fines) because of the poorer pellet quality between the different diets.

Table 3.4 *Absolute (g CO_2 eq/kg) and relative (%) effects of (partial) replacement of SBM with regionally grown protein-rich ingredients on carbon footprint (incl. LUC) of the experimental diets. Source: Nevedi CFP database, 2022.*

		Starter		**Grower1**		**Grower2**		**Finisher**	
		Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.
A	Control diet program	1582	100%	1442	100%	1382	100%	1299	100%
B	Diet program with peas	1488	94%	1122	78%	997	72%	905	70%
C	Diet program with field beans	1381	87%	1023	71%	948	69%	846	65%
D	Diet program with sunflower seed meal	1340	85%	871	60%	774	56%	795	61%
E	Diet program with rapeseed meal	1462	92%	1173	81%	1112	81%	1030	79%
F	SBM-free diet program	708	45%	668	46%	679	49%	671	52%

By using more regionally grown protein sources instead of Brazilian SBM, it is possible to reduce the carbon footprint (incl. LUC) of diets by 55 percent (Table 3.4). Complete replacement of Brazilian SBM with regionally grown protein sources reduced the carbon footprint by 45 – 52% compared to the control diet program with SBM as the main protein source. Partial replacement of soybean meal with peas reduced the carbon footprint by 6, 22, 28 and 30 percent for the starter, grower1 and grower2 and finisher diet, respectively.

Partial replacement of SBM with field beans reduced the carbon footprint in the successive phases even slightly more than with peas. Partially replacing SBM with sunflower seed meal reduced the carbon footprint even more compared to the pea and field bean diets. Partially replacing SBM with rapeseed meal also gave a reduction in carbon footprint, but of all diets with alternative protein-rich ingredients RSM diets gave the lowest carbon footprint reduction, because these diets still contained the highest level of SBM.

3.3 Performance

The obtained performance results of the broilers during the starter phase (0-9 d) are given in Table 3.5. Fast-growing broilers had higher body weight, higher daily growth, higher daily feed intake and a lower FCR than slow-growing broilers.
Within breed BW at day 0 was the same for all groups. At day 9, broilers fed the diets with the more regionally grown protein sources had lower BW and BWG than broilers fed the control diet. Broilers fed the SBM-free starter diet had the lowest BW and BWG, but the BW and BWG of these broilers did not differ significantly from the BW and BWG of the broilers fed the peas (P), sunflower seed meal (SFM) or the field bean (FB) starter diet. Broilers fed the RSM diet had a higher BW and BWG compared to broilers fed the SBM-free starter diet, but the BW and BWG did not differ significantly from the other diets with regionally grown protein-rich ingredients. Broilers fed the control and RSM starter diet had a significantly lower FCR than broiler fed with P, FB and SBM-free starter diet. The FCR of the broilers fed with the control or RSM starter diet did not differ from the achieved FCR of the broilers fed with SFM starter diet. Replacing Partial or complete replacement of SBM with more regionally grown protein sources had no effect on the mortality rate in the starter phase.
Broilers fed the diets with more regionally grown protein-rich ingredients had a significantly lower FI compared to the control group, except for the broilers fed the SFM diet. The FI of broilers fed the SFM starter diet was numerically but not significantly lower than the control group. Broilers fed the SFM diet had a significantly higher FI compared to the SBM-free group, while the FI of the other diets with regionally grown protein-rich ingredients did not differ significantly from each other.
In the starter phase, a breed*diet interaction effect was found for FI. In general, broilers fed the control diet the highest FI compared to all diets with regionally grown protein-rich ingredients except the SFM diet. However, these differences were mainly caused by the fast-growing (FG) broilers. For the slow-growing (SG) broilers, the FI of the control diet differed only significantly with the peas group while there were no further significant differences in FI between the different groups with the more regionally grown protein-rich ingredients. In the FG broilers, the highest FI was observed in the control and SFM groups. Feed intake in these groups were significantly higher compared to all other groups, except the group fed the P diet. Feeding FG broilers the SBM-free diet resulted in a significantly lower FI compared to the P, the SFM and the control diet.

Table 3.5 *Performance results starter phase (0 – 9 days).*

Effect		Body weight d0 (g)	Body weight d9 (g)	Daily gain (g/d)	Feed intake (g/d)	FCR	Mortality (%)
Breed							
Slow-growing (SG)		42.3[b]	209[b]	18.5[b]	21.8[b]	1.180[a]	0.8
Fast-growing (FG)		48.1[a]	276[a]	25.4[a]	27.8[a]	1.098[b]	1.1
Diet							
Control diet		45.1	251[a]	22.9[a]	25.6[a]	1.126 [b]	0.9
Diet with peas		45.1	241[bc]	21.7[bc]	24.8[bc]	1.148 [a]	0.3
Diet with field beans		45.1	239[bc]	21.5[bc]	24.6[bc]	1.148 [a]	1.2
Diet with sunflower seed meal		44.7	243[bc]	22.1[b]	25.0[ab]	1.141 [ab]	1.2
Diet with rapeseed meal		45.7	244[b]	22.0[b]	24.7[bc]	1.125 [b]	1.4
SBM-free diet		45.6	238[c]	21.4[c]	24.3[c]	1.146 [a]	0.6
Breed * Diet							
SG * Control		42.8	217	19.3	22.5[d]	1.166	1.2
SG * Peas		42.0	203	17.9	21.3[e]	1.189	0.0
SG * Field beans		41.7	208	18.5	21.8[de]	1.180	1.2
SG * Sunflower seed meal		41.7	207	18.4	21.6[de]	1.175	0.0
SG * Rapeseed meal		42.8	212	18.8	21.9[de]	1.168	1.7
SG * SBM-free		42.8	206	18.1	21.8[de]	1.205	0.6
FG * Control		47.4	285	26.4	28.7[a]	1.087	0.6
FG * Peas		48.3	278	25.5	28.3[ab]	1.108	0.6
FG * Field beans		48.6	270	24.6	27.4[bc]	1.116	1.2
FG * Sunflower seed meal		47.7	279	25.7	28.5[a]	1.106	2.3
FG * Rapeseed meal		48.6	277	25.3	27.4[bc]	1.083	1.2
FG * SBM-free		48.3	270	24.6	26.7[c]	1.087	0.6
Source of variation							
Breed	P-value	<0.001	<0.001	<0.001	<0.001	<0.001	0.564
	SEM (n=36)	0.25	1.2	0.12	0.13	0.0040	0.35
Diet	P-value	0.620	<0.001	<0.001	0.005	0.049	0.785
	SEM (n=12)	0.43	2.0	0.21	0.24	0.0069	0.61
Breed * Diet	P-value	0.525	0.223	0.157	0.025	0.111	0.565
	SEM (n=6)	0.60	2.9	0.30	0.33	0.0098	0.86

[a-e] Means within a column and within a source without a common superscript differ significantly ($P < 0.05$).

SEM = Standard errors of means.

Performance results in the first part of the grower phase (9-21 d) are given in Table 3.6. Fast-growing broilers showed a higher BWG and FI and had a more favourable FCR than SG broilers. No diet or breed*diet effects on performance results were found in this phase.

Table 3.6 *Performance results grower period 1 (9-21 days of age).*

Effect		Body weight d21 (g)	Daily gain (g/d)	Feed intake (g/d)	FCR	Mortality (%)
Breed						
Slow-growing (SG)		738^{b}	44.1^{b}	65.3^{b}	1.484^{a}	0.3
Fast-growing (FG)		1139^{a}	71.9^{a}	95.7^{a}	1.332^{b}	0.4
Diet						
Control diet		959	59.0	82.7	1.433	0.3
Diet with peas		938	58.1	80.0	1.398	0.0
Diet with field beans		941	58.5	81.3	1.406	0.0
Diet with sunflower seed meal		934	57.5	80.4	1.412	0.9
Diet with rapeseed meal		940	58.0	79.3	1.380	0.0
SBM-free diet		919	56.8	79.4	1.420	0.9
Breed * Diet						
SG * Control		750	44.5	68.8	1.551	0.0
SG * Peas		720	43.1	63.8	1.481	0.0
SG * Field beans		748	45.0	66.3	1.475	0.0
SG * Sunflower seed meal		731	43.7	64.2	1.469	0.6
SG * Rapeseed meal		756	45.4	64.9	1.430	0.0
SG * SBM-free		720	42.9	64.0	1.496	1.2
FG * Control		1167	73.5	96.6	1.315	0.6
FG * Peas		1156	73.2	96.2	1.314	0.0
FG * Field beans		1133	72.0	96.2	1.337	0.0
FG * Sunflower seed meal		1136	71.4	96.6	1.354	1.2
FG * Rapeseed meal		1124	70.6	93.8	1.329	0.0
FG * SBM-free		1118	70.7	94.8	1.344	0.6
Source of variation						
Breed	P-value	<0.001	<0.001	<0.001	<0.001	0.695
	SEM (n=36)	5.7	0.43	0.67	0.0113	0.17
Diet	P-value	0.153	0.380	0.310	0.497	0.091
	SEM (n=12)	9.9	0.74	1.16	0.0195	0.30
Breed * Diet	P-value	0.223	0.274	0.665	0.205	0.755
	SEM (n=6)	14.0	1.05	1.65	0.0276	0.42

$^{a-b}$ Means within a column and within a source without a common superscript differ significantly (P < 0.05).

SEM = Standard errors of means.

Table 3.7 shows the performance results achieved in the second part of the growth phase (21-29 d). As in the previous feeding phases, FG broilers had higher BWG and FI and lower FCR than SG broilers.

Broilers fed the RSM diet had the highest BWG, but the BWG of these broilers did not differ from the BWG of the broilers fed the control, P and FB diets. Broilers fed the SBM-free diet had the lowest BWG, but the BWG of the SBM-free fed broilers was only significantly lower than the BWG of broilers fed the control, FB and RSM diet. The differences found in BWG in this feeding phase resulted in differences in body weight (BW) at 29 days of age. Broilers fed the control diet had the highest BW, but the BW of these broilers was only significantly higher compared to the BW of the SFM and SBM-free groups. Broilers fed the SBM-free diet had the lowest BW. The BW of these broilers was significantly lower than the BW of all other groups, except with the BW of broilers fed the SFM diet.

A breed*diet interaction effect was observed for FCR. In general, broilers fed the control or the RSM diet had the lowest FCR in this feeding phase, but this was mainly caused by the SG broilers. Broilers fed the SBM-free diet had the highest FCR. No difference was observed in FCR between the P, FB and SFM fed broilers, but the FCR of these groups was significantly higher than the FCR of broilers fed the control or RSM diet and significantly lower than the SBM-free fed broilers.

Table 3.7 *Performance results grower period 2 (21-29 days of age).*

Effect		**Body weight d29 (g)**	**Daily gain (g/d)**	**Feed intake (g/d)**	**FCR**	**Mortality (%)**
Breed						
Slow-growing (SG)		1257^{b}	64.9^{b}	108.5^{b}	1.672^{a}	0.1^{b}
Fast-growing (FG)		1989^{a}	106.3^{a}	161.6^{a}	1.521^{b}	1.0^{a}
Diet						
Control diet		1652^{a}	86.7^{ab}	134.0	1.563^{c}	0.3
Diet with peas		1624^{ab}	85.8^{abc}	134.6	1.593^{b}	0.6
Diet with field beans		1630^{ab}	86.2^{ab}	136.1	1.604^{b}	0.0
Diet with sunflower seed meal		1607^{bc}	84.2^{bc}	134.4	1.612^{b}	0.3
Diet with rapeseed meal		1638^{ab}	87.2^{a}	135.1	1.560^{c}	0.9
SBM-free diet		1586^{c}	83.4^{c}	136.0	1.649^{a}	1.2
Breed * Diet						
SG * Control		1275	65.5	107.1	1.635^{c}	0.0
SG * Peas		1231	63.8	107.4	1.684^{bc}	0.0
SG * Field beans		1266	64.9	110.5	1.706^{ab}	0.0
SG * Sunflower seed meal		1240	63.6	106.4	1.674^{c}	0.0
SG * Rapeseed meal		1297	67.6	108.8	1.611^{d}	0.6
SG * SBM-free		1234	64.2	110.8	1.725^{a}	0.0
FG * Control		2030	107.9	160.9	1.491^{f}	0.6
FG * Peas		2018	107.7	161.9	1.503^{f}	1.2
FG * Field beans		1994	107.6	161.7	1.503^{f}	0.0
FG * Sunflower seed meal		1974	104.8	162.4	1.549^{e}	0.6
FG * Rapeseed meal		1979	106.9	161.3	1.510^{f}	1.2
FG * SBM-free		1938	102.6	162.3	1.573^{e}	2.3
Source of variation						
Breed	P-value	<0.001	<0.001	<0.001	<0.001	0.014
	SEM (n=36)	7.6	0.53	0.65	0.0039	0.24
Diet	P-value	0.015	0.035	0.704	<0.001	0.407
	SEM (n=12)	13.2	0.92	1.12	0.0068	0.42
Breed * Diet	P-value	0.099	0.263	0.511	<0.001	0.483
	SEM (n=6)	18.7	1.30	1.59	0.0096	0.59

$^{a\text{-}f}$ Means within a column and within a source without a common superscript differ significantly ($P < 0.05$).

SEM = Standard errors of means.

Table 3.8 shows the performance results achieved in the finisher phase (29-38 d). As in the previous feeding phases, FG broilers had higher BWG and FI and lower FCR than SG broilers.

In the finisher phase, a breed*diet interaction effect was found for BW, BWG, FI and FCR, indicating a difference in breed response to the fed diet on these parameters. Fast-growing broilers fed the P diet had the highest BWG in this phase, but the BWG only differed significantly from the RSM- and SBM-free-fed FG animals. While SG broilers had the highest BWG on the RSM diets and the lowest BWG on the P diets. This clearly showed that there is a difference in the response of the breed to the diet on BWG. Slower growing broilers fed the RSM diet program had significantly higher BW at 38 days of age compared to SG broilers fed the control, P, SFM and SBM-free diet programs. In contrast, FG broilers fed the control or P diet program had the highest BW at 38 days. The BW of these groups was significantly higher than the BW of the SFM, RSM and SBM-free groups. This clearly showed a difference in breed response to the given diet program on BW.
Also for the FI there was a clear difference in the response of the breed to the diet. Fast-growing broilers fed the SFM diet had the highest FI, while FG broilers fed the FB or RSM diet had the lowest FI. In contrast, the RSM-fed SG broilers had the highest FI and the SFM-fed SG-broilers were among the groups with the lowest FI. Slow-growing broilers receiving the control or RSM finisher diet had a lower FCR compared to the other groups, while FG broilers achieved the lowest FCR on the P and FB finisher diet.

