Effect of Vegetable By-Products Supplements on Coefficients of Apparent absorption from laying Hen Diets

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Abstract

The *in vivo* feeding trial was conducted on 6 weeks with 126 Tetra SL laying hens, 56-week old, assigned to 3 groups. The layers were housed in an experimental hall with controlled microclimate, in three-tier digestibility cages (2 layers per cage) which allowed the daily recording of the feed intake and excreta. All hens received a conventional compound feed (C), based on corn and soybean meal, which contained 2750 kcal/kg metabolizable energy and 17.50% crude protein. Each experimental group had as supplement one fat and one antioxidant vegetal source, rapeseed meal and grapeseed meal (RGM) respectively flaxseed meal and buckthorn meal (FBM). The balance study was conducted for 5 days, on the last experimental week. During this period, average weights from each cage (6 samples per group) of feed and excreta were recorded, collected and sampled for: dry matter (DM), crude protein (CP), ether extractives (EE), crude fibre (CF) ash and amino acids determinations from feeds. The layers from FBM group had significantly ($P<0.05$) higher concentration of ingested, excreted and digested amount of CP and the lowest ($P\leq0.05$) EE and CF absorption coefficient. Significantly higher ($P<0.05$) values for ingested, excreted, digested and absorption coefficient of CF nutrient, was registered for RGM group.

Keywords: amino acids, by-products, feeds, laying hens, nutrient digestibility

1. Introduction

In recent years, the increasing price of some raw material, like soybean has become a critical aspect for the economic sustainability of the poultry feed industry, particularly in some developing countries [1]. The evaluation of alternative feed ingredients that are affordable and locally available as substitutes for conventional meals is therefore required [2]. The use of vegetal by-products as alternative sources of fat, protein, antioxidants in animal feeds is becoming more globally appealing. Meals constitute a raw material that is included in the European Union Feed Material Register, and represent a suitable ingredient for feed manufacturing for pigs and poultry in the near future. This aspect represents a step forward to combat the severe challenges of the global capacity to supply sufficient food. By re-using the residues resulted from different manufacturing (grains, cereals, plants etc.) in animal diet, we are also helping the environment by combating the soil pollution, which is a matter of concern nowadays [3].

The meals produced from by-products have historically been viewed as products with high variability in their biochemical composition, with high levels of ash and low digestibility. In this context, different types of meals, resulted from oil extraction, have captured the interest as complementary sources of protein, amino acids, fatty acids, vitamins, antioxidants, trace elements [4]. Numerous authors have reported interesting results about the suitability of different types of meals as diet ingredients for livestock animals like poultry or pigs [5-7]. A variety of ingredients which include poultry by-products mixed with other by-products or ingredients are commercially available and a distinction must be made [8]. The label of meals shall include the raw material used

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and must guarantee minimum concentration of CP, CF, P and Ca.

Among these vegetal by-products used in poultry diets flaxseed meal is the most common source used and known as PUFA omega 3 fatty acid especially regarding the C18:3n3 fatty acid, followed by rapeseed meal which is rich in PUFA omega 6, while grapeseed meal and buckthorn meal are rich in antioxidants, polyphenols, vitamins, bioactive components and other valuable nutrients [9, 10]. According to [11] the utilization of nutrients, including amino acids, in feed ingredients may vary according to the physiological status of the bird. The absorption of nutrients of feed ingredients may depend upon the age, genotype and gender of the birds used in the experiment. From an ecological perspective, the need to improve the utilisation of dietary protein and to minimise nitrogen output in manure are increasingly important global targets. Careful attention to digestibility of nutrients in raw materials during feed formulation can make a major contribution towards meeting these objectives [12].

The present study was conducted with layers to determine the nutritional value of compound feeds, albumen quality and apparent nutrient digestibility of nutrients in three dietary feed ingredients.

2. Materials and methods

All animal experiments were conducted in compliance with the European Union Directive 2010/63/EU and in accordance with regulations set by the Ethical Commission of National Research and Development Institute for Biology and Animal Nutrition. The trial was conducted for 6 weeks on 126 Tetra SL (56 weeks old), assigned to three groups (C, RGP, FBM). Compared to the control diet (C), the diet formulations for the experimental group RGM included 9.5% rapeseed meal (RM) and 3% grapeseed meal (GM) meal, while FBM group included 8.73% flaxseed meal (FM) and 3% buckthorn meal (BM) (Table 1). The laying hens used in this experimental design have been housed in an experimental hall, with controlled microclimate Big Dutchman system type (average temperature 19.61±1.38ºC; humidity 71±23%), fitted with three-tier digestibility cages (2 layers per cage), which allow accurate weighing of the daily ingesta and excreta.

Table 1. Compound feeds formulations

Ingredients, % as feed basis	C
Corn	57.16
Soy meal	21.24
Sunflower meal	7.00
Vegetal oil	2.02
Methionine	0.10
Choline	0.05
Calcium carbonate	9.91
Phosphate	1.12
Salt	0.35
Mycofix	0.05
Premix*	1.00
Total	100.00

*1 kg premix contains: 1350000 IU/kg vit. A; 300000 IU/kg vit. D3; 2700 IU/kg vit. E; 200 mg/kg Vit. K; 200 mg/kg Vit.B1; 480 mg/kg Vit.B2; 1485 mg/kg pantothenic acid; 2700 mg/ kg nicotinic acid; 300 mg/kg vitamin B6; 4 mg/kg vitamin B7; 100 mg/kg vitamin B9; 1.8 mg/kg vitamin B12; 2500 mg/kg vitamin C; 7190 mg/kg manganese; 6000 mg/kg iron; 600 mg/kg copper; 6000 mg/kg zinc; 50 mg/kg cobalt; 114 mg/kg iodine; 18 mg/kg selenium

The light regimen was 16 h light/ 8h dark, recommended by the breeding guide [13]. Feed and water were supplied ad libitum. At the end of the trial were collected randomly 18 eggs/group (3 eggs/sample) to determine the external and internal quality parameters of the egg's albumen.

Sampling: The compound feeds samples and the average samples (6 samples per group) of excreta, were analysed for the dry matter (DM), crude protein (CP), ether extractives (EE), crude fibre (CF) and Ash (ash). By calculation we determined the organic matter (OM), and nitrogen-free extractives (NFE).

Proximate composition. The chemical analyses were performed using methods from Regulation (CE) no. 152/2009 (Sampling and analytical methods for the official inspection of feeds): gravimetric method for the DM; the Kjeldahl method for the CP; extraction in organic solvents for the EE; acid hydrolysis followed by alkaline hydrolysis for CF; gravimetric method for the ash. The amino acids determination from samples of feeds was performed by HPLC, as described by [14].
Egg albumen measurements were performed on six samples (3 abumens per sample) formed from the collected eggs (18 per group) and assayed for egg freshness using the Haugh unit and the Egg Analyzer TM).

Digestibility study
The coefficients of apparent absorption of the nutrients were determined using the balance technique in the 6th week. The hens were fed on the experimental diets for six weeks, and during the last five days, feed intake was recorded daily for the individual birds. Fresh droppings (faeces plus urine) were collected at the same hour and weighed once times a day for the individual birds, kept individually and stored in refrigerator (4^{o}C). Average samples (6 samples per group) were formed, homogenized and oven dried, for 48 h at 60^{o}C. At the end, the collected excreta per bird were pooled, mixed, grounded and stored respectively for chemical analyses.

Calculation: The recorded data on the amounts of ingested feeds and excreta, corroborated with the analytical data regarding the concentrations of nutrients in the feeds and droppings, allowed making the balance calculations. The absorbed amount was considered to be the difference between the ingested amount of nutrients and the amount of excreta, determined according to the following formula: Apparent nutrient digestibility=[(NI-NE)/NI] x100 where, NI represented the nutrient intake and NE expressed the nutrient excreted [15]. The coefficient of apparent absorption was calculated as the ratio of the absorbed amount of nutrient to the amount of ingested nutrient, multiplied by 100.

Statistical analysis
The statistical analyses were performed using STATVIEW software for Windows (SAS, version 6.0). The data were subjected to one-way ANOVA. The differences between the average values within the groups were considered significant for P<0.05.

3. Results and discussion

The nutrient profiles of the four meals (RM, GM, FM and BM) were previously reported by Panaite et al. [5]. The GE content of FM (19.31 MJ/kg) was higher than that of RM (16.94 MJ/kg), while GM (18.07 MJ/kg) and BM (18.94 MJ/kg) had close values. However, CP content was higher in RM (33.15%) than in GM (11.91%), but the BM crude fat constituted 12.19% compared with 1.04% in RM. The calculated nutrient compositions and fatty acid profile of the experimental diets are shown in Table 2.
The results of the determinations show that the compound feeds were balanced as energy and protein content. The dietary crude protein level depends on the level balance and digestibility of the essential amino acids in the finished homogenized feed [16]. The use of FM supplement in the diet formulations for the FBM group, significantly enriched in α linolenic acid (PUFA Ω 3 acid), with 88.68 higher compared to C and with 91.35 higher compared to RGM.
This difference regarding the content of α linolenic acid in the compound feeds is due to the fact that RM and GM are rich in Ω6 fatty acids, while BM has a low concentration which is similar to FM. GM and BM which act as natural antioxidants, were added in order to prevent the adverse effects of the potential antinutritional factors present in the two oleaginous meals, RM and FM, one enriched in PUFA Ω3 and the other in Ω6.
The protein of these feeds contained several essential amino acids such as threonine, glycine, methionine, valine, isoleucine, leucine, lysine, and phenylalanine (Table 2). The amino acid analysis of the compound feed revealed that the lysine concentration ranged between 0.837-1.021% (g/100 g DM), cystine between 0.288-0.311% DM and methionine between 0.331-0.390% (g/100 g DM).

