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Evaluating Dietary Red Lentil Screenings on Performance, Antioxidant Status, Caecal Environment, and Intestinal Morphometric Features in Rabbits

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Abstract: Among the goals of global environmental policies, one is to achieve a critical rethinking of the agro-industrial production chain aimed at enhancing its sustainability and resilience while addressing its environmental impact. Lentils are widespread worldwide and are consumed as part of traditional dishes, and their health-promoting benefits are currently well recognized. Their industrial processing for human consumption implies the generation of different by-products which can be used as promising alternative feedstuff. Calling for the future sustainable development of rabbit farming, the present study questions the dietary inclusion of red lentil screenings (RLS) as an upcycling strategy for this by-product, as well as to ease the challenges faced by the rabbit-farming system. A total of 120 male Bianca Italiana breed growing rabbits aged 42 days were allotted to three dietary treatments containing different levels of RLS: 0 (RLS0), 5 (RLS5), or 10% (RLS10), respectively, for 6 weeks. At 84 days (slaughter age), meat, plasma, and intestinal samples were collected. The RLS inclusion showed no adverse effects on growth performance. However, significant differences were found in the meat fatty acid profile, where both RLS5 and RLS10 groups showed reduced C18:0 percentage and higher MUFA content owing to an increase in C18:1 n-9; moreover, RLS5 showed a significant increase in C16:1 trans. On the other hand, only RLS10 recorded a significant reduction in the PUFA total content due to decreased single unsaturated fatty acid (C18:2 n-6; C20:3 n-6; C22:5 n-3). Dietary RLS significantly decreased serum total cholesterol, LDL, and HDL, along with an enhancement of the overall serum antioxidant capacity. The results regarding the rabbit caecal characteristics and microbial population were found to be similar among the groups. However, referring to histomorphometric measurements, both RLS5 and RLS10 groups displayed significant increases in villus height and an improved villus height to crypt depth ratio. In conclusion, up to 10% RLS in the diets rabbits can be considered an effort-effective feeding strategy to modulate rabbit meat fatty acid profiles, to enhance the endogenous antioxidant capacity, and to improve the serum lipid profile and intestinal morphology.

Keywords: rabbit; lentil by-product; growth; meat; gut health; histomorphology

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1. Introduction

The climate change crisis requires a critical rethinking of the agro-industrial production chain. Given the livestock sector's contribution to agricultural waste generation and environmental pollution, actual efforts should be aimed at enhancing its sustainability and resilience. Global population growth has led to an increase in protein demand and consequent higher animal protein consumption [1], leading to livestock farming intensification. This yielded environmental burdens such as greenhouse gas emissions (GHGs), biodiversity loss, natural resource utilization, and pollution [2]. In this scenario, global environmental policies [3] arose with the aim to reduce food losses by fully utilizing waste in a sustainable way to reduce their disposal.

In 2022, about 95,989,881 tonnes of pulses were produced globally, of which 6,655,827 tonnes were lentils [4] consumed either whole or after processing. Pulses are a worldwide staple food in balanced diets due to their favourable macronutrient contents, such as low fat and high protein, as well as many bioactive compounds [5]. For instance, lentils' fibre content is associated with putative cholesterol-lowering activity [6]. Recently, researchers reported a correlation between pulses' consumption and the incidence of non-communicable chronic diseases likely attributed to their content of phytochemicals [7–9]. Consequently, their consumption in human nutrition is increasingly gaining appeal among consumers who adhere to health-promoting diets and seek for alternative protein sources [8]. Agro-industrial processing leads to huge amounts of by-products [10]. Following the processing of lentils, various by-products are generated, namely straw, screening, and seed coat [11]. Lentil straw consists of the dry stems and leaves left in the field upon threshing. Screening represents ~2–4% of the total processed lentils [12] and consists of broken lentil seeds, cereal grains, weed seeds, and haulm [13], while the seed coat represents ~28% [14]. This discarded biomass outlines an imbalance between food production and consumption, which constitutes a threat to sustainability [10,15]. Moreover, considering the available literature on the nutritional and bioactive value of lentil by-products (LBs) [16,17], it has become crucial to investigate new paths for their upcycling [18] in order to maintain sustainable production.

An interesting and effort-effective upcycling strategy for them might be their inclusion in animal feeding, which may reduce feed costs as well. LBs have been investigated mainly in ruminant species, with no adverse effect on growth parameters nor ruminal fermentation [19,20], while, in monogastric species, a few studies have been conducted in growing pigs [21], poultry [12], and rabbits [13] given the high fibre content of LBs. However, rabbits, as hindgut fermenters, can efficiently digest and benefit from fibrous feedstuffs [22,23]. Therefore, the inclusion of LBs in rabbit diets may serve as a source of dietary fibre and micronutrients, supporting production and gut health.