Table 3.8 *Performance results finisher period (29-38 days of age).*

Effect		Body weight d38 (g)	Daily gain (g/d)	Feed intake (g/d)	FCR	Mortality (%)
Breed						
Slow-growing (SG)		1906^{b}	72.2^{b}	142.4^{b}	1.973^{a}	0.2
Fast-growing (FG)		2958^{a}	107.6^{a}	201.1^{a}	1.873^{b}	0.8
Diet						
Control diet		2461^{a}	90.4	170.6	1.894^{b}	0.3
Diet with peas		2440^{ab}	90.6	172.2	1.922^{b}	0.6
Diet with field beans		2445^{ab}	90.5	171.2	1.911^{b}	1.2
Diet with sunflower seed meal		2408^{bc}	89.0	173.2	1.954^{a}	0.0
Diet with rapeseed meal		2454^{a}	90.5	171.8	1.903^{b}	0.6
SBM-free diet		2382^{c}	88.4	171.4	1.952^{a}	0.3
Breed * Diet						
SG * Control		1914^{e}	72.1^{cd}	139.0^{d}	1.928^{b}	0.0
SG * Peas		1868^{e}	70.8^{d}	141.7^{cd}	2.003^{a}	0.6
SG * Field beans		1917^{de}	72.2^{cd}	144.1^{cd}	1.996^{a}	0.0
SG * Sunflower seed meal		1881^{e}	71.2^{d}	141.6^{cd}	1.988^{a}	0.0
SG * Rapeseed meal		1978^{d}	75.7^{c}	145.1^{c}	1.916^{b}	0.0
SG * SBM-free		1876^{e}	71.3^{d}	142.7^{cd}	2.004^{a}	0.6
FG * Control		3009^{a}	108.8^{ab}	202.1^{ab}	1.860^{cd}	0.6
FG * Peas		3011^{a}	110.4^{a}	202.7^{ab}	1.840^{d}	0.6
FG * Field beans		2973^{ab}	108.7^{ab}	198.2^{b}	1.826^{d}	2.3
FG * Sunflower seed meal		2935^{bc}	106.8^{ab}	204.8^{a}	1.920^{b}	0.0
FG * Rapeseed meal		2930^{bc}	105.3^{b}	198.6^{b}	1.890^{bc}	1.2
FG * SBM-free		2888^{c}	105.5^{b}	200.2^{ab}	1.900^{bc}	0.0
Source of variation						
Breed	P-value	<0.001	<0.001	<0.001	<0.001	0.093
	SEM (n=36)	9.3	0.59	0.82	0.0059	0.24
Diet	P-value	0.007	0.534	0.841	<0.001	0.481
	SEM (n=12)	16.1	1.03	1.41	0.0103	0.41
Breed * Diet	P-value	0.003	0.034	0.053	<0.001	0.186
	SEM (n=6)	22.7	1.46	2.00	0.0145	0.58

[a-e] Means within a column and within a source without a common superscript differ significantly ($P < 0.05$).

SEM = Standard errors of means.

Table 3.9 presents the performance results achieved over the entire experimental period (0-38 d). In general FG broilers had a higher BW, BWG, FI and mortality and a lower FCR than SG broilers. Due to the higher BWG and lower FCR, the EPEF of FG broilers was much higher than that of SG broilers.

Diet program had no effect on FI and mortality over the entire experimental period, but it did affect the broiler growth and feed efficiency. However, the response to these parameters depended on the breed used.

In general, broilers fed the control diet program achieved the highest BW and BWG. However, the BW and BWG of the broilers fed the control diet program differed significantly only from broilers fed the SFM or SBM-free diet program. Slow-growing broilers fed the RSM diet program had significantly higher BW at 38 days of age compared to SG broilers fed the control, P, SFM and SBM-free diet programs. In contrast, FG broilers fed the control or P diet program had the highest BW at 38 days. The BW of these groups was significantly higher than the BW of the SFM, RSM and SBM-free groups.

Broilers fed the SBM-free diet program had on average a significantly higher FCR compared to all other diet programs. However, this effect was more pronounced with FG broilers. Within FG broilers, the lowest feed efficiency, i.e. the highest FCR, was achieved on the SFM and SBM-free diet program and the highest feed efficiency on the control and P diet program. In contrast, in SG broilers, the highest feed efficiency was achieved on the RSM diet program and the lowest feed efficiency on the SBM-free diet program.

In general, feeding broilers with the control diet, P and RSM diet programs resulted in the highest EPEF, i.e. the best overall performance was achieved with these feeding programs. However, for the EPEF there was a clear difference in the breed's response to the diet. Slow-growing broilers had the highest EPEF on the RSM diet program, while FG broilers had the highest on the control, P and FB diet programs. For both breeds, the lowest EPEF was achieved on the SBM-free diet program.

Table 3.9 *Performance results over the entire experimental period (0-38 days of age).*

Effect		Body weight d38 (g)	Daily gain (g/d)	Feed intake (g/d)	FCR	FCR2300[1]	Mortality (%)	EPEF [2]
Breed								
Slow-growing (SG)		1906^{b}	49.0^{b}	82.3^{b}	1.680^{a}	1.759^{a}	1.3^{b}	288^{b}
Fast-growing (FG)		2958^{a}	76.6^{a}	118.5^{a}	1.549^{b}	1.417^{b}	3.2^{a}	480^{a}
Diet								
Control diet		2461^{a}	63.6^{a}	100.8	1.601^{cd}	1.569^{bc}	1.7	395^{a}
Diet with peas		2440^{ab}	63.0^{ab}	100.3	1.611^{c}	1.583^{b}	1.4	390^{a}
Diet with field beans		2445^{ab}	63.1^{ab}	100.7	1.612^{bc}	1.583^{b}	2.3	387^{ab}
Diet with sunflower seed meal		2408^{bc}	62.2^{bc}	100.6	1.629^{ab}	1.607^{a}	2.3	374^{bc}
Diet with rapeseed meal		2454^{a}	63.4^{ab}	100.2	1.590^{d}	1.559^{c}	2.9	393^{a}
SBM-free diet		2382^{c}	61.5^{c}	100.1	1.643^{a}	1.626^{a}	2.9	367^{c}
Breed * Diet								
SG * Control		1914^{e}	49.2^{e}	82.5	1.675^{b}	1.752^{b}	1.2	291de
SG * Peas		1868^{e}	48.1^{e}	81.4	1.693^{ab}	1.780^{ab}	0.6	282^{e}
SG * Field beans		1917de	49.3^{de}	83.5	1.693^{ab}	1.769^{ab}	1.2	288de
SG * Sunflower seed meal		1881^{e}	48.4^{e}	81.3	1.679^{b}	1.763^{b}	0.6	287de
SG * Rapeseed meal		1978^{d}	50.9^{d}	82.9	1.628^{c}	1.693^{c}	2.3	306^{d}
SG * SBM-free		1876^{e}	48.2^{e}	82.5	1.711^{a}	1.796^{a}	2.3	275^{e}
FG * Control		3009^{a}	77.9^{a}	119.1	1.528^{f}	1.386^{g}	2.3	499^{a}
FG * Peas		3011^{a}	78.0^{a}	119.2	1.528^{f}	1.386^{g}	2.3	498^{a}
FG * Field beans		2973^{ab}	77.0^{ab}	117.8	1.532^{f}	1.397^{fg}	3.5	485^{a}
FG * Sunflower seed meal		2935^{bc}	76.0^{bc}	119.9	1.579^{d}	1.452^{de}	4.0	462^{bc}
FG * Rapeseed meal		2930^{bc}	75.8^{bc}	117.6	1.551^{ef}	1.425^{ef}	3.5	480^{ab}
FG * SBM-free		2888^{c}	74.7^{c}	117.6	1.574^{de}	1.456^{d}	3.5	459^{c}
Source of variation								
Breed	P-value	<0.001	<0.001	<0.001	<0.001	<0.001	0.029	<0.001
	SEM (n=36)	9.3	0.24	0.39	0.0036	0.0043	0.57	2.9
Diet	P-value	0.007	0.007	0.973	<0.001	<0.001	0.882	<0.001
	SEM (n=12)	16.1	0.42	0.67	0.0062	0.0075	1.00	5.0
Breed * Diet	P-value	0.003	0.003	0.144	<0.001	<0.001	0.955	0.018
	SEM (n=6)	22.7	0.60	0.95	0.0087	0.0105	1.41	7.1

$^{a-g}$ Means within a column and within a source without a common superscript differ significantly ($P < 0.05$).

SEM = Standard errors of means.

[1] FCR 2300g= FCR at a weight of 2300 gram. Correction = 0.02 / 100 g weight difference

[2] EPEF = European Production Efficiency Factor = (daily BWG (g)/FCR*10) x (100 - % mortality)

3.4 Animal welfare measurements

In Table 3.10 the effects of the dietary treatment on litter quality and footpad lesions are given. At all measuring days, the visual litter quality in the pens with SG broilers was better compared to the pens with FG broilers. The litter in the pens with SG broilers was more friable and less moist compared to the pens with FG broilers. As a result SG broilers had less footpad lesions and thus a lower footpad score (FPS) than FG broilers.

The scores for litter quality increases with the age of the broilers. Which means that the litter quality (both friability and wetness) decreased with age. In general, the litter was friable and relatively dry during the whole experimental period. Due to the good litter quality hardly any footpad lesions were observed. The footpad scores are in good agreement with the litter quality scores for both breeds. A higher score for litter quality, i.e. poorer litter quality, means a higher risk of developing footpad lesions.

At day 21, the litter in the pens with the SFM-fed broilers was visually the moistest, but the moisture score of the litter differed only significantly from the litter in the pens with the control- and FB-fed broilers not from the pens with the P, RSM and SBM-free fed broilers. Of the diets with regionally grown protein-rich ingredients, broilers fed FB diets had visually the driest litter at 21 days of age. At day 29, the litter moisture score was similar to that of day 21 of age, but unlike on day 21, there was a breed*diet interaction effect. In the SG broilers, the visual litter moisture score differ significantly between the control and the SBM-free diet program. In the FG broilers there were no differences in the litter moisture score between the diets with regionally grown protein-rich ingredients and the control diet. However, there was a difference in litter moisture score between the diets with regionally grown protein-rich ingredients at day 29: the SFM diet resulted in moister litter than the FB and control diets. On day 37, visual differences in litter moisture were no longer significant.

The differences in the litter friability score were mainly due to differences in friability in FG broilers, as there were no differences in litter friability score between SG pens on all observation days.
In general, the litter friability of the broiler pens fed with FB diets was better on all observation days compared to the control diet and all other diets with regionally grown protein-rich ingredients, The litter friability of the pens fed the other diets with the regionally grown protein sources did not differ from the control.

Overall, the highest footpad lesion score (FPS) was observed with the P diet. However, there was a breed*diet interaction effect. In the SG broilers, the FPS of the P fed broilers did not differ significantly from the control, but it did differ from the FPS of the birds fed with the other diet programs with regionally grown protein-rich ingredients. Compared to the control diet program, no diet program with regionally grown protein-rich ingredients had a significant impact on the FPS in SG broilers. In the FG broilers, on the other hand, the P-fed broilers also had the highest FPS, but the FPS differed significantly from the control here. Furthermore, the FPS of the broilers that received the control or RSM diet was significantly higher than the FPS of the broilers that received the FB, SFM or SBM-free diet.

Table 3.10 *Litter quality (at d21, d29 and d37) and footpad lesion scores (d37) of birds in the different experimental treatments.*

Effect		Litter moisture [1] d21	Litter friability [2] d21	Litter moisture d29	Litter friability d29	Litter moisture d37	Litter friability d37	FPS [3] d37
Breed								
Slow-growing (SG)		1.4^{b}	1.0^{b}	1.4^{b}	1.0^{b}	1.8^{b}	1.1^{b}	8.5^{b}
Fast-growing (FG)		2.4^{a}	1.8^{a}	2.4^{a}	1.9^{a}	2.7^{a}	3.0^{a}	43.8^{a}
Diet								
Control diet		1.7^{bc}	1.4^{a}	1.8^{bc}	1.6^{a}	2.2	2.2^{ab}	29.6^{b}
Diet with peas		2.0^{ab}	1.6^{a}	2.0^{ab}	1.6^{a}	2.6	2.6^{a}	80.0^{a}
Diet with field beans		1.6^{c}	1.0^{b}	1.6^{c}	1.0^{b}	2.0	1.3^{c}	2.1^{c}
Diet with sunflower seed meal		2.2^{a}	1.4^{a}	2.2^{a}	1.4^{ab}	2.3	2.1^{b}	7.1^{c}
Diet with rapeseed meal		2.0^{ab}	1.7^{a}	2.0^{ab}	1.7^{a}	2.4	2.2^{ab}	34.6^{b}
SBM-free diet		2.1^{a}	1.4^{a}	2.1^{ab}	1.4^{ab}	2.3	1.9^{b}	3.3^{c}
Breed * Diet								
SG * Control		1.0	1.0^{c}	1.0^{d}	1.0^{b}	1.8	1.0^{d}	10.0^{de}
SG * Peas		1.5	1.0^{c}	1.5^{cd}	1.0^{b}	1.8	1.5^{cd}	32.5^{cd}
SG * Field beans		1.0	1.0^{c}	1.0^{d}	1.0^{b}	1.5	1.0^{d}	0.0^{e}
SG * Sunflower seed meal		1.5	1.0^{c}	1.5^{cd}	1.0^{b}	1.8	1.0^{d}	1.7^{e}
SG * Rapeseed meal		1.5	1.0^{c}	1.5^{cd}	1.0^{b}	2.0	1.0^{d}	5.0^{e}
SG * SBM-free		2.0	1.0^{c}	2.0^{bc}	1.0^{b}	2.0	1.0^{d}	1.7^{e}
FG * Control		2.3	1.8^{b}	2.5^{ab}	2.2^{a}	2.5	3.3^{ab}	49.2^{bc}
FG * Peas		2.5	2.2^{ab}	2.5^{ab}	2.2^{a}	3.3	3.7^{a}	127.5^{a}
FG * Field beans		2.2	1.0^{c}	2.2^{b}	1.0^{b}	2.5	1.7^{c}	4.2^{e}
FG * Sunflower seed meal		2.8	1.8^{b}	2.8^{a}	1.8^{a}	2.7	3.2^{ab}	12.5^{de}
FG * Rapeseed meal		2.5	2.3^{a}	2.5^{ab}	2.3^{a}	2.8	3.3^{ab}	64.2^{b}
FG * SBM-free		2.2	1.8^{b}	2.2^{b}	1.8^{a}	2.5	2.8^{b}	5.0^{e}
Source of variation								
Breed	P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	SEM (n=36)	0.08	0.07	0.08	0.09	0.08	0.09	3.53
Diet	P-value	0.027	0.003	0.050	0.038	0.099	<0.001	<0.001
	SEM (n=12)	0.14	0.11	0.14	0.15	0.14	0.15	6.11
Breed * Diet	P-value	0.054	0.003	0.032	0.038	0.230	0.001	<0.001
	SEM (n=6)	0.20	0.16	0.20	0.21	0.20	0.21	8.64

[a-e] Means within a column and within a source without a common superscript differ significantly ($P < 0.05$). SEM = Standard errors of means.

