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Cardoon Meal (*Cynara cardunculus* var. *altilis*) as Alternative Protein Source during Finishing Period in Poultry Feeding

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Abstract: The Food and Agriculture Organization's previsions show that by 2050 the world's population will reach 9.6 billion people, and the request for a high value protein source will increase as well. Poultry can guarantee high value protein for humans, even in the poorest regions of the world. Hence, efficient poultry production is needed, matching with sustainable development. The residual meal from cardoon seed oil (used for biodiesel and biodegradable bioplastic production) is suitable for animal feeding due to its protein content. The aim of this preliminary study was to test for a possible use of cardoon meal as a protein source in a poultry diet during the finishing period. Forty-five Kabir chickens were divided into three groups and fed three diets in which soybean meal (control) was partially (16%) or completely replaced with cardoon meal as a protein source (treated groups). In vivo performances, animal welfare, dressing out and meat color were evaluated. No statistical differences in feed efficiency, dressing out, nor in meat quality were found among groups. Moreover, birds that were fed cardoon meal showed lower perivisceral fat. Therefore, cardoon meal could be considered as an alternative for soybean meal in the finishing period in poultry feeding.

Keywords: cardoon; poultry feeding; finishing period; sustainable animal production; bio-waste

1. Introduction

Cardoon (*Cynara cardunculus* spp.) is considered to be a tolerant species that can be cultivated in non-irrigated lands of the Mediterranean area, representing a good opportunity in reclaiming and remediating marginal and unutilized lands [1]. Cardoon oil is mainly used for biodiesel and biodegradable bioplastic production [2]. The meal, derived from oil extraction, shows interesting chemical characteristics for its contents in fiber, protein, flavonoids, α-tocopherol and unsaturated fatty acids such as oleic acid, that make this byproduct suitable for animal feeding. The Food and Agriculture Organization's (FAOs) previsions show that the world population will reach 9.6 billion people by 2050, and that food production will increase to approximately 70% [3]. Hence, an efficient livestock production is needed to meet the food requirements of people, matching with sustainable development. Poultry production is strategic in supporting the livelihoods of billions of people because it guarantees high value protein for human nutrition, combined with short production cycles [4].

Chicken has a high efficiency in converting feed nutrients into body weight gains (meat), but the food vs. feed competition (one of the most important problems in the sustainability of animal production) is particularly high because many of the dietary ingredients are also mostly edible for humans. Food vs. feed competition affects the animal production sustainability under several aspects: the employment of human-edible crops in feeding practices to improve animal dietary efficiency; the soil and water exploitation; the impact of mechanical soil tillage. Soybean meal (SM) is a common ingredient of animal diets (approximate cost: EUR 345.00 per ton) due to its high protein content, however, cardoon meal (CM), containing about 25–30% protein and costing approximately EUR 180.00 per ton, could represent an interesting alternative to SM [5,6]. Up until now, cardoon meal has been used in ruminant feeding due to its high fiber content (approximately 58% in neutral detergent fiber (NDF)) and because rumen microbiota is very efficient in catabolizing cellulose [5,6]. In contrast, little information about poultry rearing is available because diets with a high fiber content have a low digestibility in monogastrics. Affecting animal welfare, performance and carcass traits, high fibrous ingredients are usually avoided in poultry formulas. The interest in testing CM in poultry feeding is focused on the high content in protein and in essential fatty acid [7]. However, breeds with a lower growth rate and a more frugal diet could exploit the CM's nutritional value. Therefore, the aim of this trial was to evaluate the effect of CM on animal welfare, performance and carcass traits, as a substitute of SM in the diet of Kabir chickens during the finishing period.

