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Effect of feeding two-grain legumes, peas and faba beans, on egg quality and laying performances

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Egg physical and nutritional quality.
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SUMMARY

Soymeal is now the main source of protein laying hens. This raw material is mainly produced abroad which makes Europe highly dependent on imports. A better protein autonomy could limit this economic impact and the environmental issue related to the cultivation of soybean. One of the options regularly studied over the past few decades to increase protein production in Europe is the cultivation of protein crops such as peas, faba beans and lupin. However, some inconvenient can limit their use in animal food as an alternative to soymeal, i.e., lower protein levels; presence of anti-nutritional factors; and non ideal amino acid profile. Therefore. te aim of this work was to assess the impact of using peas and faba beans as an alternative to soybean, on hens laying performances as well as on physical and nutritional eggs quality, including fatty acids profile. Two diets were tested, one containing only 22% of soymeal as proteins source (control group); and the second contains 45% of two leguminous plants (30% of peas and 15% faba beans) supplemented to 6% of soy meal. The egg-laying rate was significantly lower for the experimental group (51.79 vs. 60.63 %, p <0.05). However, no significant effect (P > 0.05) was found concerning physical (egg weight, weight, shell weight and weight, shell thickness and strength), and nutritional parameters (cholesterol and essential fatty acids).

Efecto de la alimentación de dos legumbres de grano, guisantes y frijoles faba, en la calidad del huevo y la puesta

RESUMEN

La harina de soja es ahora la principal fuente de gallinas ponedoras de proteínas. Esta materia prima se produce principalmente en el extranjero, lo que hace que Europa dependa en gran medida de las importaciones. Una mejor autonomía proteica podría limitar este impacto económico y la cuestión ambiental relacionada con el cultivo de soja. Una de las opciones estudiadas regularmente en las últimas décadas para aumentar la producción de proteínas en Europa es el cultivo de cultivos proteicos como guisantes, habas de faba y lupin. Sin embargo, algunos inconvenientes pueden limitar su uso en alimentos animales como alternativa a la harina de soja, es decir, niveles más bajos de proteínas; presencia de factores anti-nutricionales; y perfil de aminoácidos no ideal. por lo tanto. El objetivo de este trabajo era evaluar el impacto del uso de guisantes y frijoles faba como alternativa a la soja, en las prestaciones de puesta de gallinas, así como en la calidad de los huevos físicos y nutricionales, incluido el perfil de ácidos grasos. Se probaron dos dietas, una que contiene sólo el 22% de la harina de soja como fuente de proteínas (grupo de control); y la segunda contiene el 45% de dos plantas leguminosas (30% de guisantes y 15% de habas de faba) complementadas al 6% de la harina de soja. La tasa de puesta de huevos fue significativamente menor para el grupo experimental (51,79 frente a 60,63 %, p <0,05). Sin embargo, no se encontró ningún efecto significativo (P > 0,05) con respecto al físico (peso del huevo, peso, peso y peso de la cáscara, grosor y fuerza de la cáscara), y parámetros nutricionales (colesterol y ácidos grasos esenciales).