[1] Litter moisture: scale 1 – 5 (dry litter – very wet litter).

[2] Litter friability: scale 1 – 5 (100% friable litter - complete caked litter).

[3] FPS = Footpad score (range 0 – 200).

3.5 Carbon footprint

The carbon footprint of the diets expressed per kg body gain over the experimental period is presented in Table 3.11. The inclusion or exclusion of Land Use Change (LUC) drastically affected the effects of dietary treatments on the carbon footprint. Partial or complete replacement of Brazilian SBM with more regionally grown ingredients reduced the carbon footprint per kg BW taking LUC into account. Without considering LUC, the carbon footprint per kg body weight only decreased when SBM was replaced with rapeseed meal, while complete replacement of Brazilian SBM with more regionally grown ingredients and partial replacement of Brazilian SBM with peas or field beans even increased the carbon footprint.

Table 3.11 *Influence of partial replacement of soybean meal with more regionally grown ingredients on the carbon footprint in g CO_2 eq. per kg of body weight gain of slow and fast-growing broilers, calculated with and without taking land use change (LUC) into account. CO_2 eq based on Nevedi (2022) version 5.*

Effect		CFP excl. LUC (g CO_2 eq./kg)	CFP incl. LUC (g CO_2 eq./kg)
Breed			
Slow-growing (SG)		1025[a]	1672[a]
Fast-growing (FG)		944[b]	1539[b]
Diet			
Control diet program		964[d]	2204[a]
Diet program with peas		1064[a]	1643[c]
Diet program with field beans		982[c]	1534[d]
Diet program with sunflower seed meal		961[d]	1369[e]
Diet program with rapeseed meal		923[e]	1773[b]
SBM-free diet program		1012[b]	1109[f]
Breed * Diet			
SG * Control		1009 [d]	2307 [a]
SG * Peas		1118 [a]	1728 [d]
SG * Field beans		1031 [c]	1611 [e]
SG * Sunflower seed meal		991 [e]	1414 [h]
SG * Rapeseed meal		946 [g]	1816 [c]
SG * SBM-free		1055 [b]	1155 [j]
FG * Control		920 [h]	2102 [b]
FG * Peas		1010 [d]	1558 [f]
FG * Field beans		933 [gh]	1456 [g]
FG * Sunflower seed meal		932 [gh]	1324 [i]
FG * Rapeseed meal		901 [i]	1730 [d]
FG * SBM-free		970 [f]	1062 [k]
Source of variation			
Breed	P-value	<0.001	<0.001
	SEM (n=36)	2.2	4.8
Diet	P-value	<0.001	<0.001
	SEM (n=12)	3.8	8.22
Breed * Diet	P-value	<0.001	<0.001
	SEM (n=6)	5.3	11.6

[a-k] Means within a column and within a source without a common superscript differ significantly ($P < 0.05$).

SEM = Standard errors of means.

In general, fast-growing broilers had a lower carbon footprint with and without LUC taking into account per kg body gain compared to SG broilers. However, there was an interaction effect with diet. Partial or complete replacement of SBM reduced the carbon footprint per kg body weight gain for both broiler types when LUC was taken into account. However, in the SG birds, the carbon footprint per kg body weight gain without LUC was only reduced when SBM was partially replaced with sunflower seed meal or rapeseed meal.

Feeding SG broilers diets in which SBM was partially replaced by peas or field beans or using SBM-free diets increased the carbon footprint without considering LUC. For the FG broilers the carbon footprint was only lower when soybean meal was partially replaced with rapeseed meal, the carbon footprint of the rest of the diet increased (i.e. peas or SBM-free) or was similar to the control diet program when LUC was not taken into account. It should be noted that the SG and FG broilers were not delivered at the same weight. If the SG broilers had been delivered at the same weight, the difference in FCR and therefore the CO_2 footprint would have been greater.

3.6 Nitrogen and phosphorus efficiency

Nitrogen (N) and Phosphorus (P) retention were calculated using reference values for whole body N and P content in day-old broiler chickens and broilers at slaughter (RVO, 2022).
Fast-growing broilers had higher N and P efficiency compared to SG broilers (Table 3.12). The N and P efficiency of the SG broilers was approximately 5% lower than that of the FG broiler.

Table 3.12 *Influence of partial replacement of soybean meal with more regionally grown ingredients on N and P efficiency of slow and fast-growing broilers from 0 – 38 days of age.*

Effect		**N-efficiency (%)[1]**	**P-efficiency (%)[1]**
Breed			
Slow-growing (SG)		59.9^{b}	62.4^{b}
Fast-growing (FG)		64.9^{a}	67.7^{a}
Diet			
Control diet program		63.2^{b}	72.7^{a}
Diet program with peas		64.5^{a}	70.8^{b}
Diet program with field beans		62.5^{c}	70.4^{b}
Diet program with sunflower seed meal		63.5^{b}	56.0^{e}
Diet program with rapeseed meal		58.9^{e}	61.4^{c}
SBM-free diet program		61.8^{d}	58.8^{d}
Breed * Diet			
SG * Control		60.4^{e}	69.5^{c}
SG * Peas		61.2^{de}	67.2^{d}
SG * Field beans		59.4^{f}	67.0^{d}
SG * Sunflower seed meal		61.5^{d}	54.3^{i}
SG * Rapeseed meal		57.5^{g}	60.0^{g}
SG * SBM-free		59.2^{f}	56.4^{i}
FG * Control		66.1^{b}	75.9^{a}
FG * Peas		67.7^{a}	74.5^{b}
FG * Field beans		65.6^{b}	73.9^{b}
FG * Sunflower seed meal		65.4^{b}	57.7^{h}
FG * Rapeseed meal		60.4^{e}	62.8^{e}
FG * SBM-free		64.3^{c}	61.2^{f}
Source of variation			
Breed	P-value	<0.001	<0.001
	SEM (n=36)	0.13	0.15
Diet	P-value	<0.001	<0.001
	SEM (n=12)	0.23	0.25
Breed * Diet	P-value	<0.001	<0.001
	SEM (n=6)	0.32	0.36

$^{a-i}$ Means within a column and within a source without a common superscript differ significantly ($P < 0.05$).
SEM = Standard errors of means.

A complete replacement of SBM with more regionally grown ingredients reduced N efficiency by 1.4% units and P efficiency by almost 14% units, however these effects were more pronounced in FG broilers.

Partial replacement of SBM with rapeseed meal or field beans led to a reduction in N efficiency by 4.3% and 0.7% units, respectively, while partial replacement with peas increased the N efficiency by 1.3% units. The increased N-efficiency with the pea diet was more pronounced with the FG broilers than with the SG broilers. Partial replacement of SBM with sunflower seed meal had no effect on N efficiency. Partial replacement of SBM with regionally grown ingredients led in all cases to a reduction in P efficiency ranging from 2 – 17% units.

4 Discussion

In the present study, the effects of partial or complete replacement of SBM with peas, field beans, sunflower seed meal and rapeseed meal in diets of fast- and slow-growing broilers were studied on the growth performance, litter quality, footpad lesions, carbon footprint of the diet and the calculated N and P efficiency of broilers. The study was performed with Ross 308 (fast growing) and Hubbard JA 757 (slow growing) broilers. Ross broilers need 37 days to reach a weight of 2.45 kg, whereas Hubbard broilers need 56 days to reach the same weight. Both genotypes were fed with the same diet program, which means that the concentrations of metabolizable energy (ME) and SID EAA of the diets were set at values which were in between requirement values as recommended for Ross 308 and Hubbard JA 757 broilers (Table 4.1). The values indicate that fast growing birds received diets with a slightly higher energy value than recommended and a 2 - 7% lower SID Lys content in the different phases compared to recommendation. Conversely, slow growing broilers received diets with a somewhat higher SID Lys content (varying from 5 to 11% over the different phases) than recommended and had a 0 – 2% lower energy value than recommendations. This compromise for the dietary nutrient composition was adopted to prevent differences in (circular) ingredient composition of the diets for both genotypes of birds. Furthermore, switches of diets to a next feeding phase occurred at the same age for both genotypes, so that the duration of provision of a given diet per phase was similar for both genotypes. This approach differed from the feeding strategy applied in practice for SG birds. Furthermore, the temperature schedule used, especially in the first days of life, was more tailored to the slower growing broilers than to the fast-growing broilers.

Table 4.1 *Dietary recommendations for fast- and slow-growing broilers and concentrations of dietary energy and SID Lys per phase as used in the present experiment.*

		Fast growing [1]	**Slow growing [2]**	**Current study**
Starter				
Phase		0-7 d	0-21 d	0-9 d
Energy (ME broiler)	Kcal/kg	2850	2900	2900
SID Lysine	g/kg	12.0	10.3	11.2
Grower 1				
Phase		7-21 d	0-21 d	9-21 d
Energy (ME broiler)	Kcal/kg	2925	2900	2950
SID Lysine	g/kg	11.0	10.3	10.4
Grower 2				
Phase		21-28 d	21-35 d	21-29 d
Energy (ME broiler)	Kcal/kg	2950	3000	2975
SID Lysine	g/kg	10.2	9.5	10.0
Finisher				
Phase		28-42 d	35-56 d	29-38 d
Energy (ME broiler)	Kcal/kg	3000	3100	3025
SID Lysine	g/kg	9.7	8.5	9.4

[1] Based on CVB recommendation, 2018

[2] Based in Hubbard JA757 recommendation, 2015

In line with expectation, partial replacement of Brazilian SBM with more regionally grown protein-rich ingredients resulted in a lower carbon footprint of the diet, but only when Land Use Change (LUC) was taken into account. This is mainly due to the high LUC value of Brazilian SBM. If we do not consider the effect of LUC or if we calculate with the LUC value of North American or European SBM instead of South American SBM, then replacing SBM with regionally grown protein-rich ingredients such as peas, field beans, sunflower seed meal or rapeseed meal has hardly any effect on the calculated carbon footprint of the diets.

This study showed that partial replacement of SBM by more regionally grown protein-rich ingredients is possible without compromising broiler performance. The response of the birds, however, differed between both genotypes. In fast-growing broilers feeding diets in which SBM was partially replaced with peas, field beans or rapeseed meal did not affect the EPEF value, compared to the control treatment in which the diet contained SBM as primary protein source. Partial replacement of SBM with SFM resulted in a poorer performance (lower BWG and higher FCR). In slow-growing broilers, partial replacement of SBM for any of the more regionally grown protein-rich ingredients did not lead to a decrease in EPEF compared to the control treatment. Partial replacement of SBM with RSM resulted in a higher BWG and a lower FCR, but the EPEF did not differ significantly from the control treatment because of a numerical higher mortality rate in the RSM treatment group. Complete replacement of SBM by alternative ingredients decreased growth performance in both fast and slow-growing broilers.

The results obtained with the pea and RSM diet programs in fast-growing broilers in this study were consistent with the study by Weindl et al. (2018). They studied the effect of the (partial) replacement of SBM with RSM (10 or 15%), peas (10 or 20%) or a combination of both in fast-growing broilers. They also observed a lower body weight at the end of the trial in the diets with 10 or 15% RSM and no effect of the pea diets on final body weight. Weindl et al. (2018) assumed that the lower body weight at the end of the study in the RSM-fed broilers were due to the anti-nutritive effects of glucosinolates in rapeseed products. In the present study, no glucosinolates were found in the used RSM batch. It is therefore not likely that the negative effect on the performance of the fast-growing broilers on the RSM diets was caused by the glucosinolate content of the diet. In addition to glucosinolates, sinapine, another anti-nutritional factor present in RSM could have played a role in the reduced performance results of the fast-growing broilers (Feng and Zuo, 2003). Sinapine is bitter and reduces the palatability, which can reduce feed intake. The feed intake of the fast-growing broilers was numerically not lower compared to the control diet program. The sinapine content in the batch of RSM used was not analysed. Given the numerically higher feed intake of the slower growing broilers on the RSM diet, the effects of sinapine from RSM can be assumed to be minor. It is not clear why a positive effect was found on BW and BWG in slow-growing broilers and a negative effect in fast-growing broilers. It is possible that slow-growing broilers are less sensitive to the anti-nutritional factors present in RSM. The decreased performance results in the present study are also consistent with results of Konieczka et al. (2021). They attributed the lower production results to the 15-20% inclusion of low glucosinolate rapeseed meal with high-moisture corn preserved with organic acids to a decrease in the production of short-chain fatty acids in the caecum. This was suggested to be indicative for a lower microbial activity and reduced intestinal functionality in the hindgut. In the current study, only the performance results of the fast-growing broilers on the rapeseed meal diet decreased, while the growth performance of the slow-growing broilers was increased. The lower performance in fast-growing broilers mainly occurred in the finisher phase (29-38 d). The former might suggest that intestinal functionality and health is more at risk in this phase due to the higher absolute feed intake in the finisher phase in fast-growing compared to slow growing birds.

As with RSM, slow- and fast-growing broilers responded differently to the SFM diet program. Fast-growing broilers had lower BW and BWG at day 38 compared to the control, while the final BW and BWG of the slow-growing broilers did not differ from the control. Furthermore, fast-growing broilers fed the SFM diet program had a higher FCR than the control, while the FCR of the slow-growing broilers did not differ from the control. The results in this study were not consistent with the results of Gerzilov and Petrov (2022). They found no differences in the performance of broilers with dietary inclusion levels up to 10, 20 and 23% of SFM in the starter (0-10 d), grower (11-24 d) and finisher (25-49 d) diets, respectively. The SFM inclusion levels in their diets were largely similar to the SFM levels in our study of 10, 25 and 20% in the starter (0-9 d), grower (10-29 d) and finisher (30-38 d) phase, respectively. Based on their study, Gerzilov and Petrov recommended a dietary SFM inclusion level during the starter period of up to 10%, for the grower period of up to 20% and for the finisher period of up to 23%. Furthermore, they reported that SFM could provide more than half of required crude protein in the diet during the grower and finisher periods. The conflicting results in the present study might be due to use of higher level of SFM in the grower phase. As SFM has a high concentration of fibre which might be the reason of negative effects on different parameters of growth performance in broilers. Most research in which no effects were observed on performance when SBM was partially replaced with SFM used inclusion levels in the diets of only up to 10 percent of SFM (Berwanger et al., 2017; Horvatovic et al.,2015; Yaqoob et al., 2022).