2. Materials and Methods

2.1. Experimental Design

Animals, Diets, Management and Welfare Evaluation

The handling of the animals was carried out in compliance with the Italian government's guidelines (D.Lgs.vo 4 Marzo 2014, n. 26; comma g). Forty-five, 53-days-old Kabir male chickens (1215.78 ± 184.97 g) were provided by a local farm (Bellavista di Fulci Lajatico, PI, Italy), where they were vaccinated against Marek disease, infectious bronchitis and Newcastle disease. To avoid a sex influence of the animal yield, only male birds were used, because males have higher yields than females [8]. The birds were reared outdoors, randomly allotted into three rectangular enclosures that were delimited by metal grid walls (Keller type) with narrow meshes so as to prevent the entry of synanthropic animals but, at the same time, to guarantee excellent and natural ventilation, lighting and visual contact between the subjects of the different groups (5 m × 5 m; 15 animals per pen-1.67 m^2/animal-singularly identified by ring). Corrugated polyurethane panels were used to cover the fences. Birds belonging to the soybean meal group (SG) were fed a basal diet in which the protein source was SM, while the soybean–cardoon meal (SGC) and the cardoon meal (CG) groups were fed the same basal diet of SG, in which SM was partially (SGC) or completely (CG) substituted with CM. The diets were formulated to be isoproteic or isolipidic according to the animal requirements [9]. The ingredients of the three diets are showed in Table 1.

Feeding and water were guaranteed ad libitum (100 cm long bar feeders with 14 workstations and siphon drinkers in each pen). The litter, consisting of chipboard, was cleaned daily and, in every part of the fence it remained dry and crumbly (except for some areas near the water troughs that were a little more humid). The minimum number of animals was determined using G*Power [10], choosing α value = 0.05, power $(1 - \beta) = 0.8$ and the medium effect size = 0.30. The choice of the Kabir breed was related to its resilience to cold and heat stress. The replacement of SM with CM was considered only for the finishing period because of the high lignin content in the CM's proximate profile. After the first seven-day period of adaptation to the new feeding strategy, the trial lasted 21 days. The animal welfare evaluation of the experimental subjects was carried out using the guidelines of the ClassyFarm system [11]. Two feces samples per pen, and per inspection, were collected to assess the presence of intestinal *Coccidia* and *Nematoda*, following the McMaster method [12,13]. To evaluate intestinal activity, each enclosure was virtually divided into six equally sized areas, observing how and how many areas were used for the feces deposition, using a scoring from 1–3 (1 = little used, 2 = on average used, 3 = very used). Weekly, birds were individually weighed, and the feed intake was registered for each pen. The individual feed intake was calculated by dividing the total amount of feed consumed by the overall number of animals in the pen. Feed efficiency was calculated for each group as an estimated individual feed intake/individual weight gain ratio. On the 81st day, the animals were sacrificed in an authorized slaughterhouse in compliance with Italian government's guidelines (D.Lgs.vo 4 Marzo 2014, n. 26).

Table 1. Diet ingredients.

Ingredients g/100 g	SG [1]	SCG [1]	CG [1]
Maize	45.50	50.95	40.05
Soybean meal	9.50	8.00	-
Cardoon meal	-	20.00	40.00
Wheat	20.00	14.00	13.00
Cardoon oil	2.00	2.10	2.00
Wheat bran	18.05	-	-
Vit/Min mix	0.50	0.50	0.50
$CaCO_3$	1.50	1.50	1.50
$CaHPO_4$	1.90	1.90	1.90
$NaHCO_3$	0.25	0.25	0.25
NaCl	0.25	0.25	0.25
Lysine	0.15	0.15	0.15
Coline	0.15	0.15	0.15

[1] SG, soybean meal group fed with a basal diet in which the protein source was soybean meal (SM); SCG, soybean–cardoon meal group, in which the protein source was a mix of SM and cardoon meal (CM); CG, cardoon meal group, in which the protein source was CM.

2.2. Analysis

2.2.1. Diet Proximate Analysis

Diets were analyzed for crude protein (CP), crude fat (CF) and ash according to the Association of Analytical Chemistry (AOAC) methods 976.06, 920.39 and 942.05 [14] while, for NDF, acid detergent fiber (ADF) and acid detergent lignin (ADL) were analyzed according to Van Soest et al. [15]. Metabolisable energy (ME) was estimated according to Sauvant et al. [16]. Data are shown in Table 2.

Table 2. Diet nutritional profile.

Item	SG [1]	SCG [1]	CG [1]
Dry matter (DM) g	89.91	88.04	89.85
Crude protein g/100 g DM	19.70	19.57	19.02
Crude fat g/100 g DM	5.02	5.05	4.46
NDF g/100 g DM	27.19	31.86	38.13
ADF g/100 g DM	6.28	13.74	26.93
ADL g/100 g DM	1.94	5.04	11.48
Ca g/100 g DM	1.06	1.07	1.10
P g/100 g DM	18.05	0.81	0.83
Metabolizable energy Kcal/kg DM	2610.01	2525.65	2424.30

[1] SG, soybean meal group fed with a basal diet in which the protein source was SM; SCG, soybean–cardoon meal group, in which the protein source was a mix of SM and CM; CG, cardoon meal group, in which the protein source was CM.