PALABRAS CLAVE
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INTRODUCTION

To date, the soymeal is the most commonly used protein source in animal feed, especially for monogastric animals. In fact, it presents approximately two thirds of protein-rich materials (Laudadio & Tufarelli, 2011a; De Visser et al., 2014). In 2017, the European Union was far from being self-sufficient for producing protein-rich food, whose imports reached 65% (Terres Univia, 2018). European soybean meal production remains low, about 3% of needs (de Visser et al., 2014). The soymeal used comes mainly from the American continent ensuring the production of 87% of the 348 millions tones produced worldwide, in 2016-2017 (Terres Univia, 2018). Reducing dependence on imported soybeans whose the price is volatile (Laudadio & Tufarelli, 2010a), is important for the poultry sector because the animal food corresponds to more than half of production costs in Europe (van Horne, 2019). Moreover, as soybean is often produced from genetically modified organisms (GMOs) which are perceived rather negatively by the consumer (Laudadio & Tufarelli, 2012a). They are also not authorized in organic farming and the additional cost linked to the use of non-GMO soybean meal can reach $ 150 per tones (de Visser et al., 2014). Among the alternative sources of protein, certain grain legumes such as peas, lupin, faba beans, and chickpeas which can be cultivated in different regions of Europe (GL-Pro, 2005). However, the areas currently devoted to their culture in Europe are negligible (1 to 7%, depending on the country) and this part is even tending to decrease (Mahmood et al., 2017). It is to note that certain nutritional characteristics make protein rich leguminous plants difficult to use. Indeed, the protein percentage) varies from around 20% for peas and chickpeas, 26% for faba beans to 34% for lupine, while soybean meals generally reach 46% (INRA CIRAD AFZ, Feed tables). Compared to soy meal, the amino acid balance of these legumes is not ideal because they generally lack of sulfur-containing amino acids, i.e., Met and Cys (Perez-Maldonado, 1999). In contrast, it is possible to compensate them by adding synthetic amino acids, except in organic farming in which their use is prohibited (Bordeaux & Roinsard, 2015; Hammershoj & Steenfeldt, 2005). Protein rich legumes contain antinutritional factors (ANF) such as tannins, alkaloids, glycosides, enzymes inhibitors, and fibers (Bordeaux & Roinsard, 2015) which limit their incorporation rates into hen rations. Soybean grains also contain ANF which makes their use without heat treatment impossible; this is why it added to rations under a meal form. In addition to affect laying performance, the physical and nutritional quality of eggs can also be influence by partial or total substitution of soybean meal with rich protein legumes (Drażbo et al., 2014; Kiczorowska et al., 2016; Rutkowski et al., 2017).

Therefore, the objective of this work was to evaluate the use of peas and faba beans in laying hens diet as an alternative to a soy meal, as well as the influence on egg quality and laying performances.

MATERIAL AND METHODS

Formulation of laying hens feed ration

In the present work, peas (30%) and faba beans (15%) have been included in the experimental hens ration containing only 6% of soymeal which serves to complete protein portion required. These proportions were chosen according to some nutritional criteria. In fact, laying hens tolerate well peas whose incorporation rates can reach 50-60%, while, high proportions of faba beans had shown some adverse effects (ref). Indeed, Laudadio and Tufarelli (2010a) got satisfactory results with a rate of 24% but with using treated seeds. The laying hens ration is classically composed of wheat and maize. These two ingredients have been balanced to provide the closest possible inputs and meet the nutritional recommendations (NRC, 1994; Hans-Heinrich, 2017). Synthetic lysine and methionine were added to a control ration in order to balance a food rations. However, only Methionine was added to the experimental ration, indeed, peas and faba bean are rich in lysine. The constituents of both rations, as well as calculated inflows are reported in Table I. According to the National Research Council formula (NRC, 1994), for a 2 kg laying hen with a laying rate of 90% (eggs of 60g), and 15°C of temperature, the need for metabolized energy is about 354 kcal per day. To provide this daily energy, the hen should consume 126g of food (2800 kcal). The amount of food needed for the experiment (4 weeks of experimentation, and 10 days before corresponding to the installation of laying hens) is therefore 4.8 kg per hen (about 50kg per group).

Animals and housing

Twenty 36-weeks-old Lohmann Brown laying hens were used. They were randomly distributed in two groups of ten hens each (Experimental and control group). A total of ten lodges were used to house the 20 hens, i.e., two hens by a lodge of 2 m2, provided with 65cm long perch. Chickens were breaded in 16 hours of light, with a continuous nighttime rest period without artificial light (8 hours). The experience has been carried for 4 weeks (from February 12 to March 12) and began about ten days after the installation of the hens. The rations were distributed in "bucket" feeders, the control group received cereals and soybean meal basic ration, while, the "Experimental" group received cereals and protein rich leguminous, i.e. peas and faba beans.

Table I. Hen rations composition for control and experimental groups (Composición de raciones de gallina para grupos de control y experimentales**).**

Ingredients (%)	Control	Experimental
Corn	32.00	30.00
Wheat	38.67	15.00
Soymeal	21.68	6.00
Peas	0.00	30.00
Faba beans	0.00	15.00
Bran	4.91	0.70
L-Lys	0.03	0.00
L-Met	0.08	0.11
Chalk	2.63	2.96
Phosphate biCa	0.00	0.23
Dietary intake :		
ME kcal/kg	2800.00	2763.11
CP %	17.00	16.59
Ca %	1.11	1.28
P %	0.40	0.40
Met dig. Poultry %	0.30	0.30
Lys dig. Poultry %	0.70	0.99

