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by Pirgozliev, V.R., Whiting, I.M., Mansbridge, S. and Rose, S.P.

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Sunflower and rapeseed meal as alternative feed materials to soybean meal for sustainable egg production, using aged laying hens

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ABSTRACT

1. This study assessed the impact of replacing two thirds of a soybean meal (SBM) based diet with an alternative protein (AP) based diet (sunflower and rapeseed meal), when fed to end of production Hy-Line Brown laying hens.
2. Diets were fed in seven cages, each containing six birds aged from 75 to 83 weeks old. Measured variables included bird performance, egg production, quality variables of fresh eggs, total tract digestibility of dry matter (DMD), neutral detergent fibre (NDFD), fat (FD), and nitrogen corrected apparent metabolisable energy (AMEn).
3. No significant differences were observed ($P > 0.05$) for egg production, egg composition or egg quality. However, birds fed the AP diet had darker yolks ($P < 0.05$), possibly linked to natural pigments in AP.
4. The SBM diet had higher AMEn, DMD and NDFD ($P < 0.05$), although FD was higher in the AP diet ($P < 0.05$).
5. Diets based on AP sources can be fed to aged laying hens without deteriorating productive performance, providing care is taken in formulating diets (e.g. amino acid levels, balance etc). Using AP sources may reduce reliance on SBM associated with land use change, contributing towards sustainability for the egg industry.

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Alternative protein; poultry; sustainability; bird performance; egg quality

Introduction

Egg production worldwide exceeded 86.67 million metric tons in 2020, up from 74.14 million metric tons in 2016. Since 1990, the global egg production volume has increased by over 100% (Statista 2020). Poultry products, including eggs, play a major role in human nutrition, being relatively affordable, widely available, unaffected by religious restrictions and with high nutritional value (FAO 2013). Environmentally, poultry products are recognised as being relatively efficient with regards to land usage and with a minimal carbon footprint among the main livestock production chains (de Vries and de Boer 2010; Roma et al. 2015). In addition, consumers are influenced by different egg production systems and price point when purchasing fresh eggs (Preisinger 2018). With the increase of the world population, demand for eggs will continue to rise, therefore continuous improvement to the sustainability of egg production systems is vital (Bain et al. 2016; Costantini et al. 2021).

Meeting consumer demands for sustainable egg production systems is challenging in several ways, including the relatively high cost of soy bean meal (SBM) used traditionally as the main protein source in poultry diets. The use of imported SBM in poultry diets poses problems both economically and environmentally. Economically, the cost of SBM has continued to increase over the last few decades, making SBM one of the most expensive components in typical non-ruminant diets (IDH 2021). Environmentally, soy bean production is associated with deforestation (change of land use) resulting in a negative impact on the

environment (Grossi et al. 2022). This emphasises the need for more sustainable feed ingredients, thus strengthening the requirement to develop locally produced alternative protein (AP) sources for modern poultry production (Abdulla et al. 2016; Whiting et al. 2017). Locally grown protein sources may also be more popular, further enhancing market price and farm competitiveness (Acciani et al. 2021).

Sunflower (SFM) and rapeseed (RSM) meal are popular AP sources in poultry feeds. Sunflower meal has an established nutrient profile and is a relatively inexpensive ingredient, which can be formulated into laying hen diets as an AP source to SBM (Koçer et al. 2021). However, the use of SFM in poultry diets can be limited by variations in its chemical composition with the two main components restricting its use: namely high fibre and low lysine (Nolte et al. 2021; Saleh et al. 2021). Rapeseed meal is also used in animal nutrition as an economic AP source to SBM (Oryschak et al. 2020; Panaite et al. 2020). However, RSM inclusion rates are usually limited in poultry diets due to antinutritional factors, including glycosylates, erucic acid, sinapine and tannins (Newkirk 2009; Perez-Maldonado et al. 2003). Despite the limitations, both these AP sources can be useful ingredients in low soya diets. Both, SFM and RSM are rich in phytonutrients, many of which have biological properties, including antioxidant and immune modulatory capabilities (Panaite et al. 2020; Saleh et al. 2021). In addition, most of the published work evaluated dietary AP sources at relatively low inclusion, as SBM still comprises a significant part of dietary protein (Koçer et al. 2021; Nolte et al. 2021; Saleh et al. 2021).

