

## Grass intake and meat oxidative status of geese reared in three different agroforestry systems

Alice Cartoni Mancinelli\*, Simona Mattioli, Alessandro Dal Bosco, Claudia Ciarelli, Cesare Castellini

Department of Agricultural, Environmental and Food Science, University of Perugia, Perugia, Italy



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The agroforestry system could be considered a dynamic management of the natural resource based on the integration of trees with crops or livestock. The purpose of this study was to evaluate the grass intake and the oxidative status of meat of geese reared in three different agroforestry systems: apple orchard (AO), olives trees (OT) and vineyard (V). Eighty one-day old Romagnola geese of both sexes were divided in four homogeneous groups: control (C), with indoor density of 5 geese/m<sup>2</sup> and without pasture access, and the three agroforestry systems (AO, OT, V), with 1 hectare of pasture each. The geese were reared inside a poultry house until 20 days of age. At 21 days of age the animals belonging to AO, OT and V were allowed to outdoor access (pasture), whereas geese of the C group were kept indoor. At 150 days of age, the geese were slaughtered in a commercial slaughterhouse. After 24 h of storage at +4°C the breast and drumstick muscles were analysed to determine the fatty acid profile, the antioxidants content and the oxidative status. All the data were statistically analysed with ANOVA. The results showed that the grazing activity of geese improved the n-3 polyunsaturated fatty acids content, the n-6/n-3 ratio and the antioxidant content, especially in geese kept in the agroforestry systems enriched with trees (AO and OT). Indeed, the presence of trees make animals feel protected and stimulated them to explore the pasture and consequently to consume more grass. However, the best oxidative status was exhibited by the C geese. In the other groups the higher antioxidants intake through grass was not able to counteract the higher oxidative thrust and consequently, the meat of outdoor reared geese was characterized by a worst oxidative status. Further research is needed to identify new possible strategies to increase the antioxidant content in the muscle in order to reduce the lipid oxidation.

**Keywords:** geese, agroforestry system, PUFA, oxidative status

### 1 Introduction

The Global Forest Resources Assessment (FAO, 2016) highlighted a significant reduction of the world forest area with the loss of 129 million of hectares between 1990 and 2015. In particular, in Northern Europe the intensification of agricultural practices caused an important land use change, whereas in most southern European countries the land showed a high risk of abandonment (Plieninger et al., 2016). In Italy, orchards (mainly olive trees) are located on marginal land: the sloping terrain, the altitude and the difficulty of mechanization render about 1 million hectares of olive trees at risk of abandonment (Rosati et al., 2012). Such a state of affairs has caused a degradation of the terrain, which in turn has intensified the hydrogeological instability and has increased the risk of fire episodes, especially in the period between 2008 and 2017 (San-Miguel-Ayanz et al., 2017).

In this scenario, it is necessary to minimize the environmental impact on crop and animal productions (De Boer et al., 2011) by improving the agro-food sustainability, recovering and valorising the by-products and wastes (Tinello et al., 2017), preserving biodiversity (Bach et al., 2020), increasing animal welfare (Broom, 2019) and reducing water footprint of agricultural practices (Cosentino et al., 2015). In order to solve these environmental problems, the European Commission has developed

\* **Corresponding Author:** Alice Cartoni Mancinelli. Department of Agricultural, Environmental and Food Science, University of Perugia, Borgo XX Giugno 74, 06124 Perugia, Italy. E-mail: [acartonimancinelli@gmail.com](mailto:acartonimancinelli@gmail.com).  
ORCID: <https://orcid.org/0000-0001-9888-9079>

some strategies during the years such as the Nitrate Directive (1991; 91/676/CEE), the Water Framework Directive (2000; Directive 2000/60/EC) and the Biodiversity Strategy (2010; COM (2011) 244).

However, most of the problems due to the agricultural intensification and land abandonment still persist (Plieninger et al., 2016). Agroforestry represents a useful approach to contain problems connected with the land use (Nair and Garrity, 2012). This system is based on the consociation of trees and crops or/and trees and livestock on the same area (Torralba et al., 2016). Agroforestry is particularly interesting when animals do not require soil exclusively destined to them. Paolotti et al. (2016) demonstrated that the combination of poultry production and olive orchard provide a significant reduction in terms of environmental impact. The integrated farming system determines a closed loop between animal rearing and crop production, implementing in this way a circular agriculture. This system is based on the production of more foods (i.e. crop and meat) in the same land by reducing the intensification of agricultural practices and valorising residues (Patrizi et al., 2018).

Many poultry species (chickens, guinea fowl, geese) prefer to graze in orchards where trees give protection from predators and shade. The grazing activity of animals contributes to natural pest control (Sow et al., 2020) and fertilization, and it increases the quality of products (eggs and meat). Massaccesi et al. (2019) showed that on the upper soil horizons, the presence of geese improves the activity of the microbial biomass. Eggs of hens bred on pasture exhibited higher content of  $\alpha$ -tocopherol, carotenoids and polyphenols (Mugnai et al., 2009). The percentage of polyunsaturated fatty acids (PUFA), particularly of those with higher biological value like eicosapentaenoic (EPA) and docosahexaenoic (DHA), was higher in meat of poultry organically reared respect to the conventional ones (Dal Bosco et al., 2012). Moreover, geese are frugal animals thanks to their great grazing capacity, and do not require complex housing systems and fences (Liu and Zhou, 2013).