ME : Metabilisable Energy ; CP : Crude Proteins

Data collection

Hens were weighed at the beginning and at the end of the experimental period. The eggs were collected once a day. The number of eggs harvested, their weight and the amount of food consumed were also recorded daily during the experiment. The food conversion index to eggs (ratio between the mass of food consumed and the mass of laid eggs) was measured in periods ranging from 0 to 2 weeks; and 2 to 4 weeks. Eggs physical characteristics and cholesterol level were tested on February 26 and March 12 (on 10 eggs already laid less than 48 hours ago, from each group). Essential fatty acids (docosahexaenoic acid: DHA; and α–linolenic acid: ALA) were dosed once, at the end of the experiment (March 12) on 6 eggs from each group.

Eggs Physical Characteristics

Eggs were numbered and weighed with an electronic precision balance of 0.001g. A 0.01 mm caliper was used to measure the length (L) and the width (W) of each egg. The maximum force of egg shell rupture (Solidity of the egg shell) was supported using a device: Universal Tensile System (Moula et al., 2009), which exerts pressure on the shell and takes the measurement when the shell begins to crack. Eggs were then broken; and the yolk and the shell were weighed. The thickness of the shell was determined, using a 0.001 mm precision micrometer, from the average of 3 measurements (taken from the equatorial part). The albumen weight was then determined by subtracting the weight of the yolk and the shell from whole egg weight (Hartmann et al., 2003). The height of the albumen is obtained with a 0.001 mm precision tripod and Haugh units (HU) were calculated: HU = 100log $(H - 1.7xW0.37 + 7.6)$; which are the indicator of the egg freshness, but also the age and genetics of the hen (Roberts, 2004, Moula et al., 2013)

Egg Nutritional Characteristics

Cholesterol content

The whole egg cholesterol content was measured twice (2 and 4 weeks of the experience), on 10 eggs from each group, using spectrophotometer FoodLab ® Analyzer. Yolks and albumens were mixed; then 5 µl of samples were introduced and mixed in a spectrophotometric cuvette prefilled with a reagent. After 5 minutes of incubation, a second reagent was added and mixed and the measurement was carried 3 minutes later. The enzymes present in the reagents allow a reaction between the cholesterol and a phenol derivative, forming so a pink color complex measured at 505 nm and which is proportional to a cholesterol concentration.

Essential Fatty Acids Analysis

The dosage of two essential fatty acids was realized by the gas chromatography (GC). The yolk of 6 eggs from each group recovered at the end of the experiment was subject of these analysis three times. Before launching essential fatty acids GC analysis, a standard solution containing a methyl ester C15 fatty acid (100µg/ml) was used to test the accuracy of GC analysis. Egg yolk was first dried by lyophilization. 50 mg egg yolk powder was weighed into 20 ml Pyrex tubes for each sample, supplemented then by 1 ml of a working standard solution. The esterification of fatty acids contained in the egg yolk, was carried by 2 ml of a methanolic basic solution (0.5N) or a sodium methanolate (CH3ONa). The tubes were closed hermetically, vortexed and then incubated for 20 minutes at 75°C. Once samples were cooled, 1 ml of distilled water was added in order to separate the phases. 1 ml of hexane was added to remove the lipophilic part more easily and then dried by spelling it to tubes containing anhydrous sodium sulfate. 1 ml of this fatty acids extract was recovered, placed in an injection vial, and injected for GC analysis (Harvey, 2019).

Statistical analysis

A descriptive statistical study was conducted on the data generated in this study. The effects of the food ration, the experimentation time and their interactions were studied using the SAS software (GLM procedure, Statistical Analysis System, 2001). The Chi2 test was used to compare egg-laying rates between the two studied groups. The statistical meaning threshold was set at P<0.05.