2.2.2. Carcass Traits and Physical Analysis

After 24 h from the slaughter, the dressing out percentages were computed as the ratio between the eviscerated warm carcass and the live weights for all of the birds. Moreover, selected traits (legs, fat, breast, liver, gizzard) of all the carcasses were evaluated [17]. To evaluate the color parameters, the right section of the breast was placed into a clean glass petri dish and the color was measured on two different points by a portable colorimeter (Minolta CR 200 Chroma Meter4, Konica Minolta Chiyoda, Tokyo, Japan) and calibrated using a standard yellow calibration tile (model CRA471). Data were reported in the CIE-Lab color notation system [18]. The numerical total color difference (ΔE2000) among samples was calculated by the formula proposed by Mokrzycki and Tatol [19]. All physical measurements were carried out at the standard temperature of 25 °C.

2.2.3. Statistical Analysis

Data related to the weight gain and feed efficiency of each week were processed as a completely randomized design with repeated measures using the SAS MIXED procedure in which diet and time were fixed effects [20]. The animal was considered as the experimental unit and it was included in the model as a random effect nested within the main treatment (diet).

$$Y_{ijkl} = \mu + D_i + T_j + I_k\ (D) + (D \times T)_{ij} + e_{ijkl}$$

where Y_{ijkl} is the single observation; μ the overall mean; D_i the fixed effect of the diet (I = 1 to 3); T_j the fixed effect of the sampling time (j = 1 to 3); I_k the random effect of the bird nested within the diet (k = 1 to 15); $(D_i \times T)_{ij}$ is the interaction between the diet and sampling time and e_{ijkl} the residual error. The covariance structure was a compound symmetry, which was selected on the basis of the Akaike information criterion of the SAS MIXED model [20]. The statistical significance of the diet effect was tested against the variance of the bird nested within the diet, according to the repeated measures design theory [21]. Multiple comparisons among means were performed using the Tukey test.

Data related to the weight gain, feed efficiency of the whole period, physical parameters of the meat, dressing out and carcass traits of the slaughtered birds were analyzed by a one-way ANOVA, keeping the factor "diet" as the fixed one [20].

$$Y_{ikl} = \mu + D_i + I_k(D) + e_{ikl}$$

where Y_{ijkl} is the observation; μ the overall mean; D_i the fixed effect of the diet (i = 1 to 3); T_j the fixed effect of the sampling time (j = 1 to 3); I_k the random effect of the bird nested within the diet (k = 1 to 15) and e_{ijkl} the residual error. Multiple comparisons among means were performed using the Tukey test. The probability of a significant effect due to experimental factors was fixed for $p < 0.05$.

3. Results

3.1. Animal Performances

Observing the birds' behavior during feeding, CM seemed to be appreciated by the animals in terms of palatability. The feed intake of the diets for the whole period was 3736.00 g in SG, 3649.00 g in SCG and 4297.00 g in CG. The weekly feed intake of the diets was: in the first week 890.00 g in SG, 957.00 g in SCG and 1031.00 g in CG; in the second week 1612.00 g in SG, 1611.00 g in SCG and 1842.00 g in CG; in the third week 1234.00 g in SG, 1081.00 g in SCG and 1424.00 g in CG.

Data related to the productive performances (weekly registered) and the whole feeding period are reported in Table 3.

Table 3. Animal performances.

Days of Life		SG [1]	SCG [1]	CG [1]	SEM [2]	p [3]
60–67	gain, g	222.66a	206.67a	62.00b	14.28	<0.0001
	feed/gain	4.15b	5.88b	15.8a	1.88	0.0006
67–74	gain, g	244.00	273.00	245.67	20.84	0.5479
	feed/gain	6.97	7.34	8.68	1.06	0.4890
74–81	gain, g	155.34	174.25	180.71	15.71	0.5073
	feed/gain	8.42	7.51	8.80	0.80	0.5244
60–81	final body weight, g	1848.00	1842.33	1682.14	56.49	0.0826
	gain, g	622.00a	654.00a	481.00b	34.41	0.0021
	feed/gain	6.20b	5.86b	9.90a	0.57	<0.0001

[1] SG, soybean meal group fed with a basal diet in which the protein source was SM; SCG, soybean–cardoon meal group, in which the protein source was a mix of SM and CM; CG, cardoon meal group, in which the protein source was CM. [2] SEM, standard error mean. [3] *p*, probability of a significant effect due to experimental factors; a, b, within a row, means with different Latin letters are significantly different ($p < 0.05$).