Differences in SFM inclusion levels may be a possible explanation for the variation in response observed among studies that evaluated the suitability of SFM as ingredient for broilers.
A problem associated with the use of high inclusion rates of more regionally grown protein-rich ingredients in broiler diets may be the high fibre content of the diet. Bulky, high fibre diets may be a problem for young broilers, in particular for SFM, because their digestive system has a limited physical capacity, and the digestive tract is not yet physiologically and functionally mature at this stage. The analysed crude fibre content of the SFM diets and the SBM-free diets are the highest (Table 4.1) compared with other experimental diets. Nevertheless, in the present study, the FI in the starter phase (0-9 d) on the SFM diet was not significantly lower than that on the control diet, while the FI of broilers fed the other diets with regionally grown protein-rich ingredients was significantly lower compared to the control treatment. In the starter phase, the growth of the broilers fed the diets with regionally grown protein-rich ingredients was significantly lower than the growth of the broilers fed the control diet, resulting in a higher FCR of the birds fed the diets with regionally grown protein-rich ingredients. Unexpectedly, it was observed that over the entire experimental period (0-38 d) feed intake did not differ among treatments despite the differences in crude fibre content of the feeding programs evaluated with values up to about 60 g/kg in the diets with SFM, RSM or a combination of more regionally grown protein-rich ingredients and values below 30 g/kg for the control diets (Table 4.1).

Table 4.1 *Crude fibre concentrations (g/kg) per feeding phase of the different diet programs.*

Phase	Period (days)	Control	Pea	Field beans	SFM	RSM	SBM-free
Starter	0 – 9	24	31	33	41	34	54
Grower I	9 – 21	25	37	49	59	41	63
Grower II	21 – 29	24	36	47	64	40	59
Finisher	29 - 38	25	37	46	52	41	49

The results obtained with the diet program in which SBM was partially replaced with peas were consistent with those of the study by Janocha et al. (2022). They studied the effect of the partial replacement of SBM with 10 or 15% peas from 0 - 21 days and 20 or 25% peas from 22 - 35 days of age in fast-growing broilers. They found that broilers fed the diets with the highest pea content had similar body weight and FCR at 35 days of age compared to the control. Broilers fed the diet program with the lower pea inclusion, 10 and 20% from 0 - 21 days and 22 - 35 days respectively, had an improved FCR and a comparable body weight relative to the control treatment. In a study by Fatica et al. (2022), no effect on feed consumption and growth performance was observed in medium-growing broiler chickens in which flaked soybeans (37% CP) were completely replaced by peas. This outcome is consistent with our research with both fast- and slow-growing broilers. In the past, inclusion of peas in the diet was restricted related to the presence of antinutritional factors such as trypsin inhibitors (TIA) and tannins in peas. Both anti-nutritional factors can decrease protein and amino acid digestibility in poultry and other species. However, plant breeding efforts over the past decades have reduced levels of ANFs in peas and other legume seeds and reduced the limitation of use as protein and energy source in the diets for poultry. The analysed values for TIA and tannins in peas and field beans seem relatively low but are difficult to judge without a proper reference and further details about the analytical techniques applied.

Partial replacement of SBM with peas resulted in a poorer litter quality and higher incidence of footpad lesions. Peas contain a relatively high concentration of NSP, including pectic polysaccharides. High levels of soluble NSP are associated with increased gut viscosity and decreased activity of digestive enzymes. Insoluble NSP may have a high water-binding capacity, which results in a faster gut transit time. The latter may result in wet dropping and deteriorated litter quality, which increases the risk of skin dermatitis as hock burn and footpad lesions. The results found in the present study regarding litter quality correspond to the results of Weindl et al. (2018). They found a higher water content in the excreta of pea-fed animals, while feeding groups with a high RSM content showed a comparable dry matter content in the excreta as the control group. The higher water content in excreta could be related to the moisture content of the litter and to the footpad score. Weindl et al. (2018) found a numerically higher footpad lesion score with the 10% pea diet, but the stocking density in their study was low (6 animals/m^2).

This study shows that replacing Brazilian SBM with local or more regionally grown protein ingredients can reduce the carbon footprint (incl. LUC) per kg BW by up to 50%, depending on the protein source used. The carbon footprint (incl. LUC) per kg BW of fast-growing broilers was lower than that of slow-growing broilers. The broiler breed, however, had a marginal influence on the carbon footprint per kg BW on the response of partial replacement of SBM by the regionally grown protein ingredients. However, the limited difference in carbon footprint is linked to the study design and less relevant for practical situation were both breeds are delivered at similar body weight but 14 days difference in slaughter age.
The higher carbon footprint per kg BWG found in slow-growing broilers in the present study is consistent with the research by Leinonen et al. (2012) and Tallentire et al. (2018). Leinonen et al. (2012) compared production systems with slow-growing broilers, i.e. free-range and organic systems, with the conventional production system with fast-growing broilers, whereas Tallentire et al. (2018) compared slow-growing broilers with fast-growing broilers. These authors attributed the higher carbon footprint to the higher feed consumption per bird per kg BW in these systems or per slow-growing bird compared to the conventional system with fast growing broiler.

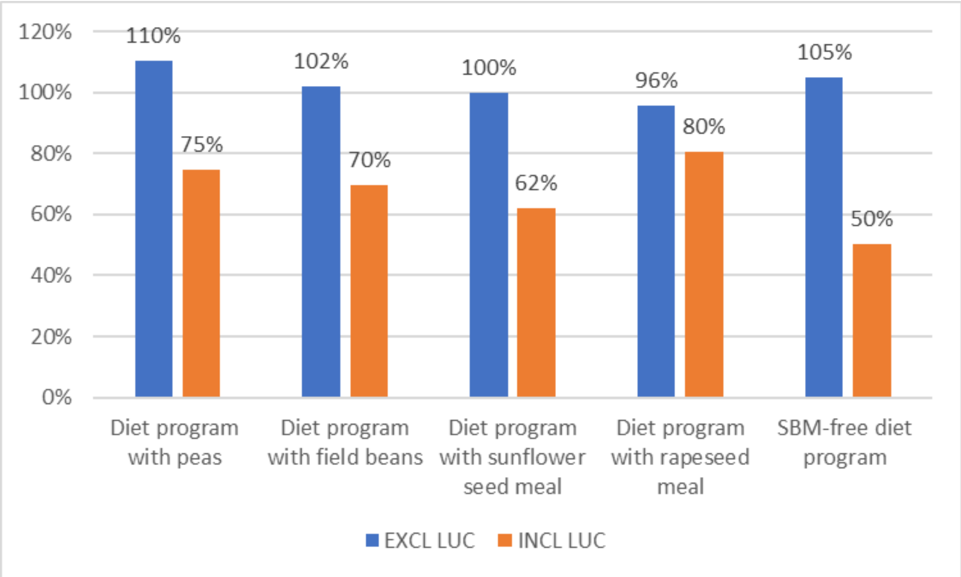

Figure 4.1 *Relative effect (Control diet program = 100%) of partial or complete replacement of Brazilian SBM with more regionally grown protein-rich ingredients on the carbon footprint per kg BWG, including and excluding LUC.*

In general, when LUC was taken into account the lowest carbon footprint per kg BWG was achieved with the SBM-free diet, followed by SFM, field beans, peas, RSM and control diet, respectively. Without LUC, the RSM diet program had the lowest carbon footprint per kg BWG, followed by SFM and the control diet (Figure 4.1). Without LUC taken into account, the peas diet had the highest carbon footprint per kg BWG. This shows the importance of including or excluding LUC emissions into the carbon footprint calculations and for achieving the CO_2-eq reduction targets. This is in-line with the results of Mostert et al. (2022) and Tallentire et al. (2018). Also, the origin of the SBM has an important effect on the calculated carbon footprint (Mostert et al., 2022). North-American SBM has a much lower carbon footprint value than SBM from South America.
It is shown that it is possible to reduce the carbon footprint of diets using more regionally grown protein-rich ingredients, but the origin of ingredients and whether or not including LUC related emissions from production of soybeans influences the outcome. Therefore origin and consideration of LUC must be taken into account when evaluating the environmental impact of diets for making broiler production more sustainable.

Partial replacement of relatively 11, 21, 25 and 13% of the SBM by peas, field beans, SFM or RSM, respectively, in diets of young broilers resulted in a lower BWG, which was caused by a lower FI. Partial replacement of SBM with peas or field beans also led to a lower feed efficiency compared to the control diet. From this it can be concluded that provision of diets with a high proportion of regionally grown protein-rich ingredients in the starter phase may not be recommended. In the present study, part of the SBM in the starter feed was replaced with 15% peas, 15% field beans, 10% SFM or 8% RSM. These inclusion levels of regionally grown protein-rich ingredients seem too high for young broilers.

Completely replacing SBM with these ingredients led to a further reduction of the performance results (BWG, FI and FCR). Therefore, complete replacement of SBM by more regionally grown protein sources in diets for young broilers is not recommended.

An interesting observation during the trial was a clear difference in litter colour, especially in the early stages of the trial, with very dark coloured litter in pens receiving the SFM diet. The darker colour of the litter and droppings are caused by the dark colour of the diet due to the inclusion of SFM.

Pellet quality could have played a role in the performance of the animals fed the diet programs with regionally grown protein-rich ingredients, since the best results were achieved with the RSM diet program. The pellets of this diet program had in all feeding phases a lower hardness and durability compared to the other diet programs with regionally grown protein-rich ingredients. The lower hardness and durability of the pellets may lead to a more rapid passage and digestion of nutrients in the intestinal tract and an improved synchronisation of nutrients in post-absorptive metabolism, which could explain the enhanced performance of the broilers fed the RSM diet program compared to the other diet programs with regionally grown protein-rich ingredients.

The use of peas, field beans, sunflower seed meal and rapeseed meal in broiler feeds is currently increasing, and knowledge about these protein sources in terms of chemical composition and nutritional value is also increasing. This indicates that we are increasingly able to formulate diets with a higher proportion of more regionally grown or sustainable ingredients, thereby reducing the ecological footprint of meat production. However, it is important to know that nutritional values depend on the type of broiler and therefore these ingredients must be tested in both slow- and fast-growing broilers.

The outcome of this study showed that the response on (partial) replacement of SBM with more regionally grown protein-rich ingredients depends on the genotype or breed of the broilers involved and must be further investigated. However, it is unknown whether the interaction effect between feeding program and breed observed in the present study would also have been found if the management and diet composition had been breed specific. In the present study both types of broilers were housed in the same room, therefore compromises were made regarding management (e.g. temperature and light schedule) and nutrient composition of the diets relative to the assumed nutrient requirements of the birds. The nutritional value of the diets for the critical nutrients energy and SID EAA were set at values which were in between the breeder nutrient recommendations for FG and SG broilers. This means that the SG broilers were fed above the breeder's recommendation and therefore might have received a somewhat luxurious diet, with higher levels of SID Lys and other essential amino acids. The FG, on the other hand, received diets with lower SID essential amino acid levels than actually necessary for maximum growth or feed conversion (Table 4.1). It is therefore not inconceivable that SG broilers react differently than the FG broilers on the diets with regionally grown protein-rich ingredients, because of a relative oversupply of nutrients. For example, it is known that body weight gain and feed conversion of ratio broilers improve further with an increasing content digestible lysine content beyond current recommendations (Kidd et al., 1998; Kerr et al., 1999; Van Harn et al, 2022). This may negate in SG broilers the possible negative effects of regionally grown raw materials on growth performance. In relation to this, it could be mentioned that the actual nutritional value of various feed ingredients classified as circular ingredients might be different from the nutritional value adopted during feed formulation related to their higher inclusion level in circular diets compared to more regular diets. Furthermore, the duration and age of switching to the next feeding phase did not correspond to regular practice. This was especially the case for the SG birds. In addition, the trial ended at 38 days of age for both FG and SG broilers. This is a normal slaughter age for FG broilers, but not for SG broilers. Slow growing broilers are normally slaughtered at an age of 49 – 56 days and a BW of 2.45 kg. It cannot be ruled out that the response to partial or entire replacement of SBM with more regionally grown feed ingredients would have been different if SG broilers had been kept to the same body weight as the FG broilers and the different feeding phases had been more in line with practice.
Complete replacement of SBM with more regionally grown protein-rich ingredients resulted in lower performance results (3,3% lower BWG, 3.6% increased FCR). However, these negative effects of a SBM-free diet on the performance results were more pronounced in the FG than SG animals.

It is not clear why results are reduced in the SBM-free diet program, as the different phase diets were iso-energetic and were formulated to be equal in the concentrations of the most limiting ileal digestible essential amino acids compared to the control diet. There could be a relative shortage of specific or total non-essential amino acids, as precursors for the synthesis of other non-essential amino acids, in the SBM-free feeding program, although evaluation of their concentrations in the diets did not reveal considerable differences between treatments. Experiences in previous digestibility studies with broilers, however, showed that omitting SBM in purified diets reduced feed intake in broilers largely and induced wet/watery droppings and a growth depression (unpublished results). Initially it was thought that this was related to a lack of fibre in purified diets, however, additionally providing oat hulls did not offer relief. In contrast, supplementing the diets with 10% SBM solved the problem. This suggests that SBM contains substances that are necessary for the bird to properly digest the diet (e.g. presence of fermentable oligosaccharides) or grow and develop normally (e.g. isoflavones). The former, however, requires further investigation, and might also explain the reduced performance results with the SFM diet program, as diets in this treatment had low inclusion levels of SBM (<6% in the grower and finisher diets) compared to the other diets programs with regionally grown protein-rich ingredients in which SBM was partially replaced by more regionally grown protein-rich ingredients.