A serious problem of rearing poultry in large open spaces is related to the intensification of the oxidation processes of the PUFA with deterioration of the oxidative status both *in vivo* and in meat (Cartoni Mancinelli et al., 2019). For this reason, the aim of this study was to evaluate the relationship between the grass intake and the oxidative status of meat of geese reared in three agroforestry systems: apple orchard (AO), olive trees (OT) and vineyard (V).

## 2 Material and methods

### 2.1 Animals housing

The trial was carried out from April to August 2019. The geese were reared according to EU Regulation 834/07 and EU Regulation 889/2008 laws on animal welfare for experimental and other scientific purposes. All the farms of this study were organic and, according to organic rules (Council Regulation: EC 834/2007 and 889/2008), the AO, OT and V were only sprayed with copper-based fungicides. Eighty one-day old Romagnola geese of both sexes were divided in four homogeneous groups: control (C), without pasture access, and the three agroforestry systems (AO, OT and V), with 1 hectare of pasture each. Geese were kept in a poultry house until 20 days of age with temperature from 20 to 32°C, relative humidity from 65 to 75% and indoor density of 5 geese/m<sup>2</sup>. At 21 days of age, the geese located in the three agroforestry systems were allowed to access the pasture during the day and each system was equipped with an overnight shelter. All the groups were fed *ad libitum* with the same organic diet (Table 1), and feeders and drinkers were distributed both inside and outside the poultry house. At the beginning of the breeding cycle two exclusion pens (50x50 cm) for each agroforestry system were used. The grass intake was estimated by applying the modified method of Lantinga et al. (2004), using the following equation:

$$\text{Grass intake} = (GMs - GMe) + \{[1 - (GMe/GMs)] / - \ln[GMe/GMs]\} \times (Gmu - GMs),$$

where GMs is the herbage mass present at the entrance of the birds in each pen; GMe is the forage that remained at the end of the trial; and Gmu is the undisturbed forage mass from the exclusion. The geese belonging to the C group were always reared indoor. At 150 days of age, all the geese of the C, AO, OT and V groups were slaughtered in a commercial slaughterhouse. Feed was withdrawn 12 h before slaughtering.

**Table 1** Ingredients of the starter and grower organic diets

Ingredient, %	Diet	
	Starter	Grower
Maize	53	63
Soy-bean meal	38	28
Fava bean	6	6
Vitamin-mineral premix	1	1
Dicalcium phosphate	1	1
Sodium bicarbonate	0.4	0.4
Calcium carbonate	0.3	0.3
NaCl	0.2	0.2
L-lysine	0.05	0.05
Methionine	0.05	0.05

## 2.2 Sampling and analytical determinations

After 24 h of storage at +4°C, 20 samples of *Pectoralis major* and *Biceps femoris* per group were dissected from the carcasses and fatty acid profile, tocopherols content, retinol amount and thiobarbituric acid reactive substances (TBARS), were determined.

## 2.3 Fatty acid profile

Fatty acids content was assessed as fatty acid methyl esters (FAME) using a gas chromatograph (Agilent Technologies 6890N Network GC System, Ca, USA) equipped with a flame ionisation detector and an automatic sampler (Agilent Technologies 7683 Series Injector). To separate and analyse the FAMES a CP-Select CB for a FAME fused silica capillary column (100 m × 0.25 mm internal diameter, film thickness 0.39 µm; J&W, Agilent technologies, Palo Alto, CA, USA) was used. The injector and the detector temperatures were 270°C and 300°C, respectively. The sample (1 µL) was injected into a split/splitless system (split ratio 1:25). Helium was used as a carrier gas, at a flow rate of 1.6 mL/min. The oven temperature followed this programme: raised from 60°C to 150°C at a rate of 30°C/min and held for 3 min. Then raised to 185°C at a rate of 0.5°C/min and after 1 min raised to 220°C at a rate of 1.5°C/min and held for 12 min. A standard mixture obtained by Sigma (PUFA No. 1, Marine Source, 37 FAMES, methyl cis-7,10,13,16,19-docosapentaenoate, Supelco, Bellefonte, PA) was used as a comparison in order to identify the individual FAME. The peak area of the samples adjusted with the respective correction factors was used to calculate the percentage of each FA (AOAC, 2012). The average amount of each fatty acid was used to calculate the sum of saturated fatty acids, monounsaturated fatty acids (MUFA) and PUFA. Furthermore, α-linolenic acid (ALA) and linolenic acid (LA) content (mg/100 g tissue) was calculated as reported by Joseph and Ackman (1992):

$$\text{Fatty acids (mg/100 g)} = [(AX \times WIS \times CRFx \times CNF_x) / (AIS - 957 \times W_s)] \times 1,000 \times WL,$$

where AX is the ALA or LA area; WIS is the weight of the internal standard added to the lipids; CRFx is the theoretical correction factor for ALA and LA; CNF<sub>x</sub> is the conversion factor from FAME to the corresponding fatty acid; AIS is the internal standard area; W<sub>s</sub> is the weight of the derivatised lipids; and WL is the percentage of sampled lipid.

## 2.4 Antioxidant content

The meat content of the tocopherols (α-tocopherol and its isoform β+γ and δ), α-tocotrