



Effects of dietary lentil seeds inclusion on performance, carcass characteristics and cecal pH of broiler chickens

GEORGETA CIURESCU¹, ANDREEA VASILACHI², MIHAELA HABEANU³ and C DRAGOMIR⁴

National Research-Development Institute for Biology and Animal Nutrition, Balotesti 077 015, Ilfov, Romania

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ABSTRACT

This study evaluated the effects of dietary replacement of soybean meal (SBM) with two cultivars of lentil seeds (cv. Eston, green-seeded and cv. Anicia, green marbled-seeded) on performance, carcass characteristics and pH of the cecal digesta in broiler chick diets. Day old, broiler chicks (1,000; Cobb 500) were randomly allocated to the following 5 treatments, viz. a diet based on corn and SBM as control; 200 g/kg of raw lentil seeds cv. Eston (LE); 400 g/kg of LE; 200 g/kg of raw lentil seeds cv. Anicia (LA); 400 g/kg of LA. The broilers fed raw lentils had comparable BW, ADG, ADFI and FCR to the control group. The digestive organ sizes (i.e. gizzard, heart, liver, pancreas, small intestine, caecum and the small intestine) and cecal pH were not affected by feeding diets with increasing levels of raw lentil seeds. Nevertheless, the data showed that cultivar (cv. Anicia) increased ($P = 0.021$) small intestine weight. A significant interaction between lentil levels and cultivars was observed for pancreas weight ($P=0.042$). Carcass, breast, thigh weight and dressing percentage were not affected by feeding diets with lentil seeds. No significant interaction between lentil levels and cultivars was noticed for growth performance, carcass characteristics as well as pH of the cecal digesta. Based on the results, it can be concluded that lentil seeds (cv. Eston or cv. Anicia) can be used as an alternative protein source to replace SBM in broiler chicken diets, at inclusion levels up to 200 g/kg.

Key words: Broiler, Carcass characteristics, Cultivar, Lentil, Performance

Utilization of soybean protein in human food and animal feeds as well, may cause problems in the future such as availability, production cost or risks associated with reliance on a single ingredient. Mostly, soybean comes from genetically modified crops and in European Union there is a tendency to limit its use in poultry feed due to potential negative effects that meat or eggs could have on human health. Compared with animal proteins, the use of legume seeds as protein sources in poultry production is limited due to the lower quality of the protein (imbalanced amino acid composition) and to the presence of so-called anti-nutritional factors (ANFs) that interfere with the nutritional utilization of diets based on these seeds as the main source of protein. Lentil (*Lens culinaris*) seeds are produced primarily for human consumption, but they occasionally become available for animal feed industry, especially when they suffer from quality problems (e.g. frost damage, discolouration or seed damage) and thus become improper

for human consumption. Lentils belong to the pulse crop family and have a chemical composition quite similar to peas, widely used in swine nutrition (Landro *et al.* 2012). No research appears to be reported on feeding lentils to broiler chickens, but lately the interest in legumes as a protein source in diets for poultry increased (Laudadio and Tufarelli 2010, Laudadio *et al.* 2011, Nalle *et al.* 2011, Dotas *et al.* 2014, Smulikowska *et al.* 2014, Zdunczyk *et al.* 2014ab, Çabuket *et al.* 2014, Koivunen *et al.* 2016). Thus, research should be conducted to assess the suitability of alternative protein sources to soybean meal (SBM) in formulated chicken diets. The aim of this study was to determine the effects of dietary lentil inclusion on performance, carcass characteristics and pH of the cecal digesta. A further aim was to find an appropriate inclusion level of raw lentil seeds in diets for broiler chickens.

MATERIALS AND METHODS

Birds were treated in accordance with Romanian legislation for handling and protection of animals used for experimental purposes. This study protocol (permission no. 5681–09–05–2016) was approved by the Ethical Committee of the National Research Development Institute for Animal Biology and Nutrition.

Animals and diets: Cobb 500 broiler chickens (1,000),

Present address: ¹Senior Researcher (ciurescu@inba.ro), ²Scientific Researcher (andryca82@yahoo.com), ³Senior Researcher and Head (mihaela.habernu@inba.ro), Animal Nutrition Laboratory. ⁴Scientific Director and Senior Researcher (catalin.dragomin@inba.ro), Laboratory of Chemistry and Nutrition Physiology.

day-old (48 ± 0.9 g/chick), purchased from a commercial hatchery were used in a 28-d feeding trial. The chicks were randomly (mixed sex) allocated to 5 dietary treatments with 4 replicate pens and 50 chick/pen. The chicks were kept in pens on litter (wood shavings) under similar managerial and hygienic conditions in a shelter with a controlled environment and with constant overhead fluorescent lighting (23L: 1D). The temperature was maintained at 33°C on d 1 and then gradually reduced to 26°C by 28 d of age. Vaccinations and a medical program were performed under the supervision of a veterinarian. Two cultivars of spring lentil seeds (cv. Eston, green-seeded and cv. Anicia, green marbled-seeded), locally grown in temperate weather conditions, were used to replace 35 or 41% of SBM. The five dietary treatments were formulated as follows: (i) a diet based on corn and SBM as control; (ii) 200 g/kg of raw lentil seeds cv. Eston (LE); (iii) 400 g/kg of LE; (iv) 200 g/kg of raw lentil seeds cv. Anicia (LA); (v) 400 g/kg of LA. All diets were formulated to be isocaloric, isonitrogenous and with similar content of total lysine, total sulfur amino acids (methionine + cysteine; TSAA), calcium and available phosphorus. The diets were formulated to meet or exceed the minimum Cobb Broiler Performance and Nutrition Supplement (Cobb Vantress 2008) requirements for broiler chickens (22.4% CP and 12.66 ME, MJ/kg). The energy value calculations were based on the chemical analyses of the feed ingredients. The diets were given in mash form, and water was offered from nipple drinker lines. Feed and water were available *ad lib.* throughout the experimental period.

Chemical analysis: Ingredients were analysed before formulation of diets (dry matter, crude protein, crude fibre, crude fat, ash and amino acids) using standard procedures according to the methods of the Commission Regulation (EC) no. 152/2009 (Official Journal of the European Union 2009). Ingredients were also analyzed for neutral detergent fibre (NDF) and acid detergent fibre (ADF), using an automatic system Foss Tecator (Fibertec 2010 apparatus). Carbohydrate content was estimated as nitrogen-free extract (NFE). Composition data are on a dry-matter basis (MJ kg/DM). Amino acids content was separated using a high performance liquid chromatography HPLC Thermo Scientific Surveyor Plus System.

Sampling, measurements and statistics: Body weight (BW) was recorded weekly. Feed intake and mortality were recorded daily. Any bird that died was weighed and the weight was used to adjust the feed conversion ratio (FCR) and was calculated by dividing total feed intake to weight gain of live plus dead birds. At 28-d of age, 8 chicks/treatment (2/replicate) representing an average pen weight were fasted for 12 h (water was allowed) to allow gut emptying. Thereafter, all chickens were weighed, and slaughtered by neck cut, severing the right carotid artery and jugular vein. The carcasses were de-feathered and eviscerated manually. The following organs were removed before being weighed: gizzard, liver, pancreas, heart, breast, thigh, abdominal fat, small intestine, and caecum. The

carcass weight of each chicken was also recorded and dressing out percentage was calculated by expressing carcass weight to the live weight. The length of small intestine (duodenum, jejunum, and ileum) was also measured and recorded. The cecum was quickly dissected out and the fresh content was gently squeezed and carefully collected in 25 ml sterilized tubes, each tube contained pooled excreta for 8 chicks per treatment. The cecal digesta pH was measured (mean of 3 readings) using a portable pH-meter (WTW pH 3310). Data were analysed using the general liner model procedure in SPSS software (2011) version 20 as a 2×2 factorial arrangement with 2 levels of lentil seeds and 2 cultivars. Differences among group means were detected using two way analysis of variance (ANOVA). Tukey's multiple range test was applied to separate means, and the results were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Nutrient composition of lentil seeds: The chemical composition of raw lentils, cv. Eston (green-seeded), and