5 Conclusions

From this study in which the effects of partial or complete replacement of soybean meal by peas, field beans, sunflower seed meal or rapeseed meal on the performance results of fast and slow-growing broilers was studied can be concluded that:

- Partial replacement of SBM in diets with regionally grown protein-rich ingredients (peas, field beans, or rapeseed meal) is possible without compromising the overall growth performance (EPEF) of fast and slow-growing broilers.
- Partial replacement of SBM with SFM resulted in impaired performance. This effect was more pronounced in fast-growing broilers.
- Complete replacement of SBM with more regionally grown protein-rich ingredients in broiler diets resulted in deteriorated performance results in both fast and slow-growing broilers.
- Partial replacement of SBM with peas resulted in more severe footpad lesions compared to the control diet program. This effect was more pronounced in fast-growing broilers.
- Complete replacement of SBM and partial replacement of SBM with FB or SFM resulted in fewer footpad lesions compared to the control diet program.
- Partial replacement of SBM with RSM had no effect on the incidence of footpad lesions compared to the control diet program.
- Complete replacement of SBM from Brazilian origin reduced the carbon footprint (incl. LUC) of the feed with approximately 50%. Partial replacement of Brazilian SBM with peas, field beans, SFM or RSM reduced the carbon footprint (incl. LUC) of the feed with 10-40 percent. Without taking the LUC into account, there were no differences in carbon footprint between the feeds.
- Fast-growing broilers had a lower carbon footprint with and without taking into account LUC and expressed per kg body gain compared to slow-growing broilers.
- Use of more regionally grown protein-rich ingredients reduced the carbon footprint (incl. LUC) per kg BWG. Complete replacement of (Brazilian) SBM reduced the carbon footprint (incl. LUC) per kg BWG with almost 50%.
- The carbon footprint (excl. LUC) per kg BWG was only reduced when SBM was replaced with rapeseed meal. Complete replacement of Brazilian SBM with regionally grown protein-rich ingredients even increased the carbon footprint (excl. LUC) per kg BWG.
- Partial or complete replacement of SBM with regionally grown protein-rich ingredients reduced P efficiency in both fast and slow-growing broilers.
- The highest N-efficiency was observed in the pea diet program, but this was more pronounced in fast-growing broilers than in slow-growing broilers. The lowest N efficiency was observed with the RSM diet program for both fast and slow-growing broilers.
- The P efficiency of all diet programs with regionally grown protein-rich ingredients was lower compared to the control diet program. The SFM diet program had the lowest P efficiency.

Conclusions per diet program with regionally grown protein-rich ingredients:

- Partial replacement of SBM with peas resulted to similar performance to the control diet in both breeds but increased the footpad lesion score.
- Partial replacement of SBM with field beans resulted in similar performance results to the control diet in both breeds and had a positive effect on litter quality and the footpad lesion score.
- Partial replacement of SBM with sunflower seed meal resulted in impaired growth and FCR compared to the control diet, but this was only the case in FG broilers. In SG broilers, partial replacement of SBM with SFM had no effect on growth and FCR compared to the control diet.
- Partial replacement of SBM with rapeseed meal in SG broilers improved growth and FCR and in FG broilers it caused impaired growth and FCR at 2300g.

- Complete replacement of SBM with more regionally grown ingredients led to impaired performance (growth and FCR) compared to the control diet, but this effect was more pronounced in FG broilers. In SG broilers the SBM-free diet resulted in SG broilers in comparable growth and an impaired FCR.

This research showed that slow-growing broilers can handle more regionally grown ingredients better than fast-growing ones. In the slow-growing broilers, partial or complete replacement of SBM had no effect on the overall production performance results expressed in the EPEF, while in the fast-growing broilers only partial replacement of SBM with peas, field beans and rapeseed meal showed comparable overall production performance to the control diet. In the fast-growing broilers, both a partial replacement of SBM with sunflower seed meal and the complete replacement of SBM with more regionally grown ingredients resulted in a reduced production performance. Although the EPEF was not significantly affected in fast-growing broilers, partial replacement of SBM with RSM resulted in a reduced growth and BW. This study also showed that it is possible to reduce the carbon footprint of diets using more regionally grown ingredients, but including or excluding LUC emissions from soybean production influences the outcome.

Recommendation

The use of peas, field beans, sunflower seed meal, rapeseed meal and other more circular or regionally grown ingredients in broiler feeds is currently increasing. Knowledge about the use of these protein sources at higher inclusion levels is expanding but requires further attention. Specific focus on the nutritional value of these ingredients at higher inclusion levels, the presence of pro- and antinutritional factors and higher concentrations of soluble (fermentable) and/or insoluble carbohydrates in some of these ingredients which might influence feed intake and the digestive process.

This means that we are increasingly able to formulate diets with a higher proportion of circular or sustainable ingredients, which reduces the ecological footprint of meat production. However, it is important to know that nutritional value of ingredients and diets may be influenced by the genotype of broiler. These ingredients should be evaluated in more detail in both slow- and fast-growing broilers.

In the present study the same diets for both genotype of birds were formulated based on "average" nutrient requirement values provided by the breeder recommendations of slow-growing and fast-growing broilers. It is recommended to tailor the nutritional values of the diets to the type of broiler to evaluate the response of the bird to a circular ingredient or diet that is nutritionally tailored to the type of broiler.

In addition, it is recommended to investigate why SBM might be a necessary ingredient in a broiler diet.

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Appendix 1 Lay-out experimental room

18 RC	19 HB		54 RC	55 HE
17 RF	20 HF		53 RD	56 HB
16 RB	21 HD		52 RE	57 HA
15 RA	22 HA		51 RF	58 HC
14 RE	23 HE		50 RA	59 HD
13 RD	24 HC		49 RB	60 HF
12 HA	25 RF		48 HE	61 RF
11 HE	26 RE		47 HB	62 RA
10 HD	27 RC		46 HD	63 RD
9 HC	28 RA		45 HC	64 RB
8 HB	29 RD		44 HA	65 RC
7 HF	30 RB		43 HF	66 RE
6 RF	31 HB		42 RC	67 HA
5 RC	32 HF		41 RA	68 HB
4 RB	33 HA		40 RE	69 HF
3 RD	34 HD		39 RB	70 HD
2 RE	35 HC		38 RD	71 HE
1 RA	36 HE		37 RF	72 HC

Front

1,2,3, ...,72: pen number.
Different colours indicated blocks.
First letter represents the breed (H= Hubbard JA757 and R= Ross 308).
Second letter represents dietary treatment / diet program.

A	Control diet (SBM as main protein source)
B	Diet program in which SBM was partly replaced with peas (P)
C	Diet program in which SBM was partly replaced with field beans (FB)
D	Diet program in which SBM was partly replaced with sunflower seed meal (SFM)
E	Diet program in which SBM was partly replaced with rapeseed meal (RSM)
F	SBM-free diet program. SBM replaced with P, FB, SFM and RSM

Appendix 2 Chemical composition of the main diet ingredients

	Method	Unit	Wheat	Maize	Soybean meal	Peas	Field beans	Sunflower seed meal	Rapeseed meal
Dry matter	Vocht 103 ºC	g/k**g**	856.3	863.9	884.4	884.7	869.6	898.9	897.5
Moisture	Vocht 103 ºC	g/kg	143.7	136.1	115.6	115.3	130.4	101.1	102.5
Ash	As 550 °C	g/kg	14.8	11.3	61.2	29.4	38.2	64.3	67.6
Crude fibre	Fibertec	g/kg	24.1	22.3	38.2	54.4	147.9	168.9	115.0
Crude fat	Na zure hydrolyse	g/kg	24.1	39.0	25.2	20.1	17.4	20.7	33.7
Crude protein	Kjeldahl	g/kg	111.7	64.9	488.3	217.0	192.0	371.9	385.2
Saccharose	NIR-analyse	g/kg	14.8	17.6	98.6	36.1	--	73.9	98.1
Starch Ewers	NIR-analyse	g/kg	607.3	657.3	46.0	470.4	--	39.2	--
Starch Brunt	Zetmeel (Brunt)	g/kg	575.4	648.1	3.4	412.4	298.6	1.4	3.8
ADF	NIR-analyse	g/kg	27.8	26.3	58.2	--	--	198.2	198.3
Nitrogen Soluble Index	NSI NIR	%			13.3				
Anti trypsin factor		g/kg				1.340	0.880		
Tannin		g/kg				4.8	13.7		
Glucosinolates	Glucosinolate NIR	mg/kg							0.0

Appendix 3 Starter and Grower I diets

Phase		Starter						Grower I					
Feed Code		**1A**	**1B**	**1C**	**1D**	**1E**	**1F**	**2A**	**2B**	**2C**	**2D**	**2E**	**2F**
Feed ingredients													
Wheat	%	32.673	20.972	23.195	27.542	26.989	20.000	41.618	20.000	20.000	28.579	28.828	20.000
Corn	%	30.000	30.001	30.001	30.000	30.000	15.000	25.000	17.494	23.383	25.000	25.000	15.000
Soybean meal 48 NGMO	%	25.341	22.486	19.878	19.092	22.109		21.745	11.459	9.880	6.167	14.472	
Peas	%		15.000				20.637		40.000				13.354
Field beans RE26	%			15.000			15.000			34.998			14.000
Sunflower seed meal Hipro 36	%				10.000		8.226				25.000		11.815
Rapeseed meal 00 RE34	%					8.000	5.000					18.000	11.000
Oat	%	3.500	3.500	3.500	3.500	3.500	3.500	3.500	3.500	3.500	3.500	3.500	3.500
Corn gluten meal RE60	%						2.551						
Poultry fat	%	2.799	2.580	2.844	4.312	4.004	4.338	3.239	2.896	3.557	7.000	5.951	6.945
Premix VLK 0-20 days	%	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000
Chalk fine <.15mm	%	1.294	1.295	1.309	1.267	1.188	1.267	0.995	1.010	1.028	0.926	0.756	0.840
Monocalcium phosphate	%	0.625	0.616	0.610	0.581	0.566	0.547	0.174	0.156	0.129	0.065	0.042	0.024
Sodium bicarbonate	%	0.245	0.198	0.216	0.290	0.221	0.298	0.293	0.204	0.208	0.431	0.239	0.277
Sodium chloride	%	0.157	0.188	0.175	0.125	0.163	0.103	0.125	0.182	0.181	0.026	0.139	0.117
L-Lysine HCl	%	0.346	0.231	0.287	0.418	0.321	0.402	0.346	0.113	0.181	0.526	0.289	0.300
DL-Methionine	%	0.290	0.312	0.330	0.248	0.254	0.307	0.251	0.325	0.339	0.148	0.172	0.221
L-Threonine	%	0.134	0.118	0.131	0.131	0.113	0.166	0.126	0.117	0.108	0.118	0.078	0.104
Tryptophane	%		0.004	0.010			0.043		0.025	0.024			0.008
Valine	%	0.089	0.079	0.090	0.069	0.071	0.120	0.076	0.087	0.064	0.028	0.036	0.050
L-Isoleucine	%			0.004			0.075		0.012		0.066		0.025
L-Arginine	%	0.087			0.005	0.081		0.092				0.078	
Xylanase 6.25%	%	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Phytase 5000L	%	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020
Maxiban 0.3%	%	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300
Nutrients													
AMEn broiler (2014)	kcal/kg	2900	2900	2900	2900	2900	2900	2950	2950	2950	2950	2950	2950
Crude protein	g/kg	195.9	197.0	197.7	197.3	199.7	194.2	184.5	182.5	193.0	189.4	192.9	191.4
Crude fat	g/kg	56.1	53.6	55.9	70.7	69.7	69.9	59.8	54.1	61.1	95.9	90.3	95.5
Crude fibre	g/kg	27.4	31.9	35.0	41.6	35.0	56.9	27.3	39.3	45.2	62.9	44.4	65.4
Crude ash	g/kg	61.1	61.8	60.8	62.3	62.4	60.4	52.9	53.7	52.5	56.0	55.9	55.8
Starch Brunt	g/kg	400.1	397.6	399.5	370.9	367.8	377.4	419.5	420.0	410.8	345.2	346.7	341.2
Calcium	g/kg	8.8	8.8	8.8	8.8	8.8	8.8	6.8	6.8	6.8	6.8	6.8	6.8
Phosphorus	g/kg	4.9	4.9	4.9	5.4	5.2	5.4	3.8	3.7	3.9	5.1	4.5	4.8
Sodium	g/kg	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4
Chloride	g/kg	2.2	2.2	2.2	2.2	2.2	2.2	2.0	2.0	2.0	2.0	2.0	2.0
Potassium	g/kg	8.4	8.7	8.4	8.3	8.4	7.3	7.7	8.2	8.0	7.5	7.7	7.6
6-Phytase E4a1640	ftu	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
Dig. Lysine	g/kg	11.2	11.2	11.2	11.2	11.2	11.2	10.4	10.4	10.4	10.4	10.4	10.4
Dig. Methionine	g/kg	5.5	5.6	5.6	5.4	5.3	5.6	4.9	5.2	5.3	4.9	4.5	4.9
Dig. Met+Cys	g/kg	8.2	8.2	8.2	8.2	8.2	8.2	7.6	7.6	7.6	7.6	7.6	7.6
Dig. Threonine	g/kg	7.2	7.2	7.2	7.2	7.2	7.2	6.7	6.7	6.7	6.7	6.7	6.7
Dig. Tryptophan	g/kg	2.1	2.0	2.0	2.1	2.1	2.0	2.0	1.9	1.9	2.1	2.1	1.9
Dig. Isoleucine	g/kg	7.1	7.2	7.1	7.1	7.1	7.1	6.6	6.6	6.6	7.2	6.6	6.6
Dig. Arginine	g/kg	12.0	12.0	12.0	12.0	12.0	12.0	11.1	11.9	12.5	12.3	11.1	12.3
Dig. Valine	g/kg	8.6	8.6	8.6	8.6	8.6	8.6	8.0	8.0	8.0	8.0	8.0	8.0
Dig. Gly+Ser	g/kg	15.0	15.2	15.0	14.9	15.1	14.0	14.1	14.0	14.5	14.0	14.4	14.0
Retainable P broiler	g/kg	4.0	4.0	4.0	4.0	4.0	4.0	3.1	3.1	3.1	3.1	3.1	3.1