3.2. Dressing out and Carcass Traits

No significant differences were found among the groups for the dressing out and the major carcass traits, with the exception of the breast weight and of the perivisceral fat content, that showed to be lower in the CG than in the other groups (Table 4).

Table 4. Means and standard error means (SEM) of dressing out, selected carcass traits and breast meat color.

Dressing out, Selected Traits	SG [1]	SCG [1]	CG [1]	SEM [2]	p [3]
Dressing out, g/100 g of live weight	88.43	89.03	87.67	0.96	0.6081
legs, g	380.10	365.26	343.34	24.42	0.5783
perivisceral fat, g	27.47a	23.02a	9.98b	4.23	0.0111
breast, g	170.50a	151.70b	108.24c	14.21	0.0256
liver, g	37.86	37.50	35.24	2.91	0.7920
gizzard, g	59.32	62.20	68.20	3.61	0.2483
Breast meat color					
L *	59.77b	59.96b	63.29a	0.77	0.0049
a *	1.55	1.21	1.07	0.29	0.4923
b *	5.28	6.12	4.49	0.55	0.1333

[1] SG, soybean meal group fed with a basal diet in which the protein source was SM; SCG, soybean–cardoon meal group, in which the protein source was a mix of SM and CM; CG, cardoon meal group, in which the protein source was CM. [2] SEM, standard error mean. [3] P, probability of a significant effect due to experimental factors; a, b, within a row, means with different Latin letters are significantly different ($p < 0.05$). "*" is part of the symbol parameter.

Considering the meat color, the highest value of L was found in CG, while the other parameters did not show any significant differences (Table 4).

3.3. Animal Welfare

The availability of space has allowed the animals to accomplish their physiological requirements and ethological characteristics of the species [22]. During the finishing period, no mortality episodes occurred and animals showed no stereotypy. No direct trauma, lameness or mutilation lesions were observed. Several subjects (three in SCG, two in CG and one in SG) presented small areas of de-draining at the lumbar area [10]. All subjects were always reactive and during the entire production cycle did not present symptoms due to respiratory or enteric diseases. The cloacal area, as well as the hocks, have always been cleaned. Positive results were found for *Coccidia*. In the SG group, 100 epg at the first control and 50 epg at the second control, and in the SCG group, 900 epg at the first control and 1000 epg at the second control. At both parasitological examinations, the CG group was always negative. The SG and SCG groups used four areas for faeces deposition (two areas scored 1, and two areas scored 2), while the CG group uniformly used the entire enclosure.

4. Discussion

Protein in poultry feeding is an essential nutrient whose failure in meeting requirements strongly affects animal growth [8]. From an economic point of view, protein is also a component that determines the cost of the feeding strategy. Soybean meal is the preferred plant protein source in poultry feeding because of its high percentage of this nutrient (from 40 to 48%). Soybean production has increased substantially to meet the market requirements of oil and meal, respectively, for human and animal nutrition. Nowadays, the soybean currently used is genetically modified to increase the herbicide tolerance, feeding debates to the opportunity to use it as an ingredient in animal diets. Hence, new protein sources, such as an SM alternative, have been considered in animal feeding strategies. An example of an SM substitute with low cost and a good nutritional profile is cardoon meal.

Data related to the first week of the trial showed a lower gain and gain/feed ratio in the CG, perhaps, due to the longer period of adaptation to the new diet, richer in fiber and lignin, compared to the control diet. Indeed, the higher dietary level of fiber in CG, due to the seed hull present in CM, induced an increase in the feed intake to meet the animal's energy requirement. As a consequence of a lower gain, the feed efficiency decreased and this negative performance hindered the whole period of finishing. The animals fed with the blend of SM and CM showed similar performances to the ones who were fed with the SM diet. The partial substitution of SM could be an interesting compromise, with the aim to diminish the use of SM in the feeding strategy and to maintain a low dietary fiber content. Similar results are reported in Manyeula et al. [23] who found that the inclusion of canola meal in place of SM had no negative impact on carcass traits, organ size or meat quality traits when inserted into the formula for slow-growing indigenous chickens that better adapt to poor quality feed resources, despite the anti-nutritional effects of glucosinolates and the high content of lignin.