Table 2. Data on nutrients content of compound feeds

Nutrient	C	RGM	FBM
ME, Kcal/kg	2750	2750	2750
∑PUFA Ω 3	1.570	1.200	13.88
DM, %	89.65	90.32	90.12
CP, %	17.43	17.00	17.90
EE, %	3.740	5.830	3.310
CF, %	4.720	6.320	4.780
Ash, %	13.13	13.86	15.20
Indispensable amino acids (g/100 g DM)			
Lysine	0.837	0.838	1.021
Cysteine	0.290	0.311	0.288
Methionine	0.331	0.345	0.390
Threonine	0.816	0.917	0.878
Valine	1.019	1.138	1.117
Phenylalanine	1.041	1.000	0.895
Isoleucine	0.896	0.854	0.898
Leucine	1.470	1.684	1.549
Dispensable amino acids (g/100 g DM)			
Aspartic acid	1.553	2.038	1.755
Glutamic acid	4.774	4.114	3.863
Serine	0.857	0.970	0.860
Glycine	0.653	0.867	0.748
Arginine	1.468	1.423	1.494
Alanine	0.901	1.073	1.024
Tyrosine	0.680	0.661	0.617
Total amino acids	17.59	18.23	17.40

where: ME=metabolizable energy; PUFA=polyunsaturated fatty acids; DM= dry matter; CP=crude protein; EE=crude fat; CF=crude fiber.

The loss of nutrients was generally lower in C and FBM diets than in RGM diet, with a range of total amino acids from 17.40% to 18.23%. Methionine and lysine had the highest values compared with other two feeds. The amino acid composition of the four test ingredients was slightly different when the oleaginous by-products was compared with the antioxidant by-products, the RM has a significant level of lysine (1.85%) and methionine (0.72%) compared to the GM, which had the lowest level of lysine (0.42%), or with the BM, which had the lowest level of methionine (0.17%), as previously shown by Panaite et al. [5]. According to Panaite et al. [5], RM and GM had a concentration of 35.86 respectively 63.23 total Ω6, while FM and BM had only 27.30respectively 25.40 Ω6 fatty acids. Similar results for GM and FM were also sustained by Vlaicu et al. [6].

The influence of dietary meals on albumen protein content, pH, Haugh Unit and freshness is presented in table 3. The crude protein (%) was not affected by the vegetable by-products used, but the albumen pH significantly decreased ($P<0.05$) in FBM group compared with C. The highest value obtained for the Haugh Unit was recoded in RGM group, but the results are not statistically sustained. Regarding the freshness degree of albumen, it was found that the highest percentage of AA egg was recorded in group C (27.78%), while FBM group had the highest level of class A (77.78%). The eggs collected from RGM group recorded 22.22% eggs for class B of freshness. As it can be noticed from table 3, the level of lysine and methionine from compound feeds did not influenced the analyzed parameters from egg albumen. According to Oomah, [17], these parameters are affected only in the case when the crude protein level from compound feed is modified, which is not in our case. Regarding the freshness classes of the egg albumen, Fernandes et al. [18] obtained similar result when the layers diets were supplemented with oleaginous vegetable by-products.

Table 3. Egg albumen quality influenced by utilised by-products

Item	C	RGM	FBM	SEM	P Value
Crude protein, %	10.35	10.30	10.25	0.065	0.8214
pH albumen	8.95a	8.83ab	8.71b	0.036	0.0245
Haugh Unit	70.23	74.48	68.28	1.623	0.2967
Freshness classes					
AA	27.78	16.67	5.56	n/a	n/a
A	66.67	61.11	77.78	n/a	n/a
B	5.56	22.22	16.67	n/a	n/a

Values with the different superscript in the same row are statistically different (P<0.05).
n/a=not applicable

The pH of the albumen had lower values in RGM group and significantly lower in FBM group, which indicated a good stability of proteins. According to Olas [19] pH values increase in time when starts the proteins degradation (such as Lysozyme and Ovomucin).