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Most published studies on laying hens and AP sources have been conducted with birds up to 52 weeks of age (Koçer et al. 2021; Nolte et al. 2021; Wang et al. 2017), 68 weeks old layers (Kuźniacka et al. 2020; Saleh et al. 2021) and up to 78 weeks of age (Star et al. 2020). However, there is lack of published information conducted in elderly laying hens (beyond 80 weeks of age) when fed residuals from oil crops as AP sources. Laying hens are extensively reared around the world, and according to breeders (Hy-Line, Studley, UK), some modern hybrids, e.g., Hy-Line Brown, can be productive up to 100 weeks of age (<https://www.hyline.com/varieties/brown>). Longer use of laying hens can potentially mitigate harm to the environment, improve profitability for producers and in general, be more sustainable. However, it is well known that egg production reduces in high performance laying hens with age, and diets must be reformulated accordingly (Hy-Line, Studley, UK). Research is therefore needed in this extended production phase (80–100 weeks) to optimise dietary formulations (Bain et al. 2016).

Albumen quality is an important indicator for egg freshness. In healthy flocks, bird age is the most important factor affecting the albumen quality of freshly laid eggs (Samli et al. 2005). Bird genetics and production environment are major factors affecting egg quality (Washburn 1979), as nutritional factors have a minor effect. Samli et al. (2005) suggested that albumen quality might be related to dietary protein source. However, there is a lack of information on feeding AP sources to laying hens towards the end of the normal production age and the impact of these AP sources on egg production and quality, dietary available energy and nutrient digestibility. Therefore, the primary objectives of the current study were to determine the effects of partial replacement of dietary SBM with RSM and SFM on the performance of Hy Line Brown laying hens between 75 and 83 weeks of age.

Materials and methods

The study was performed at the National Institute of Poultry Husbandry (NIPH) as preliminary agreed with the Research Ethics Committee of Harper Adams University (UK). This work complies with the ARRIVE 2.0 guidelines (Percie du Sert et al. 2020).

Dietary formulation

Two experimental diets were formulated by Target Feeds Limited (Whitchurch, UK), following UK commercial standards to meet or exceed breeder recommendations (Hy-Line International, Studley, UK). Diet A (soy-based control) was prepared using 175 g/kg SBM and 50 g/kg full fat soya as the main protein sources, and contained 11.56 MJ/kg AME and 172 g/kg crude protein. Diet B (alternative protein; AP) was prepared using 70 g/kg RSM, 75 g/kg SFM and 75 g/kg SBM and contained 11.53 MJ/kg AME and 158 g/kg crude protein. The diets were fed as mash for eight weeks, between 75 and 83 weeks of age and did not contain additional feed additives apart of those shown in Table 1.

Experimental design

The study involved 84 Hy Line Brown laying hens kept in 14 enriched colony cages (Hellmann Poultry GmbH & Co. KG), with six birds in each, from 75 to 83 weeks of age. After

Table 1. Dietary composition (g/kg).

Ingredients	A ²	B ³
Barley	100	160.1
Wheat	535.0	430.0
Rapeseed meal	-	70.0
Sunflower meal	-	75.0
Soya meal	175.0	75.0
Full fat soya	50.0	-
L Lysine	0.5	3.0
DL Methionine	1.5	1.5
L Threonine	-	0.8
L Tryptophan	-	0.2
L Arginine	-	4.0
Valine	-	2.4
Vegetable oil	20.0	55.0
Limestone	100.0	100.0
Monocalcium Phosphate	8.0	5.5
Salt	2.5	2.5
Sodium bicarbonate	1.5	1.5
Vitamin Mineral Premix ¹	1.0	1.0
Titanium Dioxide	5.0	5.0
Calculated analysis		
AME MJ/kg	11.56	11.53
Crude protein (g/kg)	172	158
Oil (g/kg)	43	69
Digestible lysine (g/kg)	8.26	8.13
Digestible Methionine + Cysteine (g/kg)	5.98	5.77
Digestible Threonine (g/kg)	6.01	5.99
Digestible Tryptophan (g/kg)	2.04	2.01
Calcium (g/kg)	41.7	41.5
Available phosphorus (g/kg)	31.0	27.0