Literature offers a wide range of data on the suitability of oil meals as a protein source in poultry feeding as an alternative to SM. However, many of these meals cannot be used directly in the formula, but only after chemical or physical treatments or at a very low inclusion level. For instance, jatropha meal contains about 40% of CP, but phytate, tannins, saponins, phorbol esters, and inhibitors of trypsin limit its use [24]. Raw jatropha meal causes detrimental effects on the reproductive performance of birds and decreases weight gain as a consequence of its low digestibility. These effects are due to the high content of lignin derived from shells that are not completely removed during the oil extraction [24–26]. Cottonseed and canola meal are also used in poultry feeding but, respectively, gossypol and glucosinolates exert toxic effects on birds. Several studies reviewed by Świątkiewicz et al. [27] showed that cottonseed meal worsens feed efficiency and growth performance, due to the inhibition of pepsin and trypsin, or iron and lysine deprivation caused by gossypol. Glucosinolates make canola meal unsuitable for broiler nutrition because they are detrimental to animal health, damaging the liver, negatively affecting the growth performance of birds and decreasing appetite. Canola meal results in the low growth and economic performance of broilers, and the detrimental effect of glucosinolates on liver is demonstrated in [28,29]. Another matrix is the groundnut meal which contains a high amount of tannins, decreasing the dietary protein digestibility in monogastrics, with a high risk of aflatoxin contamination. Although sesame meal is a good source of antioxidants, methionine, oleic and linoleic acids, its use is limited by the phytates presence in a not negligible amount [30].

In line with the 3R principle (Recycle, Reduce, Reuse), the possibility of recycling a byproduct of a cardoon crop, as the meal, is a very interesting opportunity. Its chemical composition makes it a good source of protein and fat, and the plant, *Cynara cardunculus* L., due to its tolerance to dry weather conditions and its resistance to diseases and parasites, is suggested as a potential alternative crop for reclaiming and remediating marginal lands [31].

Overall, the animal well-being conditions were good. In all the areas with feces deposition there were drinkers, while in the unused areas there were no drinkers. The CG group uniformly used all six areas for feces deposition, showing an average use in [32]. This also suggests a greater locomotor activity in the CG group.

No significant differences were found among the groups for the dressing out and the selected traits with the exception of the breast. The weight seems to be positively related to the SM presence in the diet, and the lower value was shown by CG ($p < 0.0256$). This finding could be related to the higher fat deposition of SM, as shown by the major content of perivisceral fat in the carcasses of birds fed the SM. However, a better feed conversion of the SM, in terms of protein digestibility, could positively have affected the final body weight. The *Pectoralis major* muscle is entirely composed of type IIB fast glycolytic fibers (white fibers) and its growth could be affected by the less N retention when CM replaced SM [33].

Meat color is one of the most important attributes used by consumers to discriminate meat quality at first glance. In this sense, the slight but significant variation in lightness seemed to cause a modification of the CG meat color that even an experienced observer may note, since the calculated ΔE was comprised between 2 and 3.5 [18]. The reasons why this change occurred remain unclear, thus further investigation on this topic could help to explain such a variation in the lightness value. Nowadays, poultry represents an excellent source of high value protein for humans [30].

Considering the amount of fiber in CM (approximately 58% in NDF, on dry matter (DM)), it is feasible that fast-growing meat birds have a low tolerance to its high inclusion level. Generally, industrial chicken hybrids are reared in indoor systems with short production cycles (45–50 days of growth) and show a high feed efficiency but, in terms of sustainability, the feed vs. food competition is very high. An alternative could be represented by the open-range production in which slow-growing genotypes, characterized by longer growing periods (45–50 days vs. 80–90 days), are used because they are particularly resilient to environmental conditions and are more tolerant to high fiber and poor quality feeds.

5. Conclusions

The current study demonstrated that CM could be considered as an alternative for SM in the finishing period of poultry feeding. In fact, no statistical differences in feed efficiency, neither in dressing out nor in meat quality, were found among groups. Considering that CM is a byproduct of oil extraction, the use of CM in a poultry feeding strategy could be a good opportunity in increasing the sustainability of animal productions. Since diets were isolipidic, the minor perivisceral fat deposition could be symptomatic of a different lipid metabolization. Thus, further investigations are needed to deepen the knowledge of CM's nutritional effects.

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