RESULTS AND DISCUSSION

Laying performances

The laying rates were significantly lower (P <0.01) in the experimental group compared to the control, during the first two weeks only (55.71 vs. 70%; and 44.9 vs. 54.29%, for the first and the second week, respectively). This significance was also found (P <0.05) for the whole experimentation period (51.79 vs. 60.63%). The negative effect of a food ration on laying rates, is according to Rutkowsky et al. (2015a, 2017b) generally observed around the 10th week of the experimentation, and mainly linked to the incorporation of high proportions of protein rich leguminous. In parallel, Hammershoj & Steenfeldt (2005) found a lower egg-laying rate when 25% of lupin was incorporated in their ration and this earlier at the 3rd week, such as in the present work (after 2.5 weeks of experimentation). In other side, the egg-laying rates were significantly affected by the age, particularly for the control group, where a significant drop was observed (from 70% in the first week to 52.86% in the fourth week). In contrast, in the experimental group, the egg laying rates were globally stable at around 54%, except for the second week where it dropped to 44.29%. This egg laying decrease is normal in 36-weeks-old hens. In fact, the egg-laying pick of this hen strain is reached around 31 weeks of age and then egg laying rate decreases a course time (Lohmann Tierzuch, 2017). However, this decrease is much lower than that observed here: about 1% in 4 weeks compared to the 17% observed for the control group (**Table II**). It is however a little difficult to link these changes to the ration. Indeed, the beginning ant the end of egg-laying can cause significant changes in egg-laying rates in one side and a small number of hens used in experiment in the other side.

Food conversion index

The feed conversion index (FCI) to eggs (ratio between the mass of food consumed and the mass of laid

Table II. Hens laying performances per a week for experimental and control groups (%) (Cuadro II. Henslaying performances per una semana para grupos experimentales y de control (%)).

(%)	Control	Exp	ESM	Age Effect (A)	Diet Effect (D)	Effet AXD
Week 1	70a	54.29b	3.66	*	**	NS
Week 2	55.71a	44.29b	3.66			
Week 3	62.86	54.39	3.66			
Week 4	52.86	54.18	3.66			
TOTAL	60.63a	51.79b	1.83		*	

* : P<0.05 ; ** : P<0.001 ; NS : P>0.05.

Table III. Hens. Average Weights, and index conversión food to eggs for control and experimental groups (Gallinas. Pesos Promedios, e indexar la conversión de alimentos a huevos para el control y grupos experimentales).

	Control	Exp	ESM
Hens Average Weight			
Begining	2070.8	2050.1	21.5
End	2024.0	1952.1	28.6
Food Conversion Index			
Weeks 0 - 2	3.27	3.56	0.15
Weeks 2 - 4	3.48	3.50	0.16
TOTAL	3.38	3.53	0.12

Non Significant Effect (P>0.05) have been observed of age, groups and the interraction groupe*age, for hens weight and food conversion index.

eggs) was not significantly (P> 0.05) different between the two groups (**Table III**).

It was however numerically higher in the experimental group compared to the control (3.53 vs. 3.38, respectively). These values are also higher than those usually found (approximately FCI=2). In the present work, the egg-laying rates were too low comparing to those of commercial hens typically reaching 90% (Lohmann Tierzuch, 2017). Consequently, it is normal for the food conversion index to be higher especially that the feed is not only used to meet production needs but also maintenance needs. For example, Rutkowsky et al. (2015) observed at the beginning of the third week (just at the beginning of egg-laying), a food conversion index close to 3 when the egg laying rate was around 65%. We can explain high values of FCI found here by the significant waste of food observed during the experiment, because of a coarsely crushed mixture form of food supplied and design of recipes used as feeders in this work. In other side, the uniformity of hen food ration is very important to avoid sorting which may exclude smallest particles less than 0.5 mm comprising synthetic amino acids (lysine and methionine for the control ration and methionine for the experimental ration); chalk and bicalcium phosphate. Ideally 80% of the particles should be between 0.5 and 3.2 mm in size (Safaa et al., 2009). Rations poor in lysine and methionine can impact laying performances by increasing the conversion index and affecting hen growth (Hammershoj et al., 2005). In fact, in the present work laying hens weight loss was also observed (**Table III**), with no significant difference-P> 0.05-between the control and experimental groups.

Physical egg characteristics

In the present work, eggs weight in control an experimental groups was not significantly different (63.19 vs. 61.38g: P>0.05). This parameter is influenced by many factors, including age, hen weight, race-strain, laying rate, livestock system, etc (Ledvinka et al., 2012). A decrease in egg weight following the incorporation faba bean in hen rations could be attributed to its content in vicine-convicine (Lessire et al., 2005). This is often reported when the faba bean rate of incorporation into rations reach 10% (Koivunen et al., 2014) and/or 16.5% (El-Hack et al., 2017; Alagawany et al., 2019). However, Igbasan and Guenter (1997) showed this negative impact at the incorporation rate of 60% which is much higher than that used here. In other there were no significant differences (P-0.05) between the groups in the length and width and (**Table IV**). Furthermore, no significant differences (P> 0.05) had been recorded between the experiment and the control group for yolk and albumen weights, in addition to Haugh units indicator of freshness (**Table IV**).

