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## Article

# Effect of Using Germinated and Fermented Lupin and Oats as a Dietary Protein Source on Laying Hen Performance and Egg Quality

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**Abstract:** Soybean meal is the dominant protein source for poultry nutrition. However, soybean is not widely grown in Europe, necessitating importation from other countries. To reduce dependency on imported soybean meal, an alternative feed material is needed. Fermentation and/or germination of grains are known to increase the value of the protein content of a diet. This study aimed to determine if they could substitute partly soybean meal in a diet. Germinated or fermented or germinated and fermented grains of lupin and oats were used in laying hen's diet (a mix of 6.50% lupin and 3.50% oat grains). Oats were dehulled or not dehulled. The hens' weight loss and the downgraded eggs rate were the lowest when using fermented grains. All trial diets reduced the egg cholesterol content. Dehulling had only a slight effect on performance. Diets containing germinated grains led to a decrease in laying performance and an increase in body weight loss. Diets containing fermented grains gave the best results in terms of quantity of amino acids, hen weight maintenance, laying performance, and egg quality. In conclusion, fermented lupin and oats can be used in laying hen diets to partly substitute sources of protein such as soya, but germinated grains cannot.

**Keywords:** laying hens; organic nutrition; amino acids; egg quality; lupin; oats; fermentation; germination



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## 1. Introduction

Eggs, with the rapid rise in the world population and increasing demand, are one of the most important and cheapest sources of animal protein in human diets [1]. There are multiple egg-production systems operating in Europe, and Germany and France are the largest producers of organic eggs in the world [2].

Organic production methods (ban of the chemical products), offering consumers a system with animal outdoor access and organic feed, are increasingly popular in Europe [2]. In organic systems, diets are based on cereals, with the ingredients being sourced from organic farms.

Soybean meal is the dominant protein source for poultry nutrition. However, soybean is not widely grown in Europe, necessitating importation from countries such as Brazil. To reduce dependency on imported soybean meal, an alternative feed material is needed. One possibility is to use microbial fermentation to improve the bioavailability of nutrients in local grains. Another possibility would be to increase the nutritional value by germinating the grains. Germination breaks down dormancy by activating the internal enzymes in

grains, increasing the digestibility and bioaccessibility of nutrients. Anti-nutritional factors as alkaloids are also degraded by germination [3]. We evaluated two grains, lupin and oats, because these plants grow on organic soils in Brittany (where the trial took place) and can be fed to laying hens. Lupin (*Lupinus angustifolius*) is a nitrogen-fixing plant that may be used to great advantage in organic farming. Ref. [4] studied several varieties of *Lupinus angustifolius* in Poland and found that lupin has a very high protein content (33%) and high contents of fat (6.8%) and crude fiber (14%). Lupin is often considered as an alternative to soybean given its elevated and high-quality protein content. This possibility was also shown by [5]. In organic farming, the inclusion of either whole lupin grains [6] or oats [7] in amounts of up to 20% of laying hen's diet has been shown to support performance. Oats' nutritional composition differs significantly from that of other cereals, with a high protein content, high amount of essential amino acids, and higher fat content than other cereals, as shown by [8,9]. The insoluble fiber fraction makes oats an unpopular feed for non-ruminants but can be counteracted by dehulling, which increases the available nutrient content (increase in energy, protein, and fat contents compared with hulled oat grains) and decreases the fiber content [6,9]. In this study, we evaluated the effect of utilizing fermentation and/or germination of the grains (lupin and oats), with or without dehulling of the oats, as a partial substitution of the soybean and sunflower meals elements of the control diet on laying hens performance and egg quality. Our hypothesis was to assess if fermentation and/or germination of grains would increase the value of the protein content of the diet and could partly substitute soybean and sunflower meals in the diet. Dehulling took place to check if dehulled oat grain increased the nutritional value of the diet.

## 2. Materials and Methods

### 2.1. Ethical Statement

The hens used were reared in compliance with regulations for the human care and use of animals in research, according to [10]. National authorization to experiment on living animals n°3502 has been delivered to M. Kouba by the French Minister of Agriculture. In this study, although the hens were weighed, ethical approval was not required due to the absence of any invasive practices such as blood sampling, surgical procedures, or slaughter. The hens were raised and kept on a farm according to [11].

### 2.2. Site of Trial and Feed Preparation

The study was conducted from November to December 2022 in an organic French commercial poultry farm (producing organic eggs) called "Lis ar Parkou" at Noyal-Pontivy. This study was a collaboration between the private company Lann Bodigen (owner of the farm), the French National Research Institute for Agriculture, Food and the Environment (INRAE), and the National Polytechnic Institute Felix Houphouët Boigny (INP-HB) at Yamoussoukro, Côte d'Ivoire.

The lupin (*Lupinus angustifolius*) and oat (*Avena sativa*) grains used in this study were produced on the farm Lis ar Parkou. To germinate the oat and lupin grains required for the study, a 2 to 3 cm thick layer of oat grains and a 10 cm thick layer of lupin grains were watered twice a day, 5 days for oats and 4 days for lupin, at  $20 \pm 2$  °C and 90% relative humidity in darkness. The germinated grains were ground with an electric blender (Ar-Teckh, Lamballe, France) and divided in two half-portions; one portion was dehydrated (ST3 Politec, Fegersheim, France) for 24 h at  $48 \pm 2$  °C and stored vacuum-packed at room temperature until chemical analysis or feeding (Germinated grains).