Meat from rabbits is a rich source of high-biological-value proteins, with low calories and cholesterol content [24]. Compared to other livestock species, rabbit meat shows high mono- and polyunsaturated fatty acids (MUFA and PUFA), although saturated fatty acids (SFAs) stand as major fatty acid group [25]. Regrettably, although showing characteristics that are highly valued by consumers [26], rabbit meat consumption shows a declining trend, even in the traditional countries mainly producing it (Spain, France and Italy), possibly owing to the price, which still higher in comparison to other species [27]. However, the rabbit farming sector still bears the potential to sustain employment and maintain socio-economic stability in several geographical rural areas, enabling the fight against depopulation and poverty [28]. As the EU is not able to sustain livestock feed demand, being dependent on protein feeds from abroad [29], recent investigations into rabbit nutrition have delved into the identification of alternative feedstuffs such as agricultural by-products that might foster sector rentability and sustainability. However, to the best of our knowledge, few studies have been conducted to investigate the effect of the dietary inclusion of lentil screenings in this species.

Therefore, the present study aimed to investigate the effect of red lentil screenings on the growth performance, meat quality, blood parameters, antioxidant defence, caecal environment, and gut morphology of rabbits.

2. Materials and Methods

2.1. Animals, Management and Feeding

Rabbits in the present study were handled and cared for in compliance with the EU legislation on animal welfare regulations (Directive 2010/63/EU, which updates and replaces the 1986 Directive 86/609/EEC) and following the research policies of the DiMePRe-J of the University of Bari Aldo Moro, Italy (Approval code 09/2022). The research was conducted, starting from February 2024, at the rabbitry of the Genetic Center of the Italian Rabbit Breeders Association (ANCI-AIA, Volturara Appula, Foggia, Italy). One hundred and twenty male Italian White (Bianca Italiana breed) growing rabbits aged 42 days (body weight 1109 ± 28.8 g, mean ± SEM) were randomly assigned to three groups of 40 animals according to the dietary treatment. Then, rabbits were fed a pelleted diet containing 0 (RLS0), 5 (RLS5), or 10% (RLS10) red lentil screenings, respectively.

The by-product obtained from red lentil (Lens culinaris, variety Crimson) screenings was provided by Agricola Piana d'Oro, Piana Cardone, Genzano di Lucania (Potenza province of Basilicata region, Italy). The by-product was mill-ground and stored in plastic bags at room temperature, then analysed in triplicate to assess the proximate chemical composition—dry matter (92.57%), crude protein (23.25%), crude lipids (2.65%), crude fibre (10.13%), and ash (3.21%)—according to [30].

The diets were formulated to be isonitrogenous and isoenergetic and to meet or exceed the nutrient requirements of growing rabbits [31]. The feeding trial lasted 6 weeks, up to 84 days of age. The rabbits were housed with four rabbits per cage in galvanized wire single cages within open-system pens 35 × 40 × 50 cm in width, height, and length (Italian battery) and at a height of 90 cm from the concrete floor. Hand feeding and an automated nipple drinker system offered constant access to fresh, clean water for every cage. The rabbits were housed under identical environmental and sanitary conditions throughout the trial. No medication was included in the feed or in the drinking water, and the rabbits' health statuses were checked through individual observations. The ingredients and chemical analysis of the diets are shown in Table 1.

Table 1. Ingredients and chemical composition of the diets fed to rabbits.

Ingredients, g/kg	Diet		
	RLS0	**RLS5**	**RLS10**
Dehydrated alfalfa meal	285.0	285.0	285.0
Dehydrated beet pulp	285.0	285.0	285.0
Corn	200.0	200.0	200.0
Soybean meal, 48% CP	100.0	85.0	70.0
Wheat middlings	85.5	50.5	15.5
Red lentil screenings	-	50.0	100.0
Cane molasses	20.0	20.0	20.0
Vitamin-mineral premix [1]	5.0	5.0	5.0
Monocalcium phosphate	5.0	5.0	5.0
Sodium chloride	4.0	4.0	4.0
Calcium propionate	2.5	2.5	2.5
L-lysine	2.5	2.5	2.5
DL-methionine	2.5	2.5	2.5
Yeast	1.0	1.0	1.0
Magnesium oxide	1.0	1.0	1.0

Magnesium carbonate	1.0	1.0	1.0
Chemical composition [2], g/kg as-fed			
Dry matter	891	892	894
Crude protein	154	153	153
Ether extract	24	25	24
Crude fibre	141	144	146
Neutral-detergent fibre	268	259	265
Acid-detergent fibre	167	168	164
Lignin	39	37	35
Ash	69	68	68
Digestible energy (MJ/kg)	10.61	10.61	10.61

RLS0: control diet without red lentil screenings; RLS5: diet containing 5% red lentil screenings; RLS10: diet containing 10% red lentil screenings. [1] Provided per kg of diet: vitamin A 12,500 IU; vitamin D_3 1500 IU; vitamin E 30 mg; vitamin B_1 1.5 mg; vitamin B_2 5 mg; vitamin B_6 2 mg; vitamin B_{12} 0.02 mg; vitamin PP 20 mg; vitamin K_3 2.5 mg; folic acid 0.75 mg; pantothenic acid 10 mg; D-biotin 0.1 mg; choline chloride 300 mg; $MnSO_4$ 150 mg; $FeSO_4$ 5 mg; $ZnSO_3$ 75 mg; $CuSO_4$ 5 mg; KI 1 mg; $CoSO_4$ 0.2 mg; Na_2SeO_3 0.1 mg. [2] Calculated.