Table 1. Chemical composition of raw lentil seeds (3)

Item	Lentil	
	Eston	Anicia
<i>Nutrient, g/kg DM⁻¹</i>		
Dry matter	890	903
Gross energy (MJ/kg)	19.45	20.33
Crude protein	292 ^a	259 ^b
Crude fat	15 ^b	32 ^a
Crude fibre	54 ^b	81 ^a
NDF ¹	283	319
ADF ²	61	72
Ash	33	37
NFE ³	639	644
Calcium	0.13	0.16
Phosphorus, total	0.48 ^b	0.70 ^a
<i>Amino acids (g/100g)</i>		
Lysine	1.97	1.79
Methionine	0.19	0.17
Threonine	1.05	1.05
Leucine	1.84	1.75
Arginine	2.21	2.14
Histidine	0.65	0.65
Isoleucine	1.21	1.07
Valine	0.99	0.92
Phenylalanine	1.29	1.20
Cystine	0.29	0.30
Alanine	1.10	1.14
Tyrosine	0.66	0.70
Aspartic acid	2.90	2.59
Serine	1.16	1.06
Glutamic acid	3.77	3.64
Proline	1.18	1.18
Glycine	0.94	0.79

^{a,b}Cow means with different superscripts differ significantly at $P < 0.05$. ¹NDF, Neutral detergent fibre; ²ADF, acid detergent fibre; ³NFE, nitrogen-free extract.

cv. Anicia (green marbled-seeded), used for this study is shown in Table 1. Raw lentil seeds showed significant ($P < 0.05$) variations among samples for crude protein, crude fat, crude fibre, and phosphorous; whereas, DM, gross energy (calculated value), ash, NDF and ADF contents, NFE and calcium were similar. The crude protein contents in both lentil seeds was lower than in soybeans (370 g/kg DM^{-1}) as recorded by NRC (1994). Similarly to the trend of protein concentrations, there were also variations between the cultivars in amino acids (AA) concentrations. The data indicated that all essential AA, except the sulphur amino acid (methionine and cysteine) were present in excessive amounts in both cultivars tested. As with the characteristics of legume, the lentil seeds were high in lysine (6.7–7% of the protein) but low in the sulphur-containing amino acids (methionine and cysteine, 2%), compared to the requirements of broilers in the starter phase. Our results were in agreement with those reported in available literatures (Ravindran and Blair 1992, Hefnawy 2011, Woyengo *et al.* 2014). Part of the variation in chemical composition can be explained by the observed differences among cultivars given by effect of drought during seed development, on the quality and nutritive value of the legume species. Protein concentration of legume seeds is known to vary with soil type and nitrogen fertilizer application (Igbasan and Guenter 1996), genotypes (Matthews and Arthur 1985), climate and year of harvest (Nell *et al.* 1992, Nikolopoulou *et al.* 2007).

Growth performance: The effect of treatments, level, cultivar or interaction between level and cultivar on the BW, average daily feed intake (ADFI), average daily gain (ADG) and FCR are summarized in Table 2. The results revealed that the broilers fed lentil seeds had comparable BW, ADG, ADFI and FCR. Mortality was not affected by the treatments (control, 200 LE, 400 LE, 200 LA and 400 LA groups was 2/50 (4.0%), 1/50 (2.0%), 1/50 (2%), 2/50 (4.0%) and 1/50 (2.0%), respectively). All deaths occurred within the first week of age and were attributed to stress due to transportation. Although there are no reports about the use of raw lentil seeds in broiler chicken diets, some are related to pig and most of them have shown that, up to 30% raw lentils have been used with success in pig diets (Bell and Keith 1986). It has also been shown that in diets for very young pigs, it is always wise to use suitable levels,