The other half-portion was fermented. The ground germinated grains were inoculated with 0.5 L of distilled water per kilo containing approximately  $10^7$  CFU/mL of *Lactiplantibacillus plantarum* CIRM-BIA2180 and *Levilactobacillus brevis* CIRM BIA 2352, both provided by the International Center for Microbial Resources-Food Associated Bacteria (CIRM-BIA, <https://collection-cirmbia.fr> accessed on 9 September 2010) for lupin and oats, respectively; the grains were mixed thoroughly and held in a tank for 24 h at 30 °C

(germinated fermented grains). The germinated fermented grains were then dehydrated (ST3 Politec, Fegersheim, France) for 24 h at  $48 \pm 2$  °C and thereafter stored vacuum-packed at room temperature until chemical analysis or feeding. Non-germinated lupin and oat grains needed for the study were similarly ground, fermented, dehydrated, and stored in the same way (fermented grains). Raw grains used in this trial were ground, dehydrated, and stored in the same conditions as the other grains. Dehulling of the oat grains needed for the trial was performed on fermented, germinated, or germinated fermented grains with a laboratory dehuller (LH 5095, Codema LLC., Maple Grove, MN, USA). The basal organic control feed was provided by Edou Breizh (Janzé, France). The grains processed in different manners were mixed with the basal feed twice a week to be as fresh as possible.

All of the diets for the study were formulated at the Animal Science Laboratory of INP-HB to meet laying hen physiological requirements and to be isoproteic and isoenergetic. Tables 1 and 2 present the ingredients and composition of the diets for laying hens. For each calculated value of nutriment in the diet, we used the following formula from [12]

$$A_i = \frac{1}{100} \sum_{j=1}^n (a_{ij} X_j)$$

$A_i$ : nutriment  $i$  content in the diet;  $i$ : nutriment to analyze;  $j$ : raw material of the diet;  $a_{ij}$ : nutriment  $i$  content in raw material  $j$ ;  $X_j$ : percentage of the raw material  $j$  in the diet; and  $n$ : number of raw materials  $j$  in the diet.  $A_i$  and  $a_{ij}$  are expressed in the same unit.

**Table 1.** Chemical composition of lupin and oat grains (raw, fermented, germinated, fermented-germinated).

Grain	Lupin Grain	Fermented Lupin Grain	Germinated Lupin Grain	Germinated Fermented Lupin Grain	Oat Grain	Fermented Oat Grain	Germinated Oat Grain	Germinated Fermented Oat Grain
Components (g/kg)								
Dry matter	958	953	975	952	969	980	984	975
Crude protein	372	363	403	422	102	95	115	117
Lipid	60	53	57	60	65	60	63	69
Ash	37	40	33	33	21	22	27	26
Insoluble fibers	394	400	402	396	194	195	193	197
Soluble fibers	20	21	24	25	129	130	130	132
Total fibers	414	421	426	421	323	325	326	329
Calcium	25.2	31.2	30.0	30.0	7.2	7.1	10	11.2
Phosphorus	4	3.5	4.0	4.7	3.6	3.7	3.5	3.4
Calculated AME <sub>n</sub> (MJ/kg)	9	8	10	10	10	9	10	10
Essential amino acids (EAAs) (g/100 g protein)								
Arginine	11.5	11.2	9.6	7.8	6.7	6.7	6.5	5.6
Cysteine	1.1	1.5	1.1	1.1	2.9	2.9	3.1	2.8
Histidine	2.6	2.9	2.6	2.5	2.0	2.5	2.1	2.0
Isoleucine	3.9	4.0	3.9	3.9	3.7	4.1	4.0	3.8
Leucine	6.5	7.1	6.4	6.3	7.1	8.1	7.6	7.0
Lysine	4.7	5.0	4.4	4.0	4.1	4.5	4.3	3.8
Methionine	0.6	0.6	0.6	0.6	1.6	1.5	1.7	1.6
Phenylalanine	3.8	4.0	3.7	1.5	5.0	5.6	5.2	4.9
Theonine	3.4	4.1	3.3	3.3	3.4	3.9	3.8	3.6
Tyrosine	3.8	3.4	3.5	3.1	3.6	3.8	3.7	3.4
Valine	3.6	4.1	3.6	3.6	4.7	5.5	5.1	5.0
Tryptophan	0.8	1.0	0.8	0.8	1.3	1.5	1.3	1.3
Total EAAs	46.5	48.9	43.6	38.5	46.2	50.7	48.4	44.8
Non-essential amino acids (NEAAs) (g/100 g protein)								
Alanine	3.1	3.6	3.7	3.8	4.6	5.2	5.9	5.4

Table 1. Cont.

Grain	Lupin Grain	Fermented Lupin Grain	Germinated Lupin Grain	Germinated Fermented Lupin Grain	Oat Grain	Fermented Oat Grain	Germinated Oat Grain	Germinated Fermented Oat Grain
Aspartate	10.0	10.3	12.5	9.9	8.3	9.1	8.0	8.4
Glutamine	21.4	20.7	18.8	17.0	18.1	20.5	18.7	17.4
Glycine	4.0	4.5	3.7	3.7	5.0	5.1	5.2	5.0
Proline	3.9	4.5	3.8	3.6	4.9	5.9	5.2	4.8
Serine	4.9	5.1	4.8	4.3	4.9	5.3	5.0	4.5
Total NEAAs	47.4	48.8	47.5	42.4	45.8	51.1	48.2	50.5
Total of all AAs	93.9	97.7	91.1	80.9	92.0	101.8	96.6	90.3

Nitrogen-corrected apparent metabolizable energy estimated from chemical composition [13]:  $AME_n$  (MJ/kg) =  $0.1551 \times \% \text{ crude protein} + 0.3131 \times \% \text{ crude fat} + 0.1669 \times \% \text{ starch} + 0.1301\% \text{ total sugar}$ . Amino acid ranking in essential and non-essential amino acids for poultry from [14].