2.2. Growth, Carcass Traits, and Meat Quality

From 6 to 12 weeks of age, rabbits were weighed individually at weekly intervals, while the feed intake was recorded daily and the feed conversion ratio was calculated. Mortality rate was considered when calculating the feed conversion ratio. At the conclusion of the 84-day fattening period, 10 rabbits per experimental group were randomly selected for slaughter in the afternoon. The following morning, these selected rabbits were transferred in small groups to a slaughter facility located adjacent to the rearing area to evaluate carcass characteristics. The animals were weighed, electrically stunned, and slaughtered within 2 h. All slaughtering and carcass dissection procedures adhered to the guidelines of the World Rabbit Science Association (WRSA), as outlined by [32]. After bleeding, the skin, gastrointestinal tract, and distal leg portions were removed. Carcasses—retaining the head, thoracic organs, liver, kidneys, and perirenal and scapular fat deposits—were weighed as "hot carcasses" and subsequently chilled at 4 °C for 24 h in a ventilated chamber. After chilling, the chilled carcasses were weighed and the slaughter yield was calculated. Samples of meat from the longissimus lumborum muscle were stored at −80 °C for assessing the lipid content, and others were individually stored in plastic bags at 4 °C for meat quality analysis.

Meat samples were analysed in triplicate for dry matter through the oven-drying method (934.01), for total ash in a muffle furnace (942.05), and for protein by the Kjeldahl method (954.01), as described by AOAC (2005). Total lipids were extracted according to the method of [33]. Fatty acid profiles were determined in raw rabbit meat from longissimus lumborum muscles according to the method described by [34]. Fatty acids were expressed as a percentage of total methylated fatty acids.

2.3. Blood Serum Lipids and Antioxidant Capacity Analysis

Blood samples of ten rabbits from each treatment were taken from the marginal ear veins at the end of the trial (84 days of age), one hour before the regular feeding time. Thereafter, the samples were gathered into sterile tubes and centrifuged, and the serum was separated and stored refrigerated (−20 °C) for further examination. The obtained serum samples were subjected to measurements of the following parameters: triglyceride (mg/dL), high-density lipoprotein (HDL; mg/dL), low-density lipoprotein (LDL; mg/dL), and total cholesterol (mg/dL). Using commercial kits, antioxidant activities including thiobarbituric acid-reactive substances (TBARS), glutathione peroxidase (GPx), glutathione S-transferase (GST), catalase (CAT), superoxide dismutase (SOD), and catalase (CAT) were measured in accordance with the manufacturer's instructions [35].

2.4. Caecal Characteristics and Microbiota

Volatile fatty acids (VFAs) were assessed in the caecal contents of slaughtered rabbits using gas chromatography, as described by [36], and ammonia was analysed using an automated distillation unit. The pH of caecal contents was also determined using a portable pH meter. To prepare a 10^{-1} dilution, 1 g of each caecal sample was homogenised in 9 mL of sterile saline–peptone solution and agitated for 30 min. Serial 10-fold dilutions were then prepared in pre-sterilized tubes up to a 10^{-6} dilution. For coliform detection, a 0.1 mL aliquot from each dilution was spread onto MacConkey agar and incubated at 37 °C for 24 h. Lactic acid bacteria were cultured on DeMan–Rogosa–Sharpe (MRS) agar at 37 °C for 48 h. Enumeration of coliforms and Lactobacillus in caecal samples was expressed as log10 colony-forming units per gram (CFU/g).

2.5. Histological Examinations

At day 84, from the same slaughtered rabbits, intestinal segment samples were collected for histomorphometric examinations. Each rabbit's duodenum was carefully excised into 2 cm long segments, approximately 4–5 cm from the pylorus, then rinsed with saline (0.9% NaCl) and preserved in 10% formalin solution. Routinary histological methods were applied to the specimens that were dehydrated, cleared, and paraffin-embedded. Serial sections (4–5 µm thick) were cut, placed on glass slides, and stained with Haematoxylin–Eosin (H&E) (Merck, Darmstadt, Germany) for morphometric studies, Azan Mallory (AM) trichrome stain (Merck, Darmstadt, Germany) to assess the extracellular matrix and collagen fibre distribution, and Periodic acid-Schiff (PAS) staining (Merck, Darmstadt, Germany) to detect the mucin-producing goblet cells (GCs) in the intestinal lining. The stained slides were observed under light microscopy supplied with a digital camera, and microphotographs were captured for morphological and morphometric measurement. The H&E-stained sections of 10 well-oriented villi of the duodenum were analysed and used to measure the villus height (VH) and the crypt depth (CD). Then, the villus height to crypt depth ratio (VH:CD) was calculated. VH was measured from the tip of the villus to the villus crypt junction, and CD was defined as the depth of the invagination between adjacent villi [37]. The apparent villus surface area was calculated by the following formula: [(villus width at one-third + villus width at two-thirds of the height of the villus) × 2 − 1 × villus height], according to [34]. In addition, PAS staining sequences were taken to detect the mucous secretion of the goblet cells and the duodenal brush border that appeared with a purple-red colour. Otherwise, the intestinal extracellular scaffold was evidenced by the Azan Mallory stain technique, which gives a blue colour to collagen fibres.

2.6. Statistical Analysis

Data were analysed by univariate ANOVA design using the GLM procedure of SAS (version 9.2; SAS, 2008), testing the diet (RLS0, RLS5, and RLS10) as the main effect. When significant differences were found among treatments, the means were separated by Duncan's multiple-range test. Results were reported as least squares mean and pooled standard error of the mean (SEM). Statistical significance was set at $p \leq 0.05$.