starting at no more than 10% in high-quality formulations. Landero *et al.* (2012) also demonstrated that green lentils can be included up to 22.5% in diets for nursery pigs without reducing growth performance. It is often recommended that diets based on lentils to be formulated on digestible and not total amino acids, but accurate values for amino acid digestibility in lentils were not available and had to be inferred from similar ingredients. A recent study from Canada has looked into this issue and determined the net energy value of lentils (Woyengo *et al.* 2014). It was determined that the digestibility of lysine was 81%, which was found to be lower than normal soybean meal (93%), with similar trends in most amino acids. In comparison with other legumes, such as soybean (*Glycine max.*), lentils (*Lens culinaris*) contain relatively small amounts of ANFs such as trypsin and chymotrypsin inhibitors (Guillamoin *et al.* 2008, Hefnawy 2011). Therefore, in our study, good performance of broiler chicks suggest that the lentil cultivars studied did not appear to contain harmful levels of ANFs. Furthermore, all diets was formulated to be similar in total lysine and TSAA content. In previous experiments with broiler chickens, where graded concentrations of raw pea was given, no negative effect on broilers' body weight and feed consumption (Igbasan and Guenter 1996, Laudadio and Tufarelli 2010, Dotas *et al.* 2014, Koivunen *et al.* 2016) was observed. Brenes *et al.* (2008) also demonstrated that the inclusion of raw chickpea in chicken diets did not affect bird's performance. On the other hand, Çabuk *et al.* (2014) showed that inclusion of 20% levels of lentil by-product in the laying quail diet significantly increased egg production without adversely affecting feed intake and feed conversion ratio, increased the yolk pigmentation and decrease malonaldehyde formation.

Carcass characteristics: Results of the carcass characteristics, digestive organ sizes and pH of the cecal digesta are presented in Table 3. The weights of gizzard, liver, heart, pancreas, small intestine, caecum and the small intestine length were not affected ($P > 0.05$) by treatments, but data showed that the cultivar increased ($P = 0.021$) small intestine weight and also, tended to increase ($P = 0.064$) gizzard weight. The increased weights of the small intestine and gizzard when feeding diets with raw lentil seeds cv. Anicia (200 LA and 400 LA, respectively) could be the result of high levels of dietary fibre (DF). In general, DF

Table 2. Effects of feeding lentil seeds on growth parameters of broiler chickens (28 d)

Parameter	Dietary treatments					SEM ¹	P-value ² Treatments	P-value ³		
	C	200 LE	400 LE	200 LA	400 LA			Level	Cultivar	L × C
BW (g)	1491.3	1472.5	1426.3	1467.5	1457.5	37.1	0.794	0.479	0.739	0.646
ADG (g/d)	51.5	51.0	49.3	50.8	50.3	1.9	0.930	0.561	0.846	0.746
ADFI (g/d)	81.8	82.5	81.8	82.0	81.8	1.8	0.998	0.790	0.894	0.894
FCR (g:g)	1.59	1.62	1.67	1.61	1.63	0.11	0.956	0.668	0.668	0.668

BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio. ¹SEM, standard error of means. ²Data were analysed as a monofactorial arrangement, including control group. ³Data were analysed as a 2 × 2 factorial arrangement, excluding control group. L × C, interaction of the effects of level of lentil inclusion and cultivar.

Table 3. Effects of feeding lentil seeds on carcass, organs weight and cecal pH of broiler chickens (28 d)