¹The Vitamin and mineral premix contained vitamins and trace elements to meet the requirements specified by the breeder. The premix provided (units/kg diet) the following: 1Premix (per kg feed): Vit A (retinyl acetate) 10,000 IE; Vit D3 (cholecalciferol) 2,000 IE; Vit E (dl- α -tocopherol) 25 mg; Vit K3 (menadione) 1.5 mg; Vit B1 (thiamin) 1.0 mg; Vit B2 (riboflavin) 3.5 mg; Vit B6 (pyridoxine-HCl) 1.0 mg; Vit B12 (cyanocobalamin) 15 μ g; Niacin 30 mg; D-pantothenic acid 12 mg; Choline chloride 350 mg; folic acid 0.8 mg; Biotin 0.1 mg; Iron 50 mg; copper 10 mg; Manganese 60 mg; Zinc 54 mg; Iodine 0.7 mg; Selenium 0.1 mg.

²Diet A (control) was prepared using 175 g/kg SBM and 50 g/kg full fat soya as the main protein sources.

³Diet B (AP) was prepared using 70 g/kg RSM, 75 g/kg SFM and 75 g/kg SBM as protein sources.

randomisation, each diet was supplied to seven cages. The birds were reared as recommended by industry (Hy Line, Studley, UK).

Hen performance, egg production and determination of egg quality and chemical composition

The hens were weighed at the beginning and the end of the study, at 75 and 83 weeks of age, respectively. Feed intake (FI) of each cage was recorded and presented on a per cage basis for the study period. Egg numbers were recorded daily and egg weight was determined once per week, assuming this represented the average egg weight for the week. Feed conversion ratio (FCR) for egg production was determined using the equation:

$$\text{FCR} = \text{feed intake (g)} / \text{eggs laid (g)}$$

Egg and shell-quality analyses were carried out on one egg per cage collected on the last day of the experiment (83 weeks old). The analyses of the eggs were completed after one day of storage at ambient temperature. All collected eggs were individually weighed and albumen height (AH), Haugh units (HU), pH of egg white and yolk, yolk colour, egg chemical composition and eggshell thickness were determined as described in other publications (Pirgozliev et al. 2010, 2022; Whiting et al. 2022).

Proximate analysis of experimental diets, excreta and eggs

The content of dry matter (DM), nitrogen (N), fat, minerals and amino acids were performed as explained elsewhere (AOAC 1997; ISO 2005; Whiting et al. 2017; Yang et al. 2020). Gross energy (GE) content of the diets and excreta was measured using microprocessor controlled, isoperibol oxygen bomb calorimeter (Parr 6200 Instrument Company, Moline, IL, 61265, United States). Starch contents were determined as described by Englyst et al. (2000).

Metabolisable energy and total tract nutrient digestibility

During the final 4 d of the experimental period, at 83 weeks of age, a representative sample of excreta was collected from the tray under each cage, dried in an air forced oven at 60°C and milled (0.75 mm mesh). The AMEn value of the experimental diets was determined following the method of Hill and Anderson (1958). Total tract nutrient digestibility coefficients, including dry matter (DMD), nitrogen (ND), fat (FD), phosphorus (PD), calcium (CaD) and neutral detergent fibre (NDFD) digestibility were determined as previously described (Abdulla et al. 2017).

Statistical analysis

Experimental data were analysed using Genstat (21st edition) statistical software (IACR Rothamstead, Hertfordshire, UK). Comparisons between the studied variables were carried out by one-way ANOVA. All tests were considered significant at $P < 0.05$. Data are expressed as means and their pooled standard errors of means (SEM).

Results

Hen performance, egg quality and composition at study end point

The diet based on SBM only (control) had a slightly higher amount of starch and crude protein, although the AP (diet B) contained more oil and 20% more NDF (Table 2). Dietary concentrations of lysine and methionine were similar, however overall, the control diet contained more dispensable amino acids.

The effect of diets on hen performance, egg quality and egg composition are shown in Tables 3 and 4, respectively.

The differences in bird weight, FI, egg production, feed efficiency and average egg weight were numerical only ($P > 0.05$). However, there were no differences between diets for egg weight, HU, AH, yolk and albumen pH, shell thickness or egg chemical composition ($P > 0.05$). Diets affected egg yolk colouration, as birds fed AP had an increased YF ($P = 0.001$), a* ($P < 0.05$) with tendencies for an increase in b* ($P = 0.101$) and decrease in L ($P = 0.051$) scores. The number of eggs with deformities was negligible, about 1% in total. There were no differences ($P > 0.05$) in numbers of eggs with deformities between the two diets (data not shown in tables).