3. Results and Discussion

To date, feeding trials regarding the administration of lentil by-products to growing–fattening rabbits are very scant, although legume seeds and the dietary inclusion of their by-products as an alternative protein source are gaining increasing interest in the livestock sector [12,20,38–41]. Recent studies have hinted at lentil screening as a potential functional ingredient in rabbit diets given its nutritional value and its rich and valuable phytochemical content [13,17].

The effects of RLS inclusion in the diets of meat rabbits on growth performance, carcass yield, and meat characteristics are shown in Table 2. The body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) of the RLS-fed groups showed no significant differences compared with the control group, nor did carcass yield or meat characteristics.

Table 2. Effect of dietary inclusion of red lentil screenings on growth performance, carcass yield, and meat composition of rabbits.

Item	Diet				
	RLS0	**RLS5**	**RLS10**	**Pooled SEM**	***p*-Value**
Growth performance					
Initial live weight [1], g	1110	1115	1109	30.9	ns
Final live weight [1], g	2122	2129	2116	61.0	ns
Daily weight gain [1], g/d	24.10	24.14	23.98	1.13	ns
Feed intake [2], g/d	107	107	106	0.44	ns
Feed conversion ratio [2], g/g	4.40	4.43	4.42	0.15	ns
Mortality, *n*	6	5	5	0.29	ns
Carcass traits					
Slaughter weight, g	2073	2079	2068	88.89	ns
Hot carcass weight, g	1256	1258	1253	51.52	ns
Carcass yield, %	60.5	60.7	60.6	1.21	ns
Meat composition, %					
Moisture	73.52	73.39	73.45	0.11	ns
Protein	23.28	23.50	23.47	0.29	ns
Lipids	1.73	1.72	1.67	0.18	ns
Ash	1.47	1.39	1.41	0.11	ns

RLS0: control diet without red lentil screenings; RLS5: diet containing 5% red lentil screenings; RLS10: diet containing 10% red lentil screenings. [1] Individual data; [2] average pen data. SEM, standard error of the mean. ns: not significant.

Similarly, [42] found no significant differences in the growth performance or carcass measurements in rabbits fed different levels of LS (15 and 30%) compared to the control group. It was recently stated by [13] that LS could be used in rabbit diets at amounts up to 15% to maintain the productive indices without negative effects on nutrient digestibility, feed efficiency, or carcass characteristics. These trials also reported improved nutrient digestibility in rabbits fed an LS-integrated diet, likably owing to the optimal protein composition and amino acid digestibility of the by-product. Accordingly, in poultry studies, [12] and [43] highlighted the superior nutritional quality of LS compared to other by-products.

Despite the fact that, in the present study, no significant differences were found in carcass traits among the diets, previous studies have observed improved carcass yields in rabbits fed diets containing LS. This could be related to the higher growth indexes and the higher feed efficiency of the diets [13] or to variations in LS chemical composition across the studies. This evidence confirms LS as a valuable alternative protein source in rabbit nutrition. Additionally, by addressing concerns on pulses' content of anti-nutritional factors, these results corroborate the conclusion reported by [44], who found the LB tannin content to be below the level required to depress animal performance.

The diet formulation deeply influences the quality of animal products. In the field of meat science, a trending research topic is modifying meat's fatty acid (FA) composition

through designed feeding management. The literature has shown that dietary manipulation is very effective in improving the lipid profile of rabbit meat [24]. To the best of our knowledge, no research has previously investigated the effects of dietary RLS inclusion on the meat FA composition of rabbits. Therefore, our data represent a novel contribution to the existing literature and will be compared with outcomes of including lentils in poultry diets.

Biochemical assays have shown that lentils are an excellent source of essential FAs [45]. In particular, lentils' lipid content ranges from 1.52 to 2.95%, of which 77.5–81.7% are unsaturated fatty acids, mainly polyunsaturated fatty acids (PUFAs, from 55.5 to 58.1% of total FAs), with a low content of saturated fatty acids (SFA, from 19.3 to 21.5%) [46]. Indeed, previous reports regarding the effects of the dietary inclusion of lentil seeds on broilers' meat characteristics have found them to positively influence the FA profile and overall meat quality [47].

In the current investigation, results regarding the effects of RLS administration on rabbits' meat FA profiles are reported in Table 3. No significative differences were found in relation to the SFA contents of *L. lumborum* among the groups, with an exception for the C18:0 (stearic acid) content, which significantly decreased in both treated groups (−6.84% and −16.06%, for RLS5 and RLS10, respectively). The observed reduction is relevant in light of the current trend in human dietary patterns emphasizing reduced consumption of SFAs in favour of PUFAs. In groups receiving RLS supplementation, significant differences were found in the mono- and polyunsaturated fatty acid (MUFA and PUFA) contents of meat. Regarding single MUFA, C16:1 trans (palmitoleic acid) in RLS5 increased by +65.57% compared to the control, while C18:1 n-9 (oleic acid) increased by +13.97% in RLS10. This also led to significant variations in the total content of MUFA (+3.61% in RLS5 and +11.16% in RLS10). The higher level of oleic acid found in the RLS10 group could be partially attributable to the correspondent reduction in the stearic acid level of the same group, owing to a hepatic desaturation process converting this SFA into the corresponding MUFA, oleic acid. The outcomes of recent investigations have related MUFA consumption with health benefits, such as a reduction in serum cholesterol and reduced lipoprotein peroxidation, both lowering the risk of atherosclerosis [48]. In addition, oleic acid prevents FAs oxidation, reducing the generation of ROS, and thus may exert an influence on meat-oxidative stability, shelf life, and sensorial properties [49,50].