However, Fru-Nij et al. (2007) observed a decrease in the proportion of yellow relative to albumen and thicker albumen when the incorporation of beans increased in a hen ration. A thinner and less resistant egg shell is sometimes found as a result of the incorporation of peas and faba beans into hen diet (Igbasan & Guenter 1997; El-Hack et al., 2017). There were no significant differences here (P> 0.05) between two studies groups in terms of shell weight, thickness or strength (Fmax). In contrast, the thickness of the egg shell decreased significantly (P-0.05) between the 2nd and the 4th week of experiment (**Table V**). Many factors can affect egg shell quality such as breed or strain, age, stress, and calcium and phosphorus intakes (Roberts, 2004). Regarding calcium levels, control and experimental rations formulated here contained a lower level of calcium than recommended. In fact, daily intakes were about 1.5 vs. 3.4g (An et al., 2016). In addition to reduce shell thickness, insufficient calcium intake can lead to osteoporosis and an increase in laying hens fractures (Webster, 2004), which could have been problematic if the experiment had been extended beyond 4 weeks.

Nutritional quality of eggs

Egg Cholesterol

The egg is considered cholesterol rich aliment; indeed, it contains about 372 mg per 100 g. However, the

Table IV. Eggs physical characteristics (Características físicas de los huevos).

		Hens Weights (g)	Height (mm)	Width (mm)	Yolk Weight (g)	Albumen Weight (g)	Haugh Units
2 Weeks	Cont.	61.75	55.9	44.23	17.43	36.55	86.08
	Exp.	60.72	56.61	43.91	16.86	36.74	89.56
	ESM	1.55	0.64	0.4	0.46	1.2	1.53
4 Weeks	Tém.	64.63	57.7	44.88	18.23	38.75	89.15
	Exp.	62.04	57.17	44.07	18.25	36.12	88.96
	ESM	1.55	0.64	0.40	0.46	1.2	1.53
TOTAL	Cont.	63.19	56.8	44.56	17.84	37.45	87.61
	Exp.	61.38	56.91	43.99	17.83	36.43	89.26
	ESM	1.1	0.45	0.28	0.33	0.85	1.29
Effects :							
Week (W)		ns	ns	ns	ns	*	ns
Group (G)		ns	ns	ns	ns	ns	ns
W*G		ns	ns	ns	ns	ns	ns

Significant differences are marqued by * (P<0.05)

link between dietary and blood cholesterol has recently been questioned (Shinn et al., 2018). In fact, current recommendations, both European (EFSA, 2010) and US (Soliman, 2018), have not yet set a limit for cholesterol intake. As a precaution, different organisms still advise to limit cholesterol intakes (not exceed 300 mg per a day) because it is not an essential nutrient and the body is able to synthesize enough (Puertas et al., 2018). In the present work no significant differences were found for whole egg cholesterol content between experimental and control group (0.334 g/ 100g an 0.345 g/ 100g, respectively). Same results were found by Krawczky et al. (2015) who used lupin in rations. The effect of protein rich leguminous in hen rations on egg cholesterol levels is rarely studied, contrary to hen chair and blood. A decrease in blood cholesterol levels was observed with a lupin-based ration by Viveros et al., (2007) and Rubio et al., (2003). The addition of peas to the guinea fowl ration was accompanied by a decrease in the amount of cholesterol in the muscle (chest) (Laudadio et al., 2012b). However, Alagawany et al., (2019) reported a significant increase on the blood LDL-cholesterol level, as a result of using beans in laying hens ration, (5.5%, 16.5% and 22% of incorporation rate)