Appendix 4 Grower II and Finisher diets

Phase		Grower II						Finisher					
Test Feed Code		**3A**	**3B**	**3C**	**3D**	**3E**	**3F**	**4A**	**4B**	**4C**	**4D**	**4E**	**4F**
Feed ingredients													
Wheat	%	43.177	20.000	20.511	31.514	30.388	20.000	45.001	20.000	22.968	35.658	32.209	23.417
Corn	%	25.000	21.446	25.000	25.000	25.000	24.999	25.000	23.795	25.000	25.000	25.000	25.000
Soybean meal 48 NGMO	%	20.161	7.766	7.845	3.078	12.889		18.029	5.275	5.093	4.476	10.757	
Peas	%		39.999				12.100		40.001				11.895
Field beans RE26	%			35.000			11.274			35.000			10.000
Sunflower seed meal Hipro 36	%				25.000		9.000				20.000		8.000
Rapeseed meal 00 RE34	%					18.000	9.000					18.000	8.000
Oat	%	3.500	3.500	3.500	3.500	3.500	3.500	3.500	3.500	3.500	3.500	3.500	3.500
Corn gluten meal RE60	%												
Poultry fat	%	3.440	2.568	3.591	7.000	6.151	5.506	3.949	2.862	4.026	6.875	6.661	5.783
Premix VLK 0-20 days	%	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000
Chalk fine <.15mm	%	0.924	0.945	0.958	0.860	0.685	0.808	1.047	1.068	1.083	0.996	0.808	0.944
Monocalcium phosphate	%	0.140	0.148	0.102	0.044	0.008	0.053	0.153	0.169	0.120	0.075	0.021	0.074
Sodium bicarbonate	%	0.238	0.199	0.162	0.446	0.184	0.350	0.297	0.243	0.211	0.477	0.242	0.404
Sodium chloride	%	0.137	0.159	0.187		0.151	0.043	0.097	0.130	0.154		0.112	0.008
L-Lysine HCl	%	0.344	0.175	0.192	0.568	0.287	0.376	0.337	0.181	0.204	0.513	0.280	0.354
DL-Methionine	%	0.234	0.326	0.326	0.238	0.155	0.238	0.210	0.306	0.306	0.135	0.131	0.215
L-Threonine	%	0.121	0.140	0.109	0.133	0.073	0.138	0.115	0.139	0.111	0.123	0.068	0.127
Tryptophane	%		0.037	0.027			0.023		0.041	0.031			0.020
Valine	%	0.072	0.120	0.068	0.049	0.031	0.099	0.064	0.119	0.070	0.043	0.024	0.086
L-Isoleucine	%		0.052	0.002	0.150		0.073		0.061	0.013	0.019		0.063
L-Arginine	%	0.092				0.078		0.091				0.077	
Xylanase 6.25%	%	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Phytase 5000L	%	0.020	0.020	0.020	0.020	0.020	0.020	0.010	0.010	0.010	0.010	0.010	0.010
Salinomycine 0.3%	%	0.300	0.300	0.300	0.300	0.300	0.300						
Nutrients													
AMEn broiler (2014)	kcal/kg	2975	2975	2975	2975	2975	2975	3025	3025	3025	3025	3025	3025
Crude protein	g/kg	178.1	168.4	184.7	179.2	186.5	172.9	169.4	157.8	174.1	169.4	177.8	165.1
Crude fat	g/kg	61.5	51.2	61.4	95.6	92.0	82.5	66.5	54.5	65.7	94.6	97.0	85.1
Crude fibre	g/kg	27.0	38.5	44.7	62.4	44.1	57.2	26.6	38.0	44.3	55.0	43.7	53.8
Crude ash	g/kg	51.8	51.8	51.2	54.4	54.8	52.9	49.2	49.1	48.4	51.5	52.3	50.3
Starch Brunt	g/kg	428.3	444.8	423.8	361.8	355.6	388.1	438.6	459.5	437.8	385.4	365.9	402.1
Calcium	g/kg	6.6	6.6	6.6	6.6	6.6	6.6	5.9	5.9	5.9	5.9	5.9	5.9
Phosphorus	g/kg	3.7	3.6	3.7	4.9	4.3	4.4	3.6	3.5	3.7	4.6	4.3	4.3
Sodium	g/kg	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.4	1.3	1.3
Chloride	g/kg	2.0	2.0	2.0	2.0	2.0	1.7	1.8	1.9	1.9	1.6	1.8	1.4
Potassium	g/kg	7.4	7.5	7.6	6.9	7.4	6.8	7.0	7.0	7.1	6.6	7.0	6.5
6-Phytase E4a1640	ftu	1000	1000	1000	1000	1000	1000	500	500	500	500	500	500
Dig. Lysine	g/kg	10.0	10.0	10.0	10.0	10.0	10.0	9.4	9.4	9.4	9.4	9.4	9.4
Dig. Methionine	g/kg	4.7	5.1	5.1	5.6	4.3	4.8	4.3	4.7	4.8	4.3	3.9	4.4
Dig. Met+Cys	g/kg	7.3	7.3	7.3	7.3	7.3	7.3	6.9	6.9	6.9	6.9	6.9	6.9
Dig. Threonine	g/kg	6.4	6.4	6.4	6.4	6.4	6.4	6.0	6.0	6.0	6.0	6.0	6.0
Dig. Tryptophan	g/kg	1.9	1.8	1.8	1.9	2.0	1.8	1.8	1.7	1.7	1.8	1.9	1.7
Dig. Isoleucine	g/kg	6.3	6.3	6.3	7.4	6.3	6.3	5.9	5.9	5.9	5.9	5.9	5.9
Dig. Arginine	g/kg	10.7	10.8	11.9	11.4	10.7	10.7	10.1	10.1	11.1	10.5	10.1	10.1
Dig. Valine	g/kg	7.7	7.7	7.7	7.7	7.7	7.7	7.2	7.2	7.2	7.2	7.2	7.2
Dig. Gly+Ser	g/kg	13.6	12.8	13.9	13.1	13.9	12.6	12.9	11.9	13.0	12.5	13.2	12.0
Retainable P broiler	g/kg	3.0	3.0	3.0	3.0	3.0	3.0	2.7	2.7	2.7	2.7	2.7	2.7

Appendix 5 Percentage SBM replaced

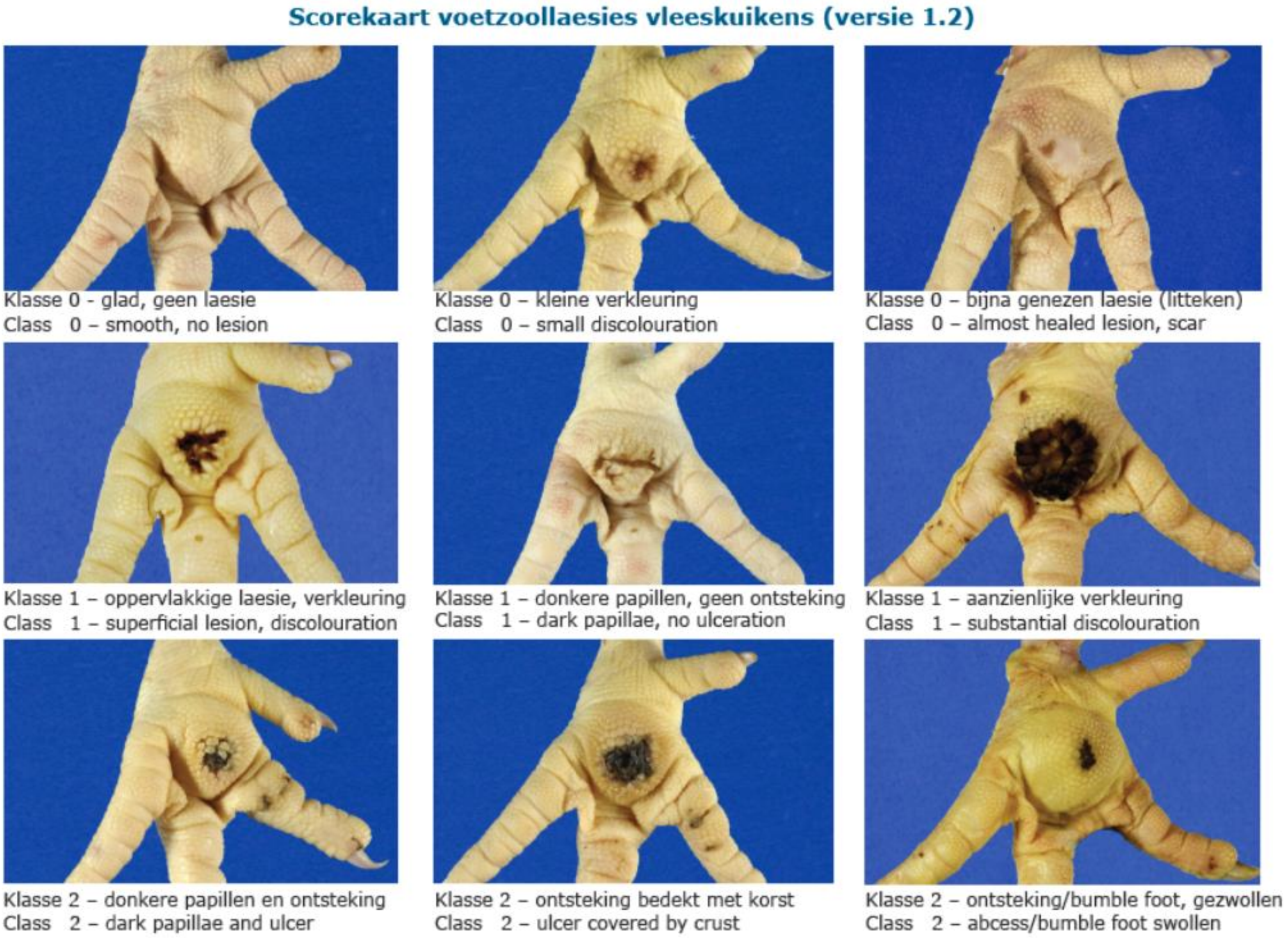
Table A5.1 *Percentage of SBM that has been replaced with more regionally grown protein sources.*

Diet		**% SBM in diet**				**% replaced with more regionally grown protein source**			
		Starter	**Grower**	**Grower II**	**Finisher**	**Starter**	**Grower**	**Grower II**	**Finisher**
A	Control	25.3	21.7	20.2	18				
B	Peas	22.5	11.5	7.8	5.3	11%	47%	61%	71%
C	Field beans	19.9	9.9	7.8	5.1	21%	54%	61%	72%
D	SFM	19.1	6.2	3.1	4.5	25%	71%	85%	75%
E	RSM	22.1	14.5	12.9	10.8	13%	33%	36%	40%

Table A5.2 *Percentage of protein-rich ingredients per feeding phase of the different diet programs.*

Diet		**% protein-rich ingredients**				**% protein-rich ingredients in SBM-free (Diet program F)**			
		Starter	**Grower I**	**Grower II**	**Finisher**	**Starter**	**Grower I**	**Grower II**	**Finisher**
A	Control/SBM	25.3	21.7	20.2	18.0	--	--	--	--
B	Peas	15.0	40.0	40.0	40.0	20.6	13.4	12.1	11.9
C	Field beans	15.0	35.0	35.0	35.0	15.0	14.0	11.3	10.0
D	SFM	10.0	25.0	25.0	20.0	8.2	11.8	9.0	8.0
E	RSM	8.0	18.0	18.0	18.0	5.0	11.0	9.0	8.0

Appendix 6 Overview of scores for footpad lesions



Appendix 7 Carbon footprint of feed and ingredients

Starter diets (0 – 9 days)

Test Feed Code		1A	1B	1C	1D	1E	1F	CFP ingredient [1]	
Feed ingredients								Excl. LUC	Incl. LUC
Wheat	%	32.673	20.972	23.195	27.542	26.989	20.000	499	513
Corn	%	30.000	30.001	30.001	30.000	30.000	15.000	514	576
Soybean meal CP48	%	25.341	22.486	19.878	19.092	22.109		617	4325
Peas	%		15.000				20.637	697	744
Field beans CP26	%			15.000			15.000	602	656
Sunflower seed meal Hipro	%				10.000		8.226	504	558
Rapeseed meal 00 CP34	%					8.000	5.000	460	555
Oat	%	3.500	3.500	3.500	3.500	3.500	3.500	866	873
Corn gluten meal CP60	%						2.551	744	856
Poultry fat	%	2.799	2.580	2.844	4.312	4.004	4.338	673	944
Premix	%	2.000	2.000	2.000	2.000	2.000	2.000	1114	1115
Chalk fine <.15mm	%	1.294	1.295	1.309	1.267	1.188	1.267	518	518
Monocalcium phosphate	%	0.625	0.616	0.610	0.581	0.566	0.547	598	599
Sodium bicarbonate	%	0.245	0.198	0.216	0.290	0.221	0.298	1284	1285
Sodium chloride	%	0.157	0.188	0.175	0.125	0.163	0.103	302	303
L-Lysine HCl	%	0.346	0.231	0.287	0.418	0.321	0.402	3155	3157
DL-Methionine	%	0.290	0.312	0.330	0.248	0.254	0.307	2584	2584
L-Threonine	%	0.134	0.118	0.131	0.131	0.113	0.166	3858	3862
L-Tryptophane	%		0.004	0.010			0.043	18455	18481
L-Valine	%	0.089	0.079	0.090	0.069	0.071	0.120	8896	8902
L-Isoleucine	%			0.004			0.075	18455	18481
L-Arginine	%	0.087			0.005	0.081		18455	18481
Xylanase 6.25%	%	0.100	0.100	0.100	0.100	0.100	0.100	1114	1115
Phytase 5000L	%	0.020	0.020	0.020	0.020	0.020	0.020	1114	1115
Maxiban 0.3%	%	0.300	0.300	0.300	0.300	0.300	0.300	1114	1115
Salinomycine 0.3%	%							1114	1115
CFP incl. LUC	g CO_2 eq/kg	1582	1488	1381	1340	1462	708		
CFP excl. LUC	g CO_2 eq/kg	611	618	606	592	601	654		

[1] CO_2 eq based on Nevedi (2022) version 5.