Regarding PUFA, while the control and RLS5 groups registered similar results, rabbits fed the RLS10 diet showed decreases in total PUFA due to the reduction in some specific fatty acids, C18:2 n-6 (−9%), C20:3 n6 (−37.1%), and C22:5 n-3 (−36.54%). Otherwise, in both the control and RLS5 groups, a significant increase in C22:5 n-3 (DPA, docosapentaenoic acid) was found. Our data partially agreed with those of [47], in which oleic acid (C18:1), palmitic acid (C16:0), and linoleic acid (C18:2) were the main FAs found in the breast muscle among broilers fed two different lentil cultivars. These results were associated with a significant increase in alfa-linoleic acid (ALA), which is the precursor of long-chain n-3 PUFA. Thus, the authors argued this finding may explain the related higher levels of long-chain n-3 PUFA (EPA, DPA and DHA) in meat. Interestingly, [47] observed a correlation between dietary lentil cultivars and meat FA profiles. As a matter of fact, the optimal LS levels in diet formulations rely upon their macro- and micronutrient composition, which in turn depend on the cultivar [17]. Moreover, genotype and environment also influence the chemical composition of lentils and their by-products, even among the same cultivar [51]. Based on the aforementioned evidence, the cultivar used in this trial and its relative composition may explain the differences in the effects of dietary inclusion of lentils on the meat lipid profiles found in rabbits. However, further investigations utilizing different lentil cultivars and by-products are needed to promote the improvement of meat quality in rabbits.

Table 3. Effects of dietary inclusion of red lentil screenings on fatty acid composition in longissimus lumborum meat muscle.

Fatty Acids, % of FAME	Diet				
	RLS0	RLS5	RLS10	Pooled SEM	*p*-Value
C4:0	0.09	0.13	0.12	0.04	ns
C6:0	0.16	0.21	0.16	0.07	ns
C8:0	0.12	0.10	0.08	0.04	ns
C10:0	0.28	0.23	0.24	0.08	ns
C12:0	0.31	0.26	0.25	0.06	ns
C14:0	1.72	1.59	2.12	0.12	ns
C15:0	0.44	0.46	0.46	0.08	ns
C16:0	27.18	26.93	27.96	0.37	ns
C17:0	0.48	0.50	0.51	0.14	ns
C18:0	9.65 [a]	8.99 [b]	8.10 [c]	0.25	*
C20:0	0.10	0.10	0.08	0.03	ns
C22:0	0.28	0.40	0.24	0.12	ns
C23:0	0.11	0.10	0.09	0.03	ns
∑ SFA	40.91	39.98	40.40	0.51	ns
C16:1 *trans*	0.61 [b]	1.01 [a]	0.63 [b]	0.23	*
C16:1 *cis*	3.27	3.25	3.00	0.35	ns
C17:1	0.12	0.14	0.16	0.07	ns
C18:1 n-9	23.05 [b]	23.61 [b]	26.27 [a]	0.31	**
C20:1 n-9	0.11	0.11	0.11	0.04	ns
∑ MUFA	27.15 [c]	28.13 [b]	30.18 [a]	0.40	*
C18:2 n-6	25.89 [a]	25.69 [a]	23.56 [b]	0.42	*
C18:3 n-6	1.20	1.24	1.47	0.15	ns
C18:3 n-3	2.99	3.05	3.10	0.22	ns
C20:2 n-6	0.25	0.25	0.21	0.09	ns
C20:3 n-6	0.62 [a]	0.61 [a]	0.39 [b]	0.10	*
C20:5 n-3 EPA	0.36	0.39	0.27	0.12	ns
C22:5 n-3 DPA	0.52 [a]	0.52 [a]	0.33 [b]	0.15	*
C22:6 n-3 DHA	0.12	0.13	0.09	0.03	ns
∑ PUFA	31.94 [a]	31.89 [a]	29.42 [b]	0.53	*
Total PUFA n-6	27.96	27.79	25.64	0.36	ns
Total PUFA n-3	3.98	4.10	3.79	0.21	ns
n-6/n-3	7.02	6.80	6.77	0.30	ns

RLS0: control diet without red lentil screenings; RLS5: diet containing 5% red lentil screenings; RLS10: diet containing 10% red lentil screenings. SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid. * = $p < 0.05$, ** = $p < 0.01$, ns = not significant; [a–c] means in the same row with different superscripts differed significantly.

The effects of feeding RLS on the blood lipids and antioxidant status of growing rabbits are reported in Table 4. The serum TC, LDL, and HDL of both treated rabbits' groups significantly decreased compared to the control. On the other hand, TG concentration, although it was not significantly reduced, showed a slightly decreasing trend among groups. Furthermore, the RLS10 group recorded the lowest values, showing a significant reduction in serum cholesterol levels even compared to RLS5. Earlier evidence has shown that lentils possess cholesterol-lowering activity linked to their high fibre content [6,9]. Recently, [13], quoting [52], concluded that the mixture of soluble and insoluble fibres derived from LS

inclusion in rabbit diets may have been the reason for the decreased TC levels observed in their investigation.

Table 4. Effect of dietary inclusion of red lentil screenings on blood serum lipids and antioxidant capacity of rabbits.

Item	Diet				
	RLS0	**RLS5**	**RLS10**	**Pooled SEM**	***p*-Value**
Serum lipids					
Cholesterol, mg/dL	189.41 [a]	160.15 [b]	157.66 [c]	0.76	***
LDL, mg/dL	158.36 [a]	142.05 [b]	135.81 [b]	0.49	*
HDL, mg/dL	63.75 [a]	61.11 [b]	60.32 [b]	0.51	*
Triglyceride, mg/dL	73.13	72.34	70.67	0.50	ns
Antioxidant capacity					
GPx, U/mL	16.35 [a]	20.55 [b]	21.61 [c]	2.67	**
GST, μmol/h/mL	1.46 [a]	1.54 [b]	1.79 [c]	0.08	*
CAT, μmol H_2O_2	60.98 [a]	67.60 [b]	71.15 [c]	3.69	**
SOD, U/mL	3.20 [a]	4.05 [b]	4.78 [c]	0.65	***
TBARS, nmol/mL	0.30 [a]	0.24 [b]	0.21 [b]	0.04	*

RLS0: control diet without red lentil screenings; RLS5: diet containing 5% red lentil screenings; RLS10: diet containing 10% red lentil screenings. SEM, standard error of the mean. LDL, low-density lipoprotein; HDL, high-density lipoprotein; GPx, glutathione peroxidase; GST, glutathione S-transferase; CAT, catalase; SOD, superoxide dismutase; TBARS, thiobarbituric acid-reactive substances. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, ns = not significant, [a–c] means in the same row with different superscripts differed significantly.

Otherwise, the lower cholesterol values found in the tissue and serum of the experimental animals may also be linked to pulses' anti-nutritional compounds, such as saponins [53,54]. In poultry, the administration of a diet containing raw lentil seeds induced a significant decrease in plasma TG and TC compared with broilers fed soybean meal [47]. The authors supposed that serum TC concentration may be influenced by the FA profile of the feedstuff, where higher levels of SFA tend to increase the plasma TC value compared to higher levels of MUFA and/or PUFA. In this trial, as mentioned before, the decreased level of the major SFA (C18:0 stearic) and the relative increased value of the major MUFA (C18:1 oleic) may have been related to the reduction in serum TC, as also supposed by [50].

The present findings point out an improvement in rabbits' antioxidant capacity owing to RLS dietary inclusion (Table 4). The GPx, GST, CAT, and SOD values significantly rose in both RLS5 and RLS10. Moreover, serum antioxidant enzymes were significant higher in rabbits from the RLS10 group compared to those in RLS5. Nevertheless, TBARS was found to be significantly lower in both RLS-treated groups compared to the control group. Recent evidence has assessed that lentils, primary the hulls [55], possess a wide range of bioactive molecules, mainly polyphenols, that may exert an influence on the endogenous antioxidant regulation. Moreover, it is noteworthy to underline that the present trial investigated the administration of a red lentil cultivar. Previous research has reported an association between the seed coat colour and the polyphenol content [51]. Additionally, insights into lentil cultivars (red, bronze and black varieties) have found darker seeds to exhibit augmented in vitro antioxidant activity correlated with higher polyphenol concentrations [56].

Given the presence of antioxidant compounds, lentils have gained increasing interest as functional feed ingredients with regard to their potential influence on the deleterious consequences of the oxidative metabolism [13]. Nevertheless, in vivo studies on the antioxidant activity of lentil polyphenols are scarce [56]. The assessment of serum antioxidant markers in this study confirmed that dietary inclusion of RLS may be considered a

sustainable way to improve rabbit antioxidant status [13,57]. Moreover, our data on TBAR levels support the assumption that bioactive molecules found in RLS may act by bolstering the endogenous and exogenous redox systems, reducing the production of ROS, and improving rabbit health status and product quality. This finding matches with those of [12], who found increased egg oxidative stability in quails treated with LS. Indeed, measurement of yolk TBAR content in the treated quails showed a linear decrease in lipid oxidation that the authors related to the content of tannins. A recent investigation highlighted the tight relationship between diet and gut health and how its comprehension can be employed to improve animal production and welfare. Based on the consulted data set, dietary administration of RLS in meat rabbits should not merely be considered to supply an alternative protein, but also to provide a source of dietary fibre, starch, polyphenols, and fatty acids, which may exert beneficial effects on the gut microenvironment and intestinal integrity.

To evaluate the influence of red lentil screenings on gut health, the present work has analysed both the rabbit caecal environment and microflora (Table 5) and the intestinal histomorphometric features (Table 6 and Figures 1 and 2). With regard to the caecal environment, the RLS groups showed no significant difference in pH or total volatile fatty acid (VFA) values compared to the control group. In addition, the microbial load showed similar outcomes in all tested groups, except for a slight reduction in Coliforms and an increasing trend of Lactobacilli in the RLS groups.