Table 2. Determined dietary energy and nutrient composition (g/kg).

Determined composition	A ¹	B ²
Dry matter	903	907
Gross energy, MJ/kg	15.40	15.20
Starch	444	403
Crude protein	172	164
Oil	34	66
Neutral detergent fibre	135	162
Calcium	30.7	36.6
Total phosphorus	5.4	5.3
Essential amino acids		
Arginine	10.79	10.66
Histidine	4.39	3.43
Isoleucine	7.48	7.77
Leucine	13.29	10.38
Lysine	9.42	9.51
Methionine	4.15	4.14
Phenylalanine	9.15	6.83
Threonine	6.23	6.46
Tryptophan	4.85	3.52
Valine	8.39	8.90
Non-essential amino acids		
Alanine	7.61	6.16
Aspartic acid	16.23	11.59
Cystine	3.39	2.94
Glutamic acid	39.23	32.62
Proline	13.57	11.53
Tyrosine	6.41	4.47
Serine	9.72	7.15
Glycine	7.78	6.93
Total essential amino acids	69.76	62.70
Total non-essential amino acids	103.95	83.37
Total AA	173.72	146.07

¹Diet A (control) was prepared using 175 g/kg SBM and 50 g/kg full fat soya as the main protein sources.

²Diet B (AP) was prepared using 70 g/kg RSM, 75 g/kg SFM and 75 g/kg SBM as protein sources.

Dietary AMEn and total tract nutrient digestibility coefficients

The control diet A had higher ($P < 0.05$) AMEn, DMD and NDFD coefficients compared to AP diet (Table 5). However, AP had a higher FD coefficient ($P < 0.05$). Birds fed either diet consumed the same amount of metabolisable energy ($P > 0.05$).

Discussion

Eggs are a common component of the human diet and with the increasing global population, demand for eggs will continue to rise (Réhault-Godbert et al. 2019). Using AP sources in poultry feed is a potential solution for reducing the economic and environmental costs of the diets, while improving egg production (Koçer et al. 2021). In addition, as hens get older, their eggs tend to get larger (Roberts 2004). This may help satisfy the consumer demand for large eggs. It should be mentioned, however, that alongside this, both external and internal egg quality reduces with age, which is not economical for the producer. However, improving nutrition, in parallel with bird genetics, health and welfare may help to increase the length of the egg production period of laying hens. Extending the period of lay and feeding balanced diets based on AP sources to soya to end of production laying hens was shown to be sustainable.

There were some small differences between calculated (Table 1) and determined (Table 2) dietary chemical composition that were probably due to the differences between the actual composition of the ingredients used in the study and the values in the software used for dietary formulation for the same ingredients. However, it was not expected that had a significant impact on the outcomes.

Table 3. Effects of diet on the performance of laying hens over the period 75 to 83 weeks of age.

	A ¹	B ²	SEM ³	P value
Body weight start (g)	2070	2001	45.1	0.323
Body weight end (g)	2109	2073	35.9	0.500
Feed intake (g/hen/day)	111	113	4.9	0.846
Egg production (%)	78.3	76.0	6.63	0.816
Egg mass (g/hen/day)	51.0	47.1	4.96	0.600
Egg weight (g)	65.1	62.0	2.13	0.585
FCR (egg production)	2.330	2.540	0.3740	0.700

¹Diet A (control) was prepared using 175 g/kg SBM and 50 g/kg full fat soya as the main protein sources.

²Diet B (AP) was prepared using 70 g/kg RSM, 75 g/kg SFM and 75 g/kg SBM as protein sources.

³SEM – pooled standard error of the mean.

Table 4. Effects of diet on egg and eggshell quality variables when fed to laying hens for 8 weeks.