Grower 1 diets (9 – 21 days)

Test Feed Code		2A	2B	2C	2D	2E	2F	CFP ingredient [1]	
Feed ingredients								Excl. LUC	Incl. LUC
Wheat	%	41.618	20.000	20.000	28.579	28.828	20.000	499	513
Corn	%	25.000	17.494	23.383	25.000	25.000	15.000	514	576
Soybean meal CP48	%	21.745	11.459	9.880	6.167	14.472		617	4325
Peas	%		40.000				13.354	697	744
Field beans CP26	%			34.998			14.000	602	656
Sunflower seed meal Hipro	%				25.000		11.815	504	558
Rapeseed meal 00 CP34	%					18.000	11.000	460	555
Oat	%	3.500	3.500	3.500	3.500	3.500	3.500	866	873
Corn gluten meal CP60	%							744	856
Poultry fat	%	3.239	2.896	3.557	7.000	5.951	6.945	673	944
Premix	%	2.000	2.000	2.000	2.000	2.000	2.000	1114	1115
Chalk fine <.15mm	%	0.995	1.010	1.028	0.926	0.756	0.840	518	518
Monocalcium phosphate	%	0.174	0.156	0.129	0.065	0.042	0.024	598	599
Sodium bicarbonate	%	0.293	0.204	0.208	0.431	0.239	0.277	1284	1285
Sodium chloride	%	0.125	0.182	0.181	0.026	0.139	0.117	302	303
L-Lysine HCl	%	0.346	0.113	0.181	0.526	0.289	0.300	3155	3157
DL-Methionine	%	0.251	0.325	0.339	0.148	0.172	0.221	2584	2584
L-Threonine	%	0.126	0.117	0.108	0.118	0.078	0.104	3858	3862
L-Tryptophane	%		0.025	0.024			0.008	18455	18481
L-Valine	%	0.076	0.087	0.064	0.028	0.036	0.050	8896	8902
L-Isoleucine	%		0.012		0.066		0.025	18455	18481
L-Arginine	%	0.092				0.078		18455	18481
Xylanase 6.25%	%	0.100	0.100	0.100	0.100	0.100	0.100	1114	1115
Phytase 5000L	%	0.020	0.020	0.020	0.020	0.020	0.020	1114	1115
Maxiban 0.3%	%	0.300	0.300	0.300	0.300	0.300	0.300	1114	1115
Salinomycine 0.3%	%							1114	1115
CFP incl. LUC	g CO_2 eq/kg	1442	1122	1023	871	1173	668		
CFP excl. LUC	g CO_2 eq/kg	606	656	611	590	584	607		

[1] CO_2 eq based on Nevedi (2022) version 5.

Grower 2 diets (21 – 29 days)

Test Feed Code		3A	3B	3C	3D	3E	3F	CFP ingredient [1]	
Feed ingredients								Excl. LUC	Incl. LUC
Wheat	%	43.177	20.000	20.511	31.514	30.388	20.000	499	513
Corn	%	25.000	21.446	25.000	25.000	25.000	24.999	514	576
Soybean meal CP48	%	20.161	7.766	7.845	3.078	12.889		617	4325
Peas	%		39.999				12.100	697	744
Field beans CP26	%			35.000			11.274	602	656
Sunflower seed meal Hipro	%				25.000		9.000	504	558
Rapeseed meal 00 CP34	%					18.000	9.000	460	555
Oat	%	3.500	3.500	3.500	3.500	3.500	3.500	866	873
Corn gluten meal CP60	%							744	856
Poultry fat	%	3.440	2.568	3.591	7.000	6.151	5.506	673	944
Premix	%	2.000	2.000	2.000	2.000	2.000	2.000	1114	1115
Chalk fine <.15mm	%	0.924	0.945	0.958	0.860	0.685	0.808	518	518
Monocalcium phosphate	%	0.140	0.148	0.102	0.044	0.008	0.053	598	599
Sodium bicarbonate	%	0.238	0.199	0.162	0.446	0.184	0.350	1284	1285
Sodium chloride	%	0.137	0.159	0.187		0.151	0.043	302	303
L-Lysine HCl	%	0.344	0.175	0.192	0.568	0.287	0.376	3155	3157
DL-Methionine	%	0.234	0.326	0.326	0.238	0.155	0.238	2584	2584
L-Threonine	%	0.121	0.140	0.109	0.133	0.073	0.138	3858	3862
L-Tryptophane	%		0.037	0.027			0.023	18455	18481
L-Valine	%	0.072	0.120	0.068	0.049	0.031	0.099	8896	8902
L-Isoleucine	%		0.052	0.002	0.150		0.073	18455	18481
L-Arginine	%	0.092				0.078		18455	18481
Xylanase 6.25%	%	0.100	0.100	0.100	0.100	0.100	0.100	1114	1115
Phytase 5000L	%	0.020	0.020	0.020	0.020	0.020	0.020	1114	1115
Maxiban 0.3%	%							1114	1115
Salinomycine 0.3%	%	0.300	0.300	0.300	0.300	0.300	0.300	1114	1115
CFP incl. LUC	g CO_2 eq/kg	1382	997	948	774	1112	679		
CFP excl. LUC	g CO_2 eq/kg	603	667	610	607	581	621		

[1] CO_2 eq based on Nevedi (2022) version 5.

Finisher diets (29 – 38 days)

Test Feed Code		4A	4B	4C	4D	4E	4F	CFP ingredient [1]	
Feed ingredients								Excl. LUC	Incl. LUC
Wheat	%	45.001	20.000	22.968	35.658	32.209	23.417	499	513
Corn	%	25.000	23.795	25.000	25.000	25.000	25.000	514	576
Soybean meal CP48	%	18.029	5.275	5.093	4.476	10.757		617	4325
Peas	%		40.001				11.895	697	744
Field beans CP26	%			35.000			10.000	602	656
Sunflower seed meal Hipro	%				20.000		8.000	504	558
Rapeseed meal 00 CP34	%					18.000	8.000	460	555
Oat	%	3.500	3.500	3.500	3.500	3.500	3.500	866	873
Corn gluten meal CP60	%							744	856
Poultry fat	%	3.949	2.862	4.026	6.875	6.661	5.783	673	944
Premix	%	2.000	2.000	2.000	2.000	2.000	2.000	1114	1115
Chalk fine <.15mm	%	1.047	1.068	1.083	0.996	0.808	0.944	518	518
Monocalcium phosphate	%	0.153	0.169	0.120	0.075	0.021	0.074	598	599
Sodium bicarbonate	%	0.297	0.243	0.211	0.477	0.242	0.404	1284	1285
Sodium chloride	%	0.097	0.130	0.154		0.112	0.008	302	303
L-Lysine HCl	%	0.337	0.181	0.204	0.513	0.280	0.354	3155	3157
DL-Methionine	%	0.210	0.306	0.306	0.135	0.131	0.215	2584	2584
L-Threonine	%	0.115	0.139	0.111	0.123	0.068	0.127	3858	3862
L-Tryptophane	%		0.041	0.031			0.020	18455	18481
L-Valine	%	0.064	0.119	0.070	0.043	0.024	0.086	8896	8902
L-Isoleucine	%		0.061	0.013	0.019		0.063	18455	18481
L-Arginine	%	0.091				0.077		18455	18481
Xylanase 6.25%	%	0.100	0.100	0.100	0.100	0.100	0.100	1114	1115
Phytase 5000L	%	0.010	0.010	0.010	0.010	0.010	0.010	1114	1115
Maxiban 0.3%	%							1114	1115
Salinomycine 0.3%	%							1114	1115
CFP incl. LUC	g CO_2 eq/kg	1299	905	846	795	1030	671		
CFP excl. LUC	g CO_2 eq/kg	598	665	609	578	576	613		

[1] CO_2 eq based on Nevedi (2022) version 5.

Table A7.1 *Absolute (g CO_2 eq/kg) and relative (%) effects of (partial) replacement of SBM with more regionally grown feed ingredients on carbon footprint (incl. LUC) – Source: Nevedi CFP table, 2022.*

		Starter		Grower1		Grower2		Finisher	
		Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.
A	Control diet	1582	100%	1442	100%	1382	100%	1299	100%
B	Diet program with peas	1488	94%	1122	78%	997	72%	905	70%
C	Diet program with field beans	1381	87%	1023	71%	948	69%	846	65%
D	Diet program with sunflower seed meal	1340	85%	871	60%	774	56%	795	61%
E	Diet program with rapeseed meal	1462	92%	1173	81%	1112	81%	1030	79%
F	SBM-free diet program	708	45%	668	46%	679	49%	671	52%

Table A7.2 *Absolute (g CO_2 eq/kg) and relative (%) effects of (partial) replacement of SBM with more regionally grown feed ingredients on carbon footprint (excl. LUC) – Source: Nevedi CFP table, 2022.*

		Starter		Grower1		Grower2		Finisher	
		Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.
A	Control diet	611	100%	606	100%	603	100%	598	100%
B	Diet program with peas	618	101%	656	108%	667	111%	665	111%
C	Diet program with field beans	606	99%	611	101%	610	101%	609	102%
D	Diet program with sunflower seed meal	592	97%	590	97%	607	101%	578	97%
E	Diet program with rapeseed meal	601	98%	584	96%	581	96%	576	96%
F	SBM-free diet program	654	107%	607	100%	621	103%	613	103%

Appendix 8 Microbiome composition-Galleon©

Effects of the dietary treatments on the microbiome composition of excreta of fast and slower growing broilers at day 7 and 21 of age

The intestinal microbiome plays a crucial role in chicken health and production performance. Different external and host factors impact the gut microbiome, which could also affect performance and health status. The interaction between environmental and host factors and its impact on the chickens' gut microbiome is complex. Indicatively, there are more than 109 bacteria per g of ileal digesta and more than 1011 bacteria per g of caecal digesta. Analysis of the gut microbiome by molecular techniques has identified bacterial populations of over 600 species from more than 100 genera. Furthermore the relationship and interactions between bacterial species and between bacteria and other factors are complex in nature. Galleon© is a practical non-invasive microbiota analysis platform to obtain insight in the microbiome composition in excreta or digesta from the digestive tract of broilers. Chicken cloaca samples are collected via swabs and the microbiome composition is quantified using a microarray chip to obtain selective information on the microbiome composition. Data are analysed using statistics and non-linear AI models.

With the combination of analytical and statistical technologies, Galleon© provides different types of outcomes. First a Principal Component Analysis (PCA) showing vectors that represent the entire microbiome. The closer the vectors are plotted in the graphs, the more similar the microbiota is. A second outcome is a Heat Map in which the composition of the microbiome is grouped based on similarity horizontally and where effects of dietary treatments are grouped based on similarity as vertical depicted clusters. Heat Maps also show relative abundances of bacterial species via differences in colour intensity in the plots. Radar Plots provide insights into the ten most important differences in microbiome composition (biomarkers) between experimental treatments and Volcano Plots show significant differences in absolute presence of bacteria between experimental treatments.

In the present trial on 7 and 21 days of age (DOA) from each treatment and bird breed cloacal swabs of 2 birds per pen (24 birds per treatment) were collected and analysed according to Cargill's Galleon© protocol. The microarray generated fluorescence readings passed data quality control and were standardized prior to further analysis. Relative intensity for each bacterial DNA probe was submitted to an ANOVA analysis with the fixed effect of dietary protein source (diet), breed, age and their interaction with "chip" as a random effect. Pairwise comparisons between standardized LS means were made for each bacterial species and variable combination adjusting for FDR (false discovery rate) test, using P=0.05 as value for statistical discrimination between treatments. When necessary, the P-value cut-off was lowered to observe tendencies.

The PCA (Figure A8.1) showed a clear effect of age of the birds (d 7 vs d 21 DOA) and an unusual relatively high spread at 7 DOA. At 7 DOA, for both breeds on the control diet, vectors were close together and were significantly different from those of the SBM-free (BLEND) treatment, which were also close for both breeds. At day 21, diet had a greater impact on the microbiota composition than the breed.

Heat Maps results (relative abundance; Figure A8.2) showed that three major vertical groups could be identified. The first group consists of the dietary treatments with field beans (HBEAN), rapeseed meal and the SBM-free (BLEND) treatment over ages and breeds. This group had a higher signal for cluster 1 bacteria (in blue). The second cluster is made of samples from the different treatments obtained on d 7 and is characterized by higher signals for lactate producing bacteria (Lactobacillus and Streptococcus). At day 7, the fast-growing chickens fed the SBM-free diet showed a unique profile high in cluster 1 bacteria. The third group consists of samples over treatments (pea, sunflower and soybean meal for both breeds) obtained at d 21 and has lower signals for cluster 1 and higher signals for bacterial clusters 5, 6 and 7 (in orange). These clusters include L. crispatus and Ruminococcus.

Considering the heat map for the pea and control treatments only (Figure A8.3), we see a clear separation by age, showing a switch from Lactobacillus in cluster 3 (blue) to Ruminococcus, Lachnospiraceae and Faecalibacterium (cluster 4), C. bartletti and Peptostreptococcus (cluster 5), and Corybacterium and S. alactolyticus (cluster 6). Based on expertise from Cargill, this shift indicates a proper development of the intestinal microbiome in birds on the pea and control treatment. Although growth performance did not differ between these treatments, feeding a pea-based diet was associated with significantly more foot pad lesions (FPL) for both breeds. It needs to be further investigated whether the observed effect on FPL is related to a higher signal for Streptococcus probes in cluster 6 (black box in Figure A8.3).

When comparing the cloaca microbiome of the rapeseed meal and control treatments (Figure A8.4), there is a clear clustering of age of the birds (d 7 and 21) with several unique clusters representing samples obtained at 21 DOA over dietary treatments. The signal for cluster 2, which includes Lactobacilli, is higher for the control treatment at 7 DOA (blue box). At 21 DOA fast-growing birds (Ross 308) on the control diet had more Ruminococcus and Lachnospiraceae in cluster 5 (red box) but when fed a rapeseed meal-based diet there was relatively higher abundance of Bacteroides and Lactobacilli (cluster 4 in orange box). A relatively high abundance of Bacteroides in this treatment could be related to the presence of more complex carbohydrates in rapeseed meal. Based on Cargill's experience, it is suggested that a delay in maturation of the microbiome is observed for the rapeseed meal compared to control treatment. Practical experience with data from the Galleon tool© by Cargill suggests that a slower maturation of the microbiome is associated with a lower growth performance.