Egg essential fatty acids

In recent publications, the link between cholesterol and saturated fatty acids had (SFA) been established. In fact, cholesterol-rich foods are often rich in SFA: saturated fatty acids (EFSA, 2010; Puertaset al., 2018). The profile of FA is modifiable according to the hen's diet. In fact, it is possible to get considerable FA amounts such as, multiplying the amount of ALA by 20 (when flax seed oil was added to the ration) or by 6 the amount of DHA, through the addition of fish oil (Soliman, 2018; Shinn et al., 2018). Zdunczyk et al. (2014); and Krawczyk et al. (2015) have shown that replacing the soy meal by lupin have caused only slight changes in fatty acids profile of eggs. Concerning the broiler meat, the inclusion of 31% of faba beans (micronized and skinned) in a ration caused important changes in fatty acids profile with differences between the muscles analyzed (white and pestle). The changes were higher levels of DHA, a slight decrease in monounsaturated fatty acids and an increase in PUFA (polyunsaturated fatty acids: oméga 3: alpha-linolénic acid - 18:3, n-3 (Laudadio et al., 2011b). In other studies, different results were found, indeed, Bosco et al. (2013) and Sirri et al. (2010) recorded a decrease in the amount of PUFA in muscles, as well as changes in omega 3 and oméga 6: linoléic acid - 18:2, n-6. In other hand, micronized peas at 25% and 49% incorporated in rations, depending on

Table V. Eggs shell physical parameters (Parámetros físicos de las cáscaras de huevos).

		Shell %	Average Thickness (mm)	Fmax (N)
2 Weeks	Cont.	12.59	0.398	39.48
	Exp.	11.75	0.39	34.89
	ESM	0.32	0.008	2.2
4 Weeks	Cont.	11.85	0.366	35.24
	Exp.	12.44	0.383	35.97
	ESM	0.32	0.008	2.2
TOTAL	Cont.	12.22	0.387	37.36
	Exp.	12.09	0.382	35.43
	ESM	0.22	0.006	2.1
Effets :				
WeeksW)		Ns	*	Ns
Group(G)		Ns	ns	Ns
S*G		*	ns	Ns

Significant differences are marqued by * (P<0,05)

Table VI. **Cholesterol and fatty acids content** (Contenido de colesterol y ácidos grasos).

		Whole egg Cholesterol (g/100g)	DHA (µg/ml)	ALA (µg/ml)
2 Weeks	Cont.	0.338	-	-
	Exp.	0.347	-	-
	ESM	0.013	-	-
4 Weeks	Cont.	0.353	269.49	167.07
	Exp.	0.321	248.65	176.08
	ESM	0.013	16.61	25.16
TOTAL	Cont.	0.345	269.49	167.07
	Exp.	0.334	248.65	176.08
	ESM	0.009	16.61	25.16
Effects :				
Week (S)		Ns	-	-
Group (G)		Ns	ns	ns
W*G		Ns	-	-

No significant differences observed (P> 0.05).

the growth stage, caused a decrease in SFA profile; an increase in n-3 (DHA and ALA); a decrease in the ratio n-6/n-3; and a significant reduction in the percentage of fats (Kiczorowska et al., 2016). Additionally, Laudadio & Tufarelli (2010b) also have assessed the same impact following incorporation 40% peas with similar results in fatty acids profiles. In most publications, changes in egg or meat fatty acids composition can be explained directly by the influence of the hen ration. Only Sirri et al. (2010) suggested that the lowest rate of PUFA after using faba beans-based ration may be caused by a decrease in their absorption due to antinutritional factors. Laudadio & Tufarelli (2010b) showed an increase in the n-3 content of the hen ration after replacing soy meal by peas (1.67 to 2.12%). In other side, when adding faba beans in hen rations, Bosco et al. (2013) noted an increase in saturated and monounsaturated fatty acids and a decrease in PUFAs while Sirri et al. (2010) observed only minor variations in fatty acid composition, which can be attributed to the diversity in faba bean variety .In the present study, no significant difference (P>0.05) had been found between control and experimental groups, for DHA and ALA content (69.49g/ml and 167.07g/ml vs. 248.65g/ml and 176.08g/ml, respectively). It is difficult to know whether this is related to a similar fatty acid profile for the two rations or whether other factors are involved because the rations fatty acid profile used here was not established (**Table VI**).

CONCLUSION

Replacing soy meal with a mixture of raw peas and faba beans led to satisfactory results because of no influence the physical and nutritional characteristics of eggs was found; on the other hand, egg-laying performance was negatively influences, with the exeption of feed conversion index.

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