Table 2. Ingredient composition and nutrient content of the control diet (C) or experimental diets containing 10% of mixed grains of lupin (6.5%) and oats (3.5%).

Ingredients (g/kg)	C	F	Fd	G	Gd	GF	GFd
Yellow corn	249	249	249	249	249	249	249
Soybean meal	171	115	115	115	115	115	115
Triticale	190	190	190	190	190	190	190
Sunflower meal	132	90	90	90	90	90	90
Wheat	97	97	97	97	97	97	97
Lucerne	30	30	30	30	30	30	30
Wheat bran	18	18	18	18	18	18	18
Soybean oil	7	5	5	5	5	5	5
Carbonate	86	86	86	86	86	86	86
Plant extract	15	15	15	15	15	15	15
Fermented grains	-	100	-	-	-	-	-
Fermented grains with dehulled oats	-	-	100	-	-	-	-
Germinated grains	-	-	-	100	-	-	-
Germinated grains with dehulled oats	-	-	-	-	100	-	-
Germinated fermented grains	-	-	-	-	-	100	-
Germinated fermented grains with dehulled oats	-	-	-	-	-	-	100
Vitamin–Mineral premix	5	5	5	5	5	5	5
Calculated content (g/kg)							
ME (MJ/kg)	11.7	11.7	11.7	11.7	11.7	11.7	11.7
Digestible lysine	11.5	11.5	11.5	11.5	11.5	11.5	11.5
Digestible threonine	5.4	5.4	5.4	5.4	5.4	5.4	5.4
Digestible methionine	2.6	2.6	2.6	2.6	2.6	2.6	2.6
Digestible methionine + cysteine	5.2	5.2	5.2	5.2	5.2	5.2	5.2
Analyzed content (g/kg)							
Dry matter	906	909	901	906	909	895	901
Ashes	161	151	138	124	138	118	130
Crude protein (CP)	180	178	177	177	180	180	177
Crude fat (CF)	55	54	55	54	57	52	54
Insoluble fibers	164	171	156	187	157	181	172
Soluble fibers	19	29	25	24	26	32	21
Total fibers	183	200	181	211	183	213	193
Calcium	460	441	450	362	381	332	373
Phosphorus	4.9	4.6	4.6	4.6	5.2	5.2	4.8

Diets: C: Control diet; F: fermented grains-supplemented diet; Fd: fermented grains-supplemented diet with dehulled oats; G: germinated grains-supplemented diet; Gd: germinated grains-supplemented diet with dehulled oats; GF: germinated fermented grains-supplemented diet; GFd: germinated fermented grains-supplemented diet with dehulled oats. The vitamin–mineral premix supplied the following per kilogram of diet: vitamin A, 10 250 IU; vitamin D3, 4100 IU; vitamin E 20 IU; vitamin K 2 mg; vitamin B1, 1.6 mg; vitamin B2, 4.5 mg; vitamin B6, 5 mg; vitamin B12, 0.03 mg; biotin, 0.1 mg; folic acid, 2 mg; niacin, 45.1 mg; choline, 50 mg; Co, 0.1 mg; Cu, 14 mg; Fe, 35 mg; I, 0.4 mg; Mn, 60 mg; Se, 0.4 mg; Zn, 109 mg; sepiolite, 726 mg.

### 2.3. Animals and Trial Design

A total of 840 organically raised Warren Brown laying hens, aged 48 weeks, were randomly distributed between 42 pens. Each pen of twenty hens was randomly allocated to one of seven dietary treatments (six replicates (pens) per diet). Each pen was 4 m<sup>2</sup> (2 m length, 2 m width), providing 2000 cm<sup>2</sup> of floor space per laying hen. Each pen also held a nest, perches, a feeder, and a drinking trough, and litter was refreshed weekly. These rearing conditions fulfilled organic laying hen requirements [15]. However, in the context of mandatory avian flu prevention and mitigation measures operable in France, during the study period, the hens were confined to the poultry house without outside access. Farmers were granted an exemption for this from the French Minister of Agriculture, so their production was still considered as organic. The lighting program was composed of 16 h of light and 8 h of darkness per day. A basal organic diet based on corn, soybean meal, and triticale (control diet C) had the soybean and sunflower meal components partially substituted for trial purposes with a mix of 10% of grains (6.5% lupin grains and 3.5% oat grains), processed in the various manners described:

The control hens therefore received diet C and the trial hens one of either:

Diet F with 10% mixed fermented grains of lupin and oats;

Diet Fd with 10% mixed fermented grains of lupin and dehulled oats;

Diet G with 10% mixed germinated grains of lupin and oats;

Diet Gd with 10% mixed germinated grains of lupin and dehulled oats;

Diet GF with 10% mixed germinated and fermented grains of lupin and oats;

or

Diet GFd with 10% mixed germinated and fermented grains of lupin and dehulled oats.

Diets in the form of mash feeds and drinking water were offered ad libitum for a total of 40 days (10 days of adaptation to the diets and the experimental conditions + 30 days of experimentation). Performance parameters were only measured after 10 days of adaptation; thus, we will only refer to the post-adaptation or experimental period. Mortality and health status were checked twice daily. Mortality was low (total mortality was 0.24%); one hen died in the control group, and a hen was culled from group F (mortality of 0.83% for control group C and for the experimental group F, not treatment-related). All of the data were corrected for mortality. Hens were weighed on days 1 and 30 of the trial on a pen basis, feed intake was measured weekly per pen, and average daily gain (ADG), average daily feed intake (ADFI), and the feed conversion ratio (FCR) were calculated.