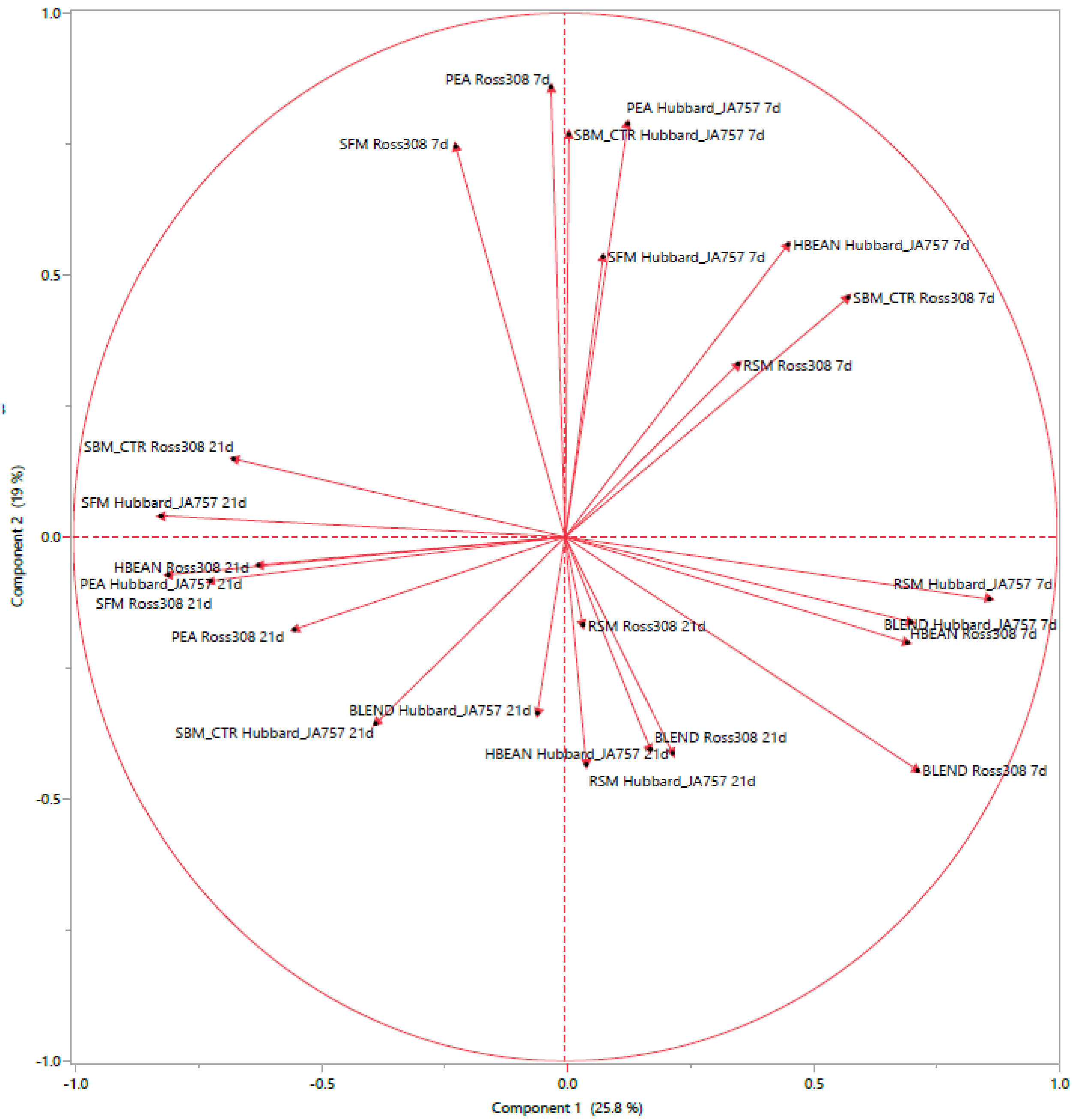


Figure A8.1 *Principal Component Analysis of the microbiome composition of cloaca swaps obtained at d 7 and 21 of age of two breeds of broilers fed different experimental diets.*

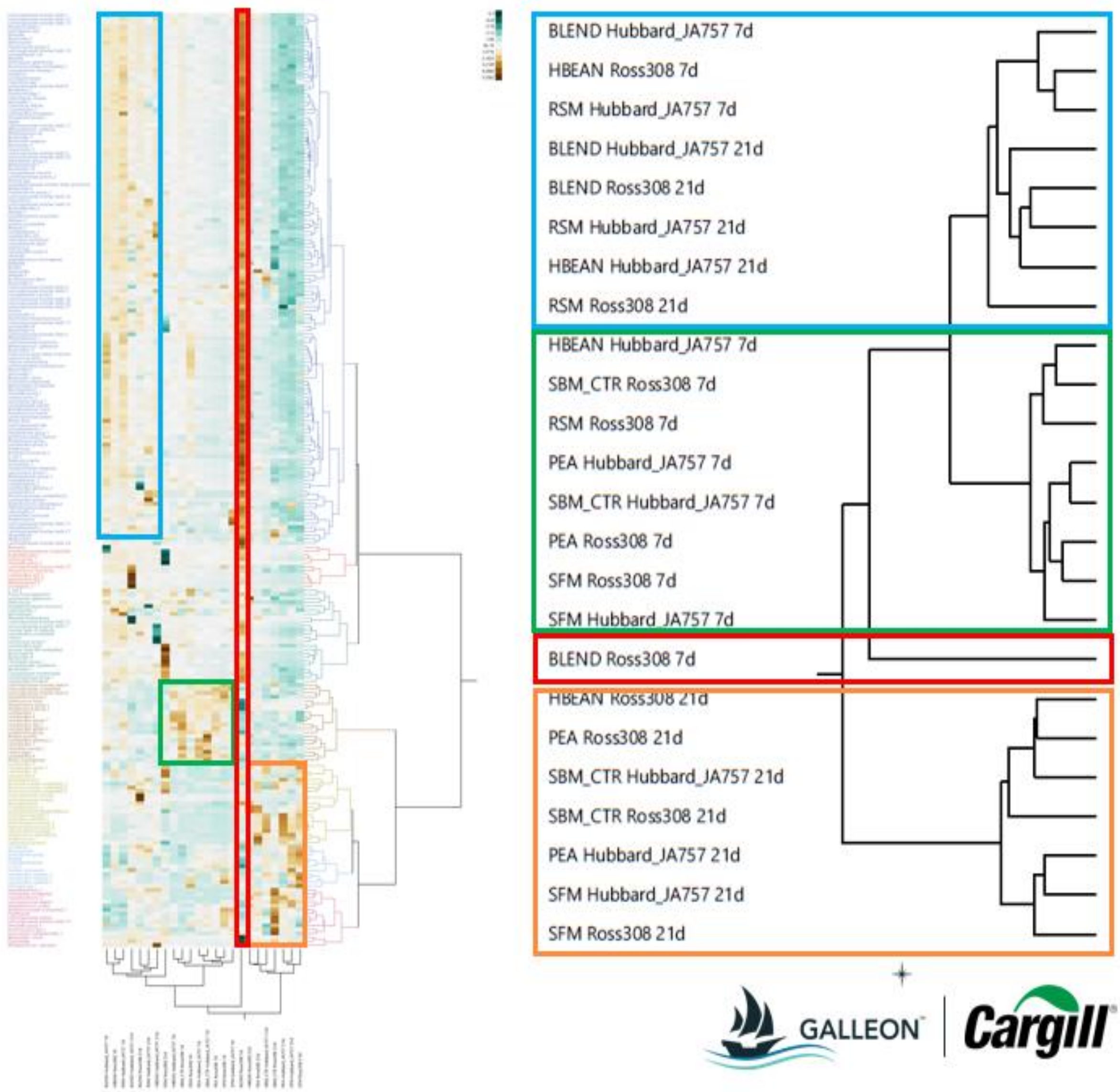


Figure A8.2 *Heat map of the microbiome composition of cloaca swaps obtained at d 7 and 21 of age of two breeds of broilers fed different experimental diets.*

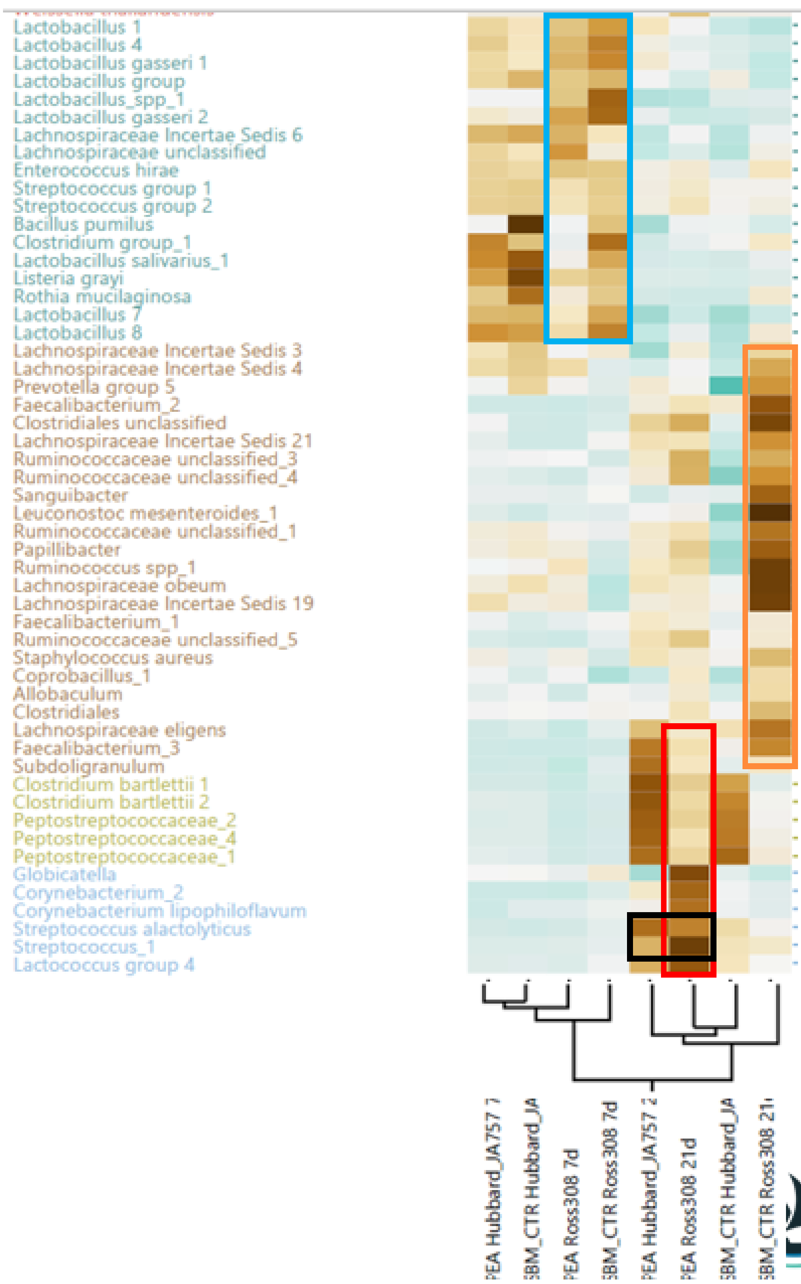


Figure A8.3 *Heat map of the microbiome composition of cloaca swaps obtained at d 7 and 21 of age of two breeds of broilers fed the control or peas diet program.*

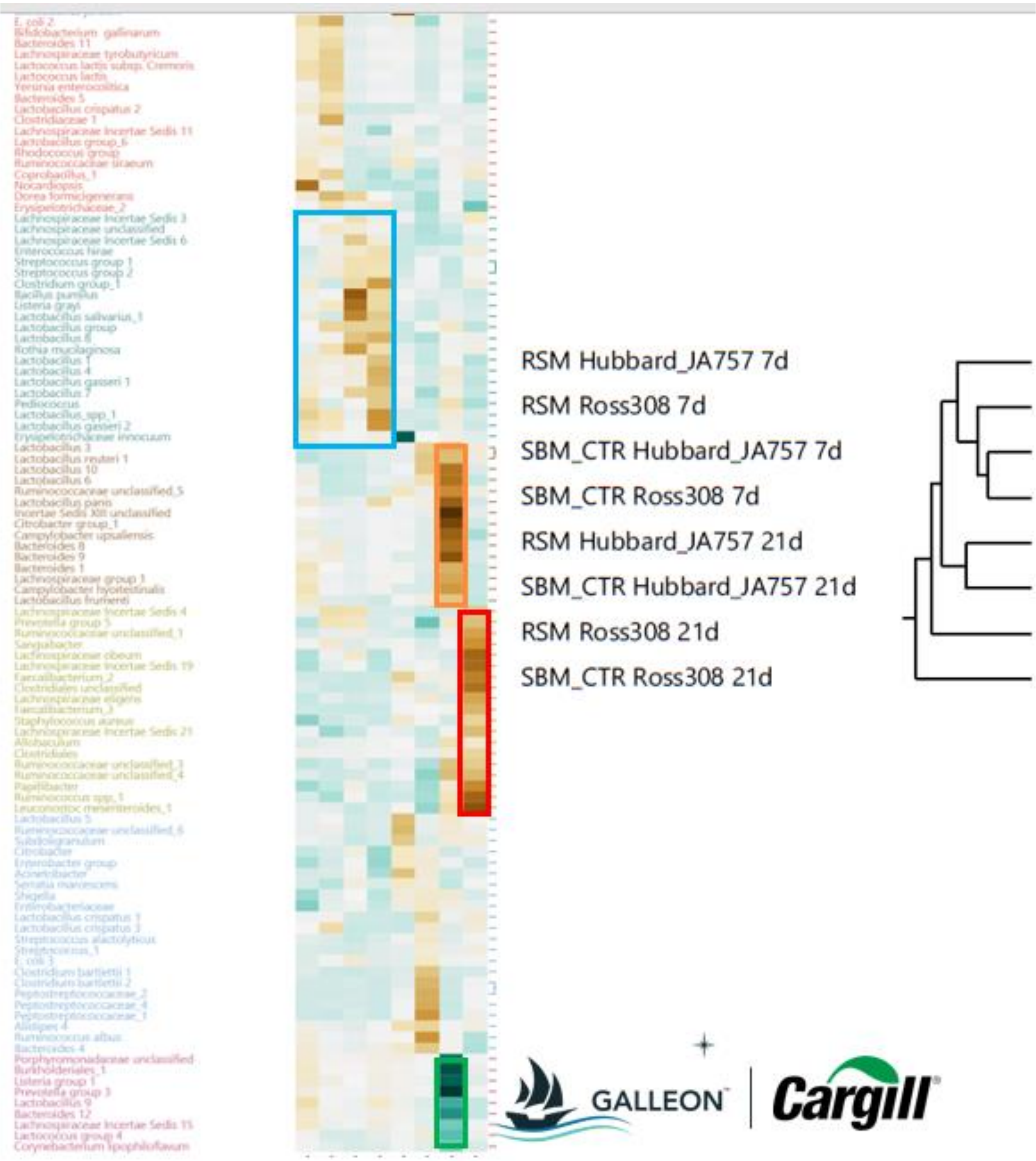


Figure A8.4 *Heat map of the microbiome composition of cloaca swaps obtained at d 7 and 21 of age of two breeds of broilers fed the control or rapeseed meal diet program.*

To explore the potential of nature to improve the quality of life

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