Table 5. Effect of dietary inclusion of red lentil screenings on caecal characteristics and microbial populations of rabbits.

Item	Diet				
	RLS0	**RLS5**	**RLS10**	**Pooled SEM**	***p*-Value**
Caecal characteristics					
pH	6.08	6.09	6.09	0.03	ns
Ammonia-N, mmol/L	3.71	3.75	3.73	1.15	ns
Total VFA, mmol/L	72.0	71.8	72.1	7.83	ns
Acetic acid, mol/100 mol VFA	82.3	82.2	82.0	1.92	ns
Propionic acid, mol/100 mol VFA	2.22	2.09	2.16	0.16	ns
Butyric acid, mol/100 mol VFA	12.0	11.5	11.7	0.39	ns
Valeric acid, mol/100 mol VFA	0.59	0.62	0.65	0.15	ns
Caecal microbial count					
Coliform, log UFC/g	6.55	6.48	6.42	0.021	ns
Lactobacilli, log UFC/g	4.49	4.55	4.63	0.023	ns

RLS0: control diet without red lentil screenings; RLS5: diet containing 5% red lentil screenings; RLS10: diet containing 10% red lentil screenings. SEM, standard error of the mean. ns, not significant.

Considering the peculiar and delicate rabbit digestive physiology, caecal microbial balance and intestinal architecture play pivotal roles in maintaining an efficient nutrient metabolism network. LBs can be considered a rich source of dietary fibres and phytochemicals that concur in the maintenance of rabbit gut health [13,58,59], because to sustain the digestive system, a balanced diet including fibre is required. Indeed, a fraction of dietary fibre undergoes caecal microbial fermentation and subsequent production of VFA, which, in turn, improves the gut environment while promoting the growth of beneficial bacteria [60]. Therefore, dietary supplementation with LS fibre may be considered a nutritional strategy aimed to sustain intestinal development and the gut microbiota community. In animals, several reports have evidenced that lentil seeds and lentil by-products are good sources of prebiotic carbohydrates that aid in preserving the gut microbial environment and mucosa physiology [13,61,62]. The findings of this study confirm that RLS integration

was able to maintain a balanced caecal microenvironment, with a positive effect on gut morphometric features.

Morphometric assay values are provided in Table 6, while Figures 1 and 2 display representative microphotographs of the intestines of the different rabbit treatment groups.

Table 6. Effect of dietary inclusion of red lentil screenings on duodenal histomorphometry of rabbits [1].

Item	Diet				
	RLS0	RLS5	RLS10	Pooled SEM	*p*-Value
Villus height, μm	675 [b]	633 [b]	692 [a]	35.04	*
Crypt depth, μm	123.5	125.1	120.2	4.56	ns
Villus height/crypt depth	5.47 [b]	5.05 [c]	5.76 [a]	1.109	**
Villus surface area, mm^2	977	980	1033	41.0	ns

RLS0: control diet without red lentil screenings; RLS5: diet containing 5% red lentil screenings; RLS10: diet containing 10% red lentil screenings. [1] Each value represents the mean of ten rabbits per group. SEM, standard error of the mean. * = $p < 0.05$, ** = $p < 0.01$, ns = not significant, [a–c] means in the same row with different superscripts differed significantly.

Morphologically, no difference was found among the diets. Duodenum mucosa analysis showed well-organized structures in all tested groups, without any signs of alterations (Figure 1).

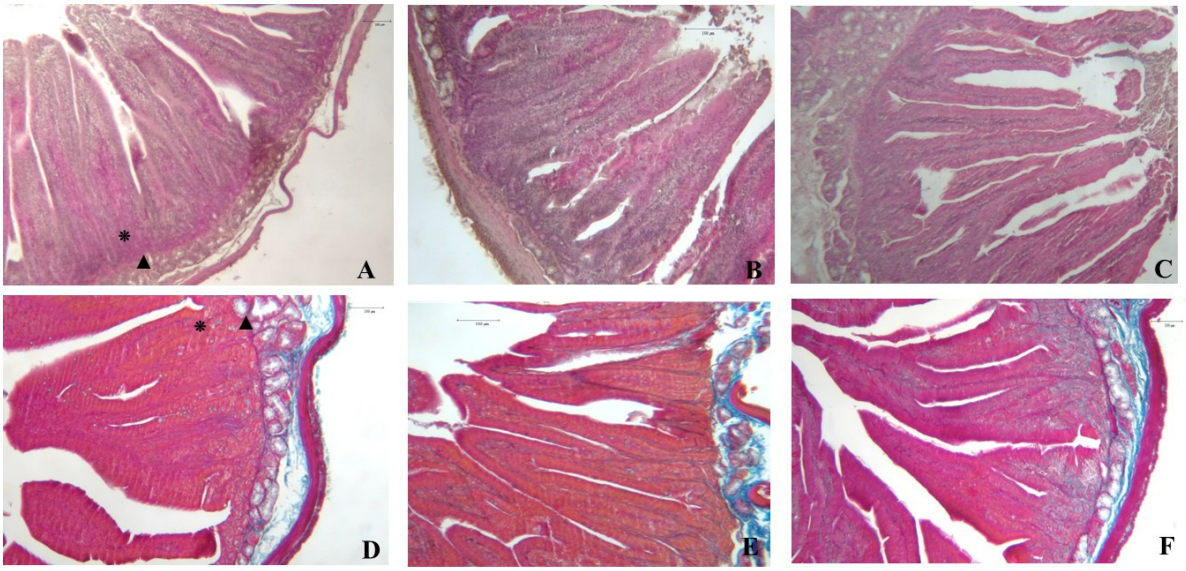

Figure 1. Representative photomicrographs of duodenum showing normally arranged intestinal villi with normal goblet cells and connective structures (at 40×). Crypts of Lieberkühn (*); glands of Brunner (▲). RLS0 diet (**A**–**D**); RLS5 diet (**B**–**E**); RLS10 diet (**C**–**F**). Panels (**A**–**C**): H&E-stained; panels (**D**–**F**): Azan–Mallory-stained.