### 2.4. Egg Physical Parameters

On day 30, the eggs collected from each individual pen were held separately for 1 day at 15 °C and 70% RH, pending analyses. The following morning, the collected eggs were visually examined, and soft eggs (eggs without shell because of a lack of calcification), eggs of abnormal shape (for example eggs not really in oval form), eggs with abnormal shell (calcium carbonate deposition that was not homogeneous), and broken eggs were excluded. The percentage of these downgraded eggs was calculated for each treatment. Three eggs of the remaining intact eggs per pen were put forward to be analyzed (18 eggs in total per diet).

The length and the equatorial diameter of each egg was measured using a micrometer marked at 0.01 mm intervals (Preciva Professional Caliper, Wenzhou, Zhejiang, China) to calculate the egg shape index using the formula shape index (SI) = (egg width/egg length) × 100. The egg, vitellus, albumen, and shell weights were recorded. The shell thickness of eggs was determined (with shell membrane) using the mean value of measurements from three locations (air cell, equator, and sharp end) of the egg with a Neoteck 0–25, 4 mm digital measuring gauge (Neoteck Technology, Hong Kong) with a 0.001 mm accuracy. Haugh units were measured with a Haugh tester, Bröring EggQuality 3.0 (Bröring Technology GmbH, Saint-Laurent-du-Var, France). A QCH albumen height gauge from Technical Services and Supplies, York, UK, was used to determine height of albumen in mm. Yolk color was determined using the DSM Yolktan<sup>TM</sup> lineal (DSM Nutritional Products France, La

Garenne-Colombes, France), and colors were scored according to 15 sample colors ranging from 1 (the lightest) to 15 (the darkest).

### 2.5. Chemical Analyses

The determination of total, soluble, and insoluble fibers for the lupin and oat grains and the diets were in accordance with Official Methods of Analysis of AOAC International [16], based on enzymatic AOAC Method 991.43. Samples of grains, diets, and selected eggs (yolks analyzed individually without any pooling) were submitted to analyses for dry matter, ash, and crude protein (N X 6.25): AOAC 950.46 for the dry matter analysis; AOAC 920.153 for the ash content determination; and AOAC 928.08 for total protein (crude protein, N 6.25) content determination by the Kjeldahl method. Total egg cholesterol content was quantified using a commercial kit (Giese Diagnostics SRL, Guidonia Montecelio, Italy) according to a colorimetry method. Mineral macro-elements calcium and phosphorus were analyzed in grains and diets by an atomic absorption spectrometer (VarianSpectraa 20, IET, Mundelein, IL, USA).

Lipids were extracted from the grains, the diet samples, and the selected eggs by the chloroform/methanol procedure of [17]. For lipid extraction and fatty acids analysis, we used yolk (albumen contains no lipids). We did not pool the yolks; all of the eggs were analyzed individually. Fatty acid composition was measured after methylation of samples. Fatty acid methyl esters were prepared with boron trifluoride-methanol according to [18]. These esters were analyzed on an Agilent Technologies 7890A gas chromatograph (Bios Analytic, Toulouse, France), with an internal standard (C21:0, Sigma-Aldrich, Darstadt, Germany being used to quantify fatty acids (g/100 g of total fatty acids). Nutritional quality was described by the polyunsaturated fatty acids (PUFAs)/saturated fatty acids (SFAs) ratio, expressed as:  $(18:2n - 6 + 18:3n - 3)/(14:0 + 16:0 + 18:0)$ . The 18:2n - 6/18:3n - 3 ratio is relevant to the competition for synthesis of longer-chain PUFAs. The results were also expressed as sum of n - 6 PUFAs/sum of n - 3 PUFAs.

Amino acids (AAs) were released from the grains and the diets by acid hydrolysis for 23 h at 110 °C under reflux. Total methionine and cysteine were hydrolyzed after oxidation by performic acid. Total AA contents were analyzed by ion exchange chromatography and ninhydrin derivatization (JLC-500/V AminoTac Amino Acid Analyzer; Jeol, Croissy-sur-Seine, France; method NF EN ISO 13903:2005). Total tryptophan was analyzed by HPLC processing with a fluorescence detector (RF 10AXL; Shimadzu, Bonneuil sur Marne, France) after an alkaline hydrolysis with barium hydroxide.

### 2.6. Statistical Analysis

Data were subjected to the Shapiro–Wilk and Levene tests to check normality and equality of variances, respectively. Data were then analyzed by the one-way analysis of variance ANOVA test with diet as the main effect. A multiple comparison of means was performed using the Tukey test when differences revealed by ANOVA were significant. All analyses were performed using R 4.2.1 software (Copyright © 2022, R Foundation for Statistical Computing Platform, Vienna, Austria). Significance implies  $p < 0.05$ , unless stated otherwise.

## 3. Results

### 3.1. Composition of the Grains and the Diets

The composition of grains is presented in Table 1. The lupin grains showed a higher protein content than the oat grains. Fermented lupin and fermented oats showed the highest content of amino acids with a high content of essential amino acids. The total amino acid content was the lowest in germinated fermented lupin and oats compared with diet C and the other diets. The ash, Ca, and P contents were also higher in lupin, and the lipid percentage was the lowest in fermented lupin compared with diet C and the other diets. Crude protein percentage was increased by germination compared with raw grains

of lupin and oats. The contents of total and insoluble fibers were higher in lupin grains compared with oat grains, while the content of soluble fibers was higher in oat grains.