Parameter	Dietary treatments					SEM ¹	P-value ² Treatments	P-value ³		
	C	200 LE	400 LE	200 LA	400 LA			Level	Cultivar	L × C
Gizzard (g)	32.3	31.5	31.0	35.3	35.8	2.2	0.440	0.987	0.064 ^T	0.814
Liver (g)	40.8	47.0	43.5	50.5	45.5	3.6	0.412	0.244	0.443	0.832
Pancreas (g)	4.5	3.0	4.5	4.8	4.0	0.53	0.192	0.463	0.230	0.042 [*]
Heart (g)	9.3	9.5	9.3	9.8	9.0	0.54	0.885	0.258	0.989	0.563
SIW ⁴ (g)	47.5	45.8	45.3	56.0	52.3	3.3	0.158	0.524	0.021 [*]	0.625
SIL ⁵ (cm)	182.5	178.3	185.8	180.5	189.5	3.9	0.319	0.075 ^T	0.492	0.862
Caecum (g)	4.5	5.0	5.3	4.5	5.3	0.44	0.573	0.199	0.510	0.510
Cecal (pH)	6.72	6.59	6.61	6.57	6.52	0.20	0.476	0.589	0.447	0.736
Breast (g)	544.5	536.5	488.3	529.8	518.3	19.9	0.348	0.167	0.578	0.384
Thigh (g)	402.5	390.3	384.3	408.0	406.5	8.4	0.233	0.683	0.045 [*]	0.806
Abdominal fat (g)	25.5	25.0	19.3	25.5	23.3	1.7	0.080 ^T	0.043 [*]	0.228	0.343
Carcass weight (g)	1133.0	1112.3	1109.8	1178.3	1150.8	28.7	0.442	0.619	0.094 ^T	0.678
Dressing (%)	77.8	78.2	78.1	79.3	79.4	0.7	0.375	0.689	0.512	0.869

¹SEM, standard error of means. ²Data were analysed as a monofactorial arrangement, including control group. ³Data were analysed as a 2×2 factorial arrangement, excluding control group. L × C, interaction of the effects of level of lentil inclusion and cultivar. ⁴Small intestine weight. ⁵Small intestine length. T, tendency to be influenced by the treatment. *Significant differences.

ingestion leads to increased size and length of the digestive organs, including the small intestine, caecum and colon of chickens (Van der Klis and Van Voorst 1993, Iji *et al.* 2001). These effects are often associated with modification of the gut epithelium morphology, and consequently with the hydrolytic and absorptive functions of the epithelium. The observed increase in liver weight could be related to the nutritional status of the chickens fed lentil. The mobilization of body reserves to meet the needs of the rapidly growing tissues might increase the liver activity, thus causing hypertrophy. Viveros *et al.* (2001) and Arija *et al.* (2006) observed an increase in the relative weight of the liver in birds fed with other legumes, such as chickpea and kidney bean, respectively. The replacement of SBM with lentil seeds (cv. Eston and cv. Anicia) at higher inclusion levels (400 LE and 400 LA) tended to increase (P=0.075) small intestine length of broiler chickens. No significant interaction between lentil levels and cultivars was noticed for small intestine and gizzard weight as well as liver, heart, and caecum weight, cecal pH and small intestine length. A significant interaction between lentil levels and cultivar was observed for pancreas weight (P=0.042), indicating a low response that could be attributed to the effects of ANFs (Arija *et al.* 2006). Raw lentil seeds inclusion or cultivars had no effect on the breast, thigh weight and dressing percentage of the broilers. No differences were observed between the treatments in terms of abdominal fat weight, but there was a tendency to be influenced by the treatment (P=0.080) and a significant difference of the level (P=0.043). Broiler chickens' carcass weight was not affected by feeding diets with different levels of raw lentil seeds, as well. However, the replacement of SBM with lentil seeds (cv. Eston and cv. Anicia) tended to increase (P=0.094) carcass weight of broiler chickens. No significant interaction between lentil levels and cultivars was noticed for carcass, breast and thigh weight as well as abdominal fat weight.

The lack of effect of lentil seed inclusion levels on carcass weight, dressing percentage, thigh and breast weight is consistent with findings from similar studies elsewhere (Farrell *et al.* 1999, Nikolakakis *et al.* 2005, Laudadio and Tufarelli 2010, Dots *et al.* 2014).

The present study showed that the chemical composition of the 2 lentil cultivars (*Lens culinaris* cv. Eston and cv. Anicia) varies considerably. Replacement of SBM in broiler chickens diets with these two lentil cultivars at levels less than 400 g/kg resulted in similar productive performance, digestive organ sizes and pH of the cecal digesta. Moreover, the results from this study suggested that, where lentils can be grown locally, low input farming systems would benefit from the use of this source of protein for chickens feed.

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