	A ¹	B ²	SEM ³	P value
Albumen height (mm)	6.65	6.83	0.512	0.810
Haugh unit	77.7	80.3	6.16	0.598
Albumen pH	8.22	8.07	0.153	0.516
Yolk pH	5.91	5.87	0.046	0.566
Eggshell thickness (mm)	0.342	0.376	0.0158	0.190
Internal egg chemical composition:				
Egg Crude protein (g/kg)	497	492	10.2	0.759
Egg Crude fat (g/kg)	348	360	8.6	0.382
Yolk colour:				
DSM YolkFan™ (YF)	2.10	3.43	0.138	0.001
L* ⁴	17.58	16.37	0.335	0.051
a* ⁴	0.51	0.74	0.049	0.024
b* ⁴	17.89	19.28	0.491	0.101

¹Diet A (control) was prepared using 175 g/kg SBM and 50 g/kg full fat soya as the main protein sources.

²Diet B (AP) was prepared using 70 g/kg RSM, 75 g/kg SFM and 75 g/kg SBM as protein sources.

³SEM – pooled standard error of the mean.

⁴L* = lightness; a* = redness; b* = yellowness.

Table 5. Dietary AMEn and total tract nutrient digestibility coefficients.

	A ¹	B ²	SEM ³	P value
AMEn (MJ/kg)	11.84	11.10	0.151	0.013
AMEn intake (MJ/d)	1.31	1.27	0.056	0.596
Dry matter digestibility	0.719	0.680	0.0056	0.004
Nitrogen digestibility	0.491	0.472	0.0211	0.545
Fat digestibility	0.816	0.913	0.0150	0.006
Phosphorus digestibility	0.338	0.310	0.0260	0.487
Calcium digestibility	0.278	0.309	0.0961	0.829
Neutral detergent fibre digestibility	0.542	0.453	0.0229	0.040

¹Diet A (control) was prepared using 175 g/kg SBM and 50 g/kg full fat soya as the main protein sources.

²Diet B (AP) was prepared using 70 g/kg RSM, 75 g/kg SFM and 75 g/kg SBM as protein sources.

³SEM – pooled standard error of the mean.

Laying performance

The production and egg quality results were within the expected range for Hy Line Brown laying hens between 75 and 83 weeks of age. Saleh et al. (2021) reported no adverse effects when feeding 68-week-old hens 5 or 10% dehulled SFM, in fact the authors found that egg FCR and production improved when fed at the higher inclusion. The dehulling process of SFM has been commercially improved to allow the production of high-protein SFM which may contain over 40% crude protein (CP) and less than 10% fibre (Waititu et al. 2018). Research by Karkelanov et al. (2020) demonstrated a strong relationship between the reduction in SFM fibre content and increases in metabolisable energy. Furthermore, Chobanova (2019) showed that SFM may partially be used as a substitute for SBM in poultry diets, when balanced with synthetic amino acids.

Perez-Maldonado et al. (2003) suggested that 10% RSM was the maximum recommended level in laying hen diets, thus the included 7% dietary RSM in the reported study was

within limits. Cheva-Isarakul et al. (2001) found that RSM could be incorporated in laying hen diets up to 10% without any adverse effects but increasing dietary RSM beyond 10% reduced egg production. However, research by Oryschak et al. (2020) demonstrated that Brassica cakes and meals could be fed to laying hens at dietary inclusions up to 20% without adverse effects on hen productivity or egg quality. The reduced antinutrients, e.g., erucic acid and glucosinolate in modern rapeseed varieties, and the improved processing techniques of oil extraction likely contributes most for the improved feeding value of RSM (Watts et al. 2020, 2021).

Since feed comprises over 70% of the production cost in eggs, FCR determination is an important measure used to assess the economics of egg production. Soleimani and Gilbert (2020) reported high correlations between FCR and all impact categories, thus emphasising the importance of improving feed efficiency to reduce negative environmental impacts. In a study with layers between 60 and 68 weeks of age, Saleh et al. (2021) reported a 9% difference in FCR when feeding isocaloric and isonitrogenic diets. The current study

used hens between 75 and 83 weeks of age, which may explain the variation in the observed FCR values. The lack of difference in egg weight and feed intake between treatment groups further confirm that SBM can at least partially (67%) be replaced by SFM and RSM in diets for older laying hens.