Tables 2 and 3 present the composition of the diets. Diets were isoproteic and isoenergetic. The Ca proportion was lower in all the diets having germinated grains (diets G, Gd, GF, GFd) compared with the control diet C and diets F and Fd. Diets having dehulled oats showed less insoluble fibers than diets having entire oat grains. There was no difference in the fatty acid profile in the experimental diets, but the control diet had higher saturated fatty acid and polyunsaturated fatty acid percentages and a lower monounsaturated fatty acid percentage than experimental diets. Diet F (and to a lesser extent diet Fd) exhibited the highest content of non-essential amino acids, essential amino acids, and total amino acids compared with the other diets. There was no effect of germination on the content of amino acids in diets G, Gd, GF, and GFd.

**Table 3.** Composition in the fatty acids and amino acids of control diet (C) or experimental diets containing 10% of mixed grains of lupin (6.5%) and oats (3.5%).

Ingredients (g/kg)	C	F	Fd	G	Gd	GF	GFd
<b>Fatty acids (FAs), g/100 g total FAs</b>							
SFAs	16.5	14.4	14.9	14.6	14.3	14.5	14.5
MUFAs	39.4	45.6	44.6	45.2	45.1	44.9	44.8
PUFAs	44.1	40.0	40.5	40.2	40.6	40.6	40.7
n – 3	3.4	3.0	2.8	3.0	3.0	3.0	3.1
n – 6	40.7	37.0	37.6	37.2	37.6	37.6	37.6
PUFAs/SFAs	2.7	2.8	2.7	2.7	2.8	2.9	2.8
n – 6/n – 3	11.8	12.3	13.2	12.5	13.0	12.9	12.0
<b>Essential amino acids (EAAs) (g/100 g protein)</b>							
Arginine	6.3	7.1	6.2	6.1	6.8	6.2	6.1
Cysteine	1.4	1.6	1.6	1.6	1.5	1.6	1.5
Histidine	2.3	2.5	2.4	2.4	2.2	2.4	2.3
Isoleucine	3.7	4.0	3.8	3.9	3.7	3.8	3.3
Leucine	7.0	7.5	7.2	6.3	6.8	7.2	7.2
Lysine	4.5	4.3	4.3	4.0	4.0	4.3	4.2
Methionine	1.2	1.3	1.3	1.2	1.2	1.3	1.1
Phenylalanine	4.4	4.6	4.4	4.3	4.2	4.4	4.3
Threonine	3.4	3.6	3.4	3.5	3.5	3.4	3.7
Tyrosine	2.9	3.2	3.1	3.1	2.8	3.1	3.0
Valine	4.3	4.7	4.5	4.4	4.3	4.2	3.9
Tryptophan	1.1	1.2	1.1	1.1	1.1	1.1	1.1
Total EAAs	42.6	45.6	43.4	42.1	42.2	43.1	42.0
<b>Non-Essential amino acids (NEAAs) (g/100 g protein)</b>							
Alanine	4.2	4.5	4.4	4.5	4.1	4.3	4.5
Aspartate	9.3	10.2	9.6	9.7	9.8	9.2	9.1
Glutamine	17.6	19.1	17.8	16.8	17.3	16.8	16.9
Glycine	4.1	4.5	4.3	4.4	4.1	4.3	4.3
Proline	5.0	5.2	5.2	5.2	5.2	5.2	5.1
Serine	4.4	4.7	4.4	4.6	4.5	4.4	4.4
Total NEAAs	44.6	48.1	46.3	45.2	44.9	44.2	45.3
Total of all AAs	87.3	93.7	89.7	87.3	87.1	87.3	87.3

Diets: C: Control diet; F: fermented grains-supplemented diet; Fd: fermented grains-supplemented diet with dehulled oats; G: germinated grains-supplemented diet; Gd: germinated grains-supplemented diet with dehulled oats; GF: germinated fermented grains-supplemented diet; GFd: germinated fermented grains-supplemented diet with dehulled oats. SFAs: Saturated fatty acids; MUFAs: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids. Ranking in essential and non-essential amino acids for poultry from [14]. Values are the means of three analyses per sample.

### 3.2. Animal Performance

The laying hen performance are presented in Table 4. There was a very low mortality rate during the trial. All the laying hens lost weight (from 1.2% with diet C to 4.9% with



diet G) regardless of the diet. The body weight loss was not significantly different with diets F and Fd compared with the control diet. In the other diets containing germinated grains, G, Gd, GF, and GFd, the weight loss was higher than the weight loss observed with the control diet ( $p = 1.34 \times 10^{-12}$ ). The daily feed intake was similar with diets C, F, and Fd and lower with the other diets compared with the control diet ( $p = 1.09 \times 10^{-7}$ ). Diets G, Gd, GF, and GFd led to a lower laying rate compared with the other diets, for which laying rate was not significantly different from the laying rates obtained with the control diet ( $p = 3.52 \times 10^{-3}$ ). The proportion of downgraded eggs was the lowest with diets F and Gd and the highest with diet G ( $p = 2.39 \times 10^{-7}$ ). Egg weight was not significantly affected by the diet ( $p = 6.13 \times 10^{-2}$ ). The feed to egg ratio was not affected by the diet, except for diet GF, which increased the feed to egg ratio ( $p = 3.73 \times 10^{-3}$ ).

**Table 4.** Production parameters of hens fed the control diet (C) or experimental diets (g/kg) containing 10% of mixed grains of lupin (6.5%) and oats (3.5%).