Well-oriented finger-like villi were lined with a columnar epithelium composed of enterocytes and GCs that were regularly distributed among columnar cells, and were revealed using the PAS technique in darker pink colour. At the base of the villi, invaginations of the epithelium form the crypts of Lieberkühn; these glands, together with GCs, were positive to PAS staining (Figure 2). The structural features of the intestinal wall were also analysed by the Azan Mallory trichrome method, which evidenced a harmonic distribution of extracellular matrix and collagen fibres stained in blue (Figure 1).

The dietary treatments had a significant effect on gut histomorphometry values, with an improvement of RLS10 duodenal VH and VH:CD ratio ($p < 0.05$ and $p < 0.01$,

respectively) in rabbits that received RLS10 compared to the other diets. Conversely, rabbits in the RLS0 diet group showed greater VH and VH:CD ratio compared to rabbits fed the RLS5 diet. With regard to CD and villus surface area measurement, they were similar in all the dietary treatments, except for a slight increase in the villus surface area in RLS-supplemented groups.

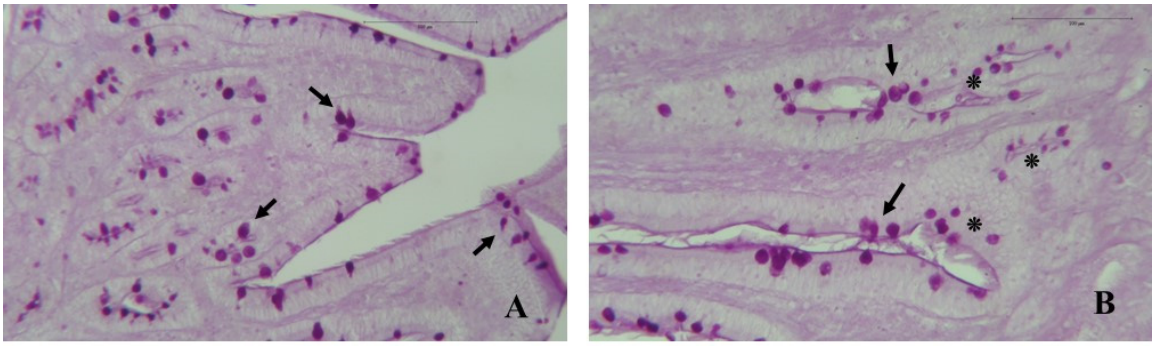

Figure 2. Details of rabbit duodenum apex (panel **A**) and base of villus (panel **B**) stained by the PAS method at 100× magnification. The PAS staining revealed goblet cells (arrows) and crypts of Lieberkühn (*), which were normally distributed on the mucosa layer in all tested dietary groups.

To date, no previous studies have evaluated the influence of LS dietary integration on duodenal histomorphometric traits. As already mentioned, lentils contain bioactive compounds, such as phenols, tannins, and saponins, with attested antioxidant and anti-inflammatory activity, potentially leading to beneficial effects on gut health [16,63,64]. Moreover, phenols bound to fibre and released after fermentation are reputed to exert in situ antioxidant and anti-inflammatory activity, being responsible for better nutrient absorption and an enhanced intestinal barrier [56,65,66]. Thus, the harmonic development of digestive tract architecture and the morphometric changes found in rabbits fed RLS may be tied to a restoration of redox balance in the intestinal environment promoted by lentil phenols. Notably, such effects have been postulated to positively influence gastrointestinal morphology. With this in mind, the improved VH and VH:CD ratio found in the RLS10 diet vs. the RLS5 and control diets could be related to the increased amount of dietary antioxidants delivered by the higher levels of dietary RLS implementation.

Lastly, data collected in this feeding trial show that dietary RLS exerted a role in preserving the gut environment and maintaining balanced gut homeostasis, as well as functionality through adequate gut morphometry and morphology and a stable microbiota community. These findings confirm that RLS may be considered a safe feedstuff in rabbit nutrition to support adequate gut functionality and proper anatomical development.

4. Conclusions

According to the findings of the present study, it may be concluded that red lentil screenings could serve as an unconventional feed ingredient in rabbit diet formulations with no adverse effects on productive performance, blood parameters, antioxidant status, or histomorphometric features. The possibility to further investigate the effects of lentil by-products in rabbit diets seems to be an effort-effective way to enhance animal products, reduce feed costs, and meet consumer demand. Furthermore, the upcycling of red lentil screenings addresses the environmental concerns derived from agricultural by-product disposal, hence reducing valuable biomass loss.

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Data Availability Statement: Data are available upon request.

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