Although there were differences in some of the dietary amino acid concentrations between diets in this study, the diets met or exceeded the commercial amino acid recommendations for Hy Line brown layers. These differences may have a pronounced performance effect, especially as diets are typically formulated for persistent egg production, so that in practice recommendations may be lower than required for optimal egg production (Macelline et al. 2021). In addition, Gu et al. (2021), showed that there was higher amino acid excretion from elderly birds compared to younger birds, which supported the potential for dietary amino acid differences to impact performance. Further research is therefore recommended into the amino acid requirements for elderly laying hens.

Egg quality and chemical composition variables

The egg quality variables were different to those of young birds, *e.g.*, albumen quality (Whiting et al. 2022), but similar to elderly hens (Star et al. 2020), which was expected (Roberts 2004). Feeding SFM and RSM to laying hens did not influence overall egg quality and chemical composition compared to SBM fed birds. This was in accordance with Saleh et al. (2021) who fed 10% SFM. However, when feeding 10% RSM to layers, Panaite et al. (2020) found an increased albumen pH and reduced eggshell weight compared to SBM fed layers. In agreement with Saleh et al. (2021) and Laudadio et al. (2014) dietary SFM supplementation increased the yolk colour score. However, Panaite et al. (2020) did not report an increase in yolk colour score of laying hens fed 10% dietary RSM. The effect of SFM on yolk colour score may be linked to the number of natural pigments it contains (Laudadio et al. 2014).

In addition, dietary fat accelerates the absorption of pigment and fat-soluble vitamins (Costa et al. 2008; De Morais Oliveira et al. 2016), with higher fat content being a feature of AP in this study. Oryschak et al. (2020) reported similar darkening in egg yolk from RSM fed birds, using the CIELAB colour system, further supporting the current results. However, it should be noted that some other studies did not find changes in egg colour when feeding SFM in diets (Shi et al. 2010; Tsuzuki et al. 2003) and described no positive effect of dietary SFM on egg yolk colour.

Differences in yolk colour is a factor in consumer perception and acceptance of eggs produced using AP. There was no difference in protein and egg albumen quality, thus not supporting the view that dietary protein source may influence it (Samli et al. 2005). Bird genetics and rearing environment are the major factors affecting egg quality (Roberts 2004), thus supporting the lack of differences in protein and fat contents of eggs in this study.

Dietary AMEn and total tract nutrient digestibility coefficients

Determined AMEn in the diets had a similar range to previous reports for wheat-based diets in layers (Whiting

et al. 2019). The low AMEn of AP can be linked to its higher fibre content (Abdulla et al. 2017; Choct et al. 1999), which may increase digesta viscosity and inhibit the digestion of nutrients in the small intestine (Choct and Annison 1990; Pirgozliev et al. 2015). This agreed with the reduced DMD and NDFD and numerically lower ND in AP. Dietary FD for AP was higher, possibly due to the higher dietary fat content. Research by Karkelanov et al. (2021) found that pelleting low fibre SFM increases its metabolisable energy value for broilers. Watts et al. (2020, 2021) demonstrated that some mild processing techniques and protease enzyme application can improve the feeding value of RSM by possibly improving access to dietary protein. The use of fibre degrading enzymes may improve energy and nutrient availability in high fibre diets (Bedford 2018; Pirgozliev et al. 2019, 2023). The coefficients for mineral digestibility were similar to those reported by Saleh et al. (2021), but there was no difference between diets. Although there were some differences in the determined AMEn of the two diets, all birds consumed a similar daily amount of about 1.3 MJ, which suggested that diets were providing enough nutrients to satisfy the requirements of the hens, further explaining the lack of difference in egg performance variables.

Conclusions

Replacing 67% of dietary SBM with a mixture of SFM and RSM did not adversely impact bird body weight, egg production or quality variables when fed to laying hens between 75 and 83 weeks of age. The soy bean-based diet had higher metabolisable energy, however the birds fed the alternative diet consumed the same amount of energy. Whilst diets based on AP sources were considered suitable to be fed to aged laying hens without any loss in performance in this study, there were other factors that should be considered before feeding at farm level. For example, diets based on AP sources or designed for end of production laying hens may be further optimised with the use of synthetic amino acids to ensure balance. The use of AP sources may contribute to sustainable egg production, avoiding deforestation and maintaining good animal health and welfare. Finding an optimum inclusion level of alternatives to soya protein sources in laying hen diets requires more research. Further assessments of AP sources and increased length of production of layers should be continued to evaluate improved sustainability.

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