Item	C	F	Fd	G	Gd	GF	GFd	SEM	p-Value
Initial body weight (g)	1923	1986	1954	1977	1958	1948	1945	33.9	0.60
Final body weight (g)	1901	1954	1922	1881	1873	1868	1857	35.3	0.13
Body weight loss (g)/hen	22.5 <sup>b</sup>	31.7 <sup>b</sup>	31.5 <sup>b</sup>	96.2 <sup>a</sup>	85.0 <sup>a</sup>	79.2 <sup>a</sup>	87.5 <sup>a</sup>	5.4	$1.14 \times 10^{-12}$
Body weight loss (%)/hen	1.2 <sup>b</sup>	1.6 <sup>b</sup>	1.6 <sup>b</sup>	4.9 <sup>a</sup>	4.3 <sup>a</sup>	4.1 <sup>a</sup>	4.5 <sup>a</sup>	0.3	$1.34 \times 10^{-12}$
Daily feed intake (g)	137.8 <sup>a</sup>	137.4 <sup>a</sup>	137.0 <sup>a</sup>	130.2 <sup>b</sup>	127.3 <sup>b</sup>	131.2 <sup>b</sup>	130.0 <sup>b</sup>	2.3	$1.09 \times 10^{-7}$
Laying rate (%)	86.1 <sup>a</sup>	85.9 <sup>a</sup>	78.6 <sup>ab</sup>	76.3 <sup>b</sup>	74.5 <sup>b</sup>	76.0 <sup>b</sup>	75.0 <sup>b</sup>	3.5	$3.52 \times 10^{-3}$
% downgraded eggs	1.4 <sup>b</sup>	0.6 <sup>d</sup>	1.0 <sup>c</sup>	2.2 <sup>a</sup>	0.8 <sup>cd</sup>	1.6 <sup>b</sup>	1.2 <sup>c</sup>	0.2	$2.39 \times 10^{-7}$
Egg weight (g)	63.5	62.3	62.6	61.6	61.7	62.1	61.8	0.6	0.061
Feed to egg ratio	2.5 <sup>b</sup>	2.6 <sup>b</sup>	2.7 <sup>ab</sup>	2.7 <sup>ab</sup>	2.9 <sup>ab</sup>	3.0 <sup>a</sup>	2.8 <sup>ab</sup>	0.1	$3.73 \times 10^{-3}$

Diets: C: Control diet; F: fermented grains-supplemented diet; Fd: fermented grains-supplemented diet with dehulled oats; G: germinated grains-supplemented diet; Gd: germinated grains-supplemented diet with dehulled oats; GF: germinated fermented grains-supplemented diet; GFd = germinated fermented grains-supplemented diet with dehulled oats. Values are the means of 120 laying hens per treatment (n = 120). SEM = Standard error of the mean. Mean value within rows of diet with different superscript letters differ significantly at  $p < 0.05$ .

### 3.3. Egg Physical Parameters

There were no diet effects on the different parameters (height; diameter; shape index; contents and proportions of albumen, vitellus, and shell; shell thickness; Haugh units; and yolk color) (Table 5).

**Table 5.** Egg physical parameters of hens fed the control diet (C) or experimental diets (g/kg) containing 10% of mixed grains of lupin (6.5%) and oats (3.5%).

Item	C	F	Fd	G	Gd	GF	GFd	SEM	p-Value
Height (mm)	57.7	57.9	57.8	58.0	57.0	58.5	57.6	0.5	0.15
Diameter (mm)	44.7	44.6	44.7	44.1	44.3	44.6	44.7	0.3	0.45
Shape index (SI)	78	77	77	76	78	76	76	0.7	0.07
Albumen (g)	40.7	40.7	41.2	40.1	40.1	41.5	39.4	0.8	0.15
Vitellus (g)	16.9	16.8	16.7	16.1	16.1	16.5	16.1	0.4	0.06
Shell (g)	7.8	7.9	7.8	7.6	7.6	7.8	7.65	0.2	0.29
Albumen proportion (%)	62.2	62.2	62.6	62.8	62.8	62.9	62.4	0.6	0.66
Vitellus proportion (%)	25.8	25.6	25.4	25.2	25.2	25.1	25.4	0.4	0.64
Shell proportion (%) <sup>c</sup>	12.0	12.1	11.9	11.9	12.0	11.9	12.1	0.2	0.89
Shell thickness (mm)	0.42	0.43	0.43	0.43	0.43	0.42	0.43	0.0	0.53
Haugh unit	81.1	78.5	81.3	81.4	81.6	80.7	79.9	1.6	0.50
Yolk color (1–15)	11.2	11.7	11.7	11.7	11.1	11.5	10.6	0.5	0.18

Diets: C = Control diet; F: fermented grains-supplemented diet; Fd = fermented grains-supplemented diet with dehulled oats; G = germinated grains-supplemented diet; Gd = germinated grains-supplemented diet with dehulled oats; GF = germinated fermented grains-supplemented diet; GFd = germinated fermented grains-supplemented diet with dehulled oats. Values are the means of 18 eggs per treatment (n = 18). Shape index (SI) = (egg width/egg length) × 100. The yolk color scales from 1, pale yellow, to 15, dark yellow. SEM = Standard error of the mean.

### 3.4. Egg Composition

The chemical composition of albumen and vitellus is shown in Table 6. For the albumen, there was no diet effect on the dry matter and protein proportions. For the vitellus, the dry matter proportion was similar to the control diet for diet G and lower for diets F, Fd, Gd, and GF, and the highest dry matter proportion was obtained with diet GFd ( $p = 6.81 \times 10^{-4}$ ). In albumen and vitellus, the protein proportion was similar for all diets ( $p = 0.25$  and  $p = 0.10$ , respectively). The lipid percentage of vitellus was decreased with diet F compared with the control diet and the other experimental diets ( $p = 2.72 \times 10^{-4}$ ). The cholesterol percentage was decreased in all experimental diets compared with the control diet ( $p = 2.91 \times 10^{-11}$ ). There was no dietary effect on the fatty acid profiles.