Apparent digestibility coefficients (ADC) of C and experimental diets, as obtained by standard calculation are presented in Table 4. ADC data show the OM did not had any significant differences between the groups. The ingested and excreted ADC for CP was significantly higher (P≤0.05) in RGM group compared with C and FBM groups. Also, the digested values were significantly higher in the same group (P=0.0081). Regarding the EE, RGM group had a significantly higher (P≤0.05) digestibility values of ingested nutrient compared with C and FBM, being with 39.89% respectively 48.43% higher. Same trend was observed also for the CF nutrient. RGM group also had the highest values (P≤0.05) for the ingested, excreted and absorption coefficient of CF compared with both C and FBM. The ADC of ingested NFE was significantly (P≤0.05) lower in RGM group compared to C and FBM group, while the excreted and absorbed amount was in range with the other two groups. Usually NFE is affected by a high level of uric acid that the layers need to eliminate, caused by an imbalance between energy and nitrogen content in the diet [20]. In our case BM contribute to a decrease of NFE. Observations derived from this experiment regarding the absorption coefficient of EE in RGM was significantly (P<0.05) influenced by the rate of inclusion level of by-products (Table 4). Thus, the ADC of EE increased in this group, ranged between 69.10% (FBM group) to 83.38% (RGM), while the C group was 76.14%. The values obtained for the digestibility of EE in experimental groups are in range with those reported for other oilseeds used in poultry [21] or pigs' diets [22] and at the same time higher compared with those reported by same author in other study [23]. CP absorption coefficient was higher in FBM group but not significantly influenced. These observations are in agreement with those reported by Ortiz et al. [23], who used flax seeds in broiler diets. In our study we noticed higher values for the ADC of OM (74.25%) and ash (52.68%) than those reported by the other researchers: 75.84 % and 36.43% [24]. Contrary, Panaite et al. (2017) [25] obtained different result when the same experimental design was tested on broilers, but with a slightly different inclusion rate of these by-products. This difference may be due to the fact that the birds' age, sex and type of hybrid has a major influence on nutrients, amino acids digestibility and ADC. Batal and Parsons [26], stated in a study conducted on broilers that some ADC and amino acids have increased linear with the age of birds. Others [27], made the same statement when the age was correlated with nutrient absorption in broilers experimental design.

Table 4. Influence of the supplemented by-products on the level of apparent absorption of digestibility coefficients (ADC)

Nutrient	C	RGM	FBM	SEM	P value
Ingested (g/layer/day)					
Organic matter OM)	92.15	90.45	94.65	1.185	0.3680
Crude protein (CP)	19.85a	20.11a	22.87b	0.418	0.0006
Ether extractives (EE)	4.52a	6.90b	4.18a	0.301	P<0001
Crude fiber (CF)	5.70a	7.47b	6.04a	0.202	P<0001
Nitrogen free extractives (NFE)	62.08a	55.96b	61.57a	0.981	0.0005
Ash	15.86a	16.40a	18.83b	0.363	P<0001
Excreted (g/layer/day)					
Organic matter OM)	23.98	22.98	26.81	1.121	0.1753
Crude protein (CP)	2.71a	2.64a	3.05b	0.065	0.0136
Ether extractives (EE)	1.07	1.16	1.40	0.086	0.1781
Crude fiber (CF)	3.43a	4.13b	3.96ab	0.136	0.0841
Nitrogen free extractives (NFE)	17.16	18.09	18.73	0.411	0.3163
Ash	8.87a	8.42a	9.80b	0.206	0.0200
Digested (g/layer/day)					
Organic matter OM)	68.17	67.47	66.79	1.778	0.9667
Crude protein (CP)	17.13a	17.46a	19.36b	0.336	0.0081
Ether extractives (EE)	3.45a	5.74b	2.78c	0.321	P<0001
Crude fiber (CF)	2.55a	3.20b	2.23a	0.139	0.0034
Nitrogen free extractives (NFE)	45.32	40.90	42.24	1.549	0.5195
Ash	7.56a	7.97ab	8.61b	0.177	0.0624
Absorption coefficient (%)					
Organic matter OM)	73.82	74.25	70.57	1.276	0.4637
Crude protein (CP)	86.26	86.86	86.31	0.309	0.7069
Ether extractives (EE)	76.14a	83.38b	65.39c	2.131	P<0001
Crude fiber (CF)	43.94ab	44.70a	37.21b	1.493	0.0730
Nitrogen free extractives (NFE)	72.84	72.54	68.62	1.936	0.6388
Ash	43.75a	48.59	46.97	1.229	0.2760

*Values with different superscript in the same row are statistically different (P<0.05).

4. Conclusions

There is a need for the evaluation of the nutrient digestibility of vegetable by-products as a feed ingredient. The current study revealed that the oilseed meals used in layers diets are valuable sources of amino acids, complying with nutritional requirements for laying hens uses. The group feed with RGM diet had a significantly higher (P≤0.05) ingested and absorbed crude fat and crude fiber. FBM group, had the highest ingested, excreted and absorbed concertation of crude protein and a medium level of nitrogen free extractives compared with other two groups.

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