**Table 6.** Chemical composition of egg from hens fed the control diet (C) or experimental diets (g/kg) containing 10% of mixed grains of lupin (6.5%) and oats (3.5%).

Item	C	F	Fd	G	Gd	GF	GFd	SEM	<i>p</i> -Value
Albumen									
Dry matter (%)	11.9	12.0	12.1	11.7	11.9	12.0	11.9	0.1	0.17
Protein (%)	10.4	10.6	10.9	10.7	10.6	10.9	10.5	0.3	0.27
Vitellus									
Dry matter (%)	51.9 <sup>b</sup>	51.4 <sup>cd</sup>	51.3 <sup>d</sup>	51.9 <sup>b</sup>	51.7 <sup>c</sup>	51.3 <sup>d</sup>	52.0 <sup>a</sup>	0.2	$6.84 \times 10^{-4}$
Protein (%)	16.7	16.8	17.2	17.1	17.1	17.1	16.9	0.2	0.102
Lipids (%)	33.9 <sup>a</sup>	31.8 <sup>b</sup>	32.8 <sup>ab</sup>	32.9 <sup>ab</sup>	33.7 <sup>ab</sup>	34.4 <sup>a</sup>	34.5 <sup>a</sup>	0.6	$2.72 \times 10^{-4}$
Cholesterol (mg/g of vitellus)	19.7 <sup>a</sup>	16.4 <sup>b</sup>	16.8 <sup>b</sup>	17.2 <sup>b</sup>	17.0 <sup>b</sup>	16.5 <sup>b</sup>	17.4 <sup>b</sup>	0.5	$2.91 \times 10^{-11}$
Fatty acid (FA) composition (% of total FA)									
SFAs	32.4	31.3	31.3	31.5	33.0	31.7	32.2	0.7	1.34
MUFAs	46.5	48.0	47.2	47.3	48.0	47.8	47.0	0.6	1.12
PUFAs	21.1	20.7	21.5	21.2	18.9	20.4	20.8	1.1	1.83
n – 6	19.3	19.0	19.7	19.4	17.3	18.7	19.0	1.0	1.70
n – 3	1.8	1.7	1.8	1.8	1.6	1.8	1.8	0.1	0.17
PUFAs/SFAs	0.6	0.7	0.7	0.7	0.6	0.6	0.6	0.1	0.08
18:2n – 6/18:3n – 3	23.3	26.9	26.2	24.8	28.1	28.0	25.1	2.3	3.88
n – 6/n – 3	10.7	10.8	11.0	10.7	11.0	10.5	10.4	0.5	0.67

Diets: C = Control diet; F: fermented grains-supplemented diet; Fd: fermented grains-supplemented diet with dehulled oats; G: germinated grains-supplemented diet; Gd = germinated grains-supplemented diet with dehulled oats; GF: germinated fermented grains-supplemented diet; GFd: germinated fermented grains-supplemented diet with dehulled oats. Values are the means of 18 eggs per treatment (n = 18). SFAs: Saturated fatty acids; MUFAs: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids. SEM = Standard error of the mean. Mean value within rows of diet with different superscript letters differ significantly at  $p < 0.05$ .

## 4. Discussion

A clear comparison of our results to the published literature is difficult because, to the best of our knowledge, there is no study comparing protein and lipid values in raw and fermented oats. The ash, lysine, methionine, threonine, and cysteine contents of the raw lupin grains were in the same range as results of [3], but in the present study, lipid and protein contents were higher. Fermentation increased total essential amino acid and total amino acid contents for lupin and oats. Furthermore, fermentation led to a higher content in essential amino acids, non-essential amino acids, and total amino acids in diet F compared with the other diets. The microorganism used for the fermentation and the hydrolytic breakdown of the nutrient components during fermentation may have caused the increase in AA content. In the present study, we found a decrease in essential amino acids (43.6 vs. 46.5 g/100 g protein, respectively, in germinated and raw lupin) and total amino acid content (91.1 vs. 93.9 g/100 g protein, respectively, in germinated and raw lupin). Ref. [3] found that germination resulted in a decrease in the content of several amino acids in lupin. However, there was an increase in total amino acid content by germination of oats (96.6 vs. 92.0 g/100 g protein, respectively, in germinated and raw oats), as seen in [19] for germinated oats. There was no effect of germination on the content of amino

acids in diets G, Gd, GF, and GFd. Soluble fibers composed 60% of the total fibers in raw oat grain, which concurred with the results of [5]. Oat hulls are a source of insoluble fibers. In our study, diets with dehulled oats showed less insoluble fibers than the same diet having hulled oat grains. This result was also observed by [5]. This study shows the impact of fermentation on increasing the nutritive value of the grains and the impact of the diets F and Fd containing these fermented grains.

Regardless of their dietary treatment, all hens showed a loss in body weight at the end of the trial, which was possibly due to their feed intake. Indeed, regardless of diet, germination of the grains led to the lowest feed intake and the highest body weight loss, while the daily feed intake was not significantly different in diets C, F, and Fd. There was a strong correlation between weight loss and feed intake ( $R^2 = 0.95$ ). In fact, studies on the effect of germination on feed intake are very controversial in poultry. The benefits of germination on poultry performance have been proven in some studies, such as the study on the use of germinated papaya seed in broilers [20]; however, others have revealed either no significant benefits in laying hens, as is the case for the study of [21] using pre-germinated fenugreek seeds, or a decreased performance of laying hens, as in the study of [22] using germinated malted sorghum, leading to a decrease of feed intake or a decrease in feed efficiency in broilers fed germinated common vetch seeds [23]. According to [3], the negative effect of germination on feed intake can be due to a reduction in palatability or due to the duration of germination (a long duration can activate antinutritional factors in lupin). In their study, the duration of germination of lupin was 4 days, as was the case in the present study. In lupin, the major antinutritional factor is alkaloid. Alkaloids usually reduce palatability and feed intake because of their bitter taste [3].

Regardless of diet type, all hens lost weight; this was likely exacerbated by the concomitant heavy infestation of poultry red mite (PRM, *Dermanyssus gallinae*) in the poultry house. In organic production, parasitism is a considerable health and welfare issue because none of the effective treatments against parasites can be used. Heavy infestations of red mite cause stress in poultry, a decline in production, weight loss, anemia, and in extreme, severe cases of blood sucking, lead to the death of the birds [24].

The best result for laying rate was obtained in the control group and groups having fermented grains (diets F and Fd), while poorer results were obtained for groups whose diets contained germinated grains. This result concurred with those of [22], who showed a decrease in egg production with increasing levels of malted sorghum sprouts introduced in laying hen diet. There are many studies on the use of fermented products in poultry nutrition [25]. Most of these studies show a positive effect of fermented feed on laying hen performance.

Fermentation led to a decrease in egg loss (broken eggs, abnormal eggshell color or egg shape, soft eggs) (respectively, 0.59 and 1.45% for diet F and diet C). The beneficial effect of fermented feed on downgraded egg percentage has already been described in laying hens [26,27]. This could be due to an increase in the bioavailability of feed nutrients and fermented microorganisms. The use of dehulled oats affects the percentage of downgraded eggs, improving it for diet Gd compared with diet G and for diet GFd compared with diet GF. Even if several authors show a link between dehulling and the percentage of downgraded eggs [5,9,26,27], the mechanism is not described. Dehulling normally increases the available nutrient content (increase in energy, protein, and fat contents compared with hulled oat grains) and decreases the fiber content [5,9,26,27]. That could explain the best results with naked grains. However, diet Fd led to a higher percentage of downgrading compared with diet F.

There is no diet effect on egg physical parameters. The lack of effect of diets containing fermented ingredients on egg component proportions has already been described by [28] with fermented rapeseed cake. The lack of an observed effect has not been elucidated, and the mechanism is still unclear. Another study using germinated and fermented soybean showed only an effect on eggshell thickness that was increased with the increased level of germinated and fermented soybean in the diet [29]. The Haugh unit is an important

item in evaluating albumen quality and egg freshness. The Haugh unit, which indicates the relationship of the height of the thick white to the weight of the egg in grams, is the most widely used measure of albumen quality and freshness of eggs. Haugh units were intermediate between the results of [26,27] (higher) and those of [29,30] (lower).

In monogastric animals, the composition of fatty acids stored largely reflects that of the ingested lipids, and the fatty acid composition of hen eggs can be changed by dietary means [31]. The fatty acid profiles of the experimental diets were quite similar and in the same range as those of [32] for their control group. Thus, there was no diet effect on the fatty acid profile in the eggs.

Egg cholesterol has always been a concern in relation to human health [1]. Our results on the albumen and vitellus composition of the control group are in the range of the results of [1] except for the cholesterol value, which is higher in the present study. The yolk cholesterol values for hens fed the control diet in the current study were higher than those found by [32], using fermented fish, but similar to those of [21], using pre-germinated fenugreek seeds. Yolk cholesterol content was lower in all the trial groups compared with the control group. Thus, cholesterol was decreased by fermentation and by germination. The reduction in egg cholesterol content, following dietary intervention in laying hens, has already been shown as a consequence of fermentation, using fermented Ginkgo-leaves, [33], and of germination, using malted sorghum sprouts [22]. The hypocholesterolemic effect of lupin is also well known and has been shown in several poultry species, and the mechanism of this effect is a reduction in plasma LDL cholesterol [34]. Oat grains are also hypocholesterolemic and reduce the serum total cholesterol in animals by a modulation of plasma LDL cholesterol [35]. This hypocholesterolemic effect could be due to the fiber content of lupin and oats, as it has been demonstrated in the past [36]. Therefore, the lupin and oats present in all experimental diets could also contribute to the decrease in cholesterol in the yolk.

## 5. Conclusions

In conclusion, these results showed that the inclusion of germinated grains in the diet (diets G, Gd, GF, and GFd) led to negative effects on laying performance and hen weight maintenance. Therefore, germinated lupin and oats cannot be considered as suitable for laying hens, regardless of the treatment they receive (germinated or germinated and fermented, with or without dehulled oats). However, diet F, containing fermented lupin and fermented oat grains, and to a lesser extent diet Fd, containing fermented lupin and fermented dehulled oat grains, have a positive effect on amino acid content, laying performance, hen weight maintenance, feed efficiency, and egg quality. Therefore, fermented lupin and oats as a mix of grains of 10% can be used in laying hen diet to partly substitute other sources of protein such as soya.

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**Institutional Review Board Statement:** The hens used were reared in compliance with regulations for the humane care and use of animals in research in Europe, according to [7]. National authorization to experiment on living animals (n°3502) has been delivered to M. Kouba by the French Minister of Agriculture. In this study, although the hens were weighed, ethical approval was not required due to the absence of any invasive practices such as blood sampling, surgical procedures, or slaughter. The hens were raised and kept on a farm according to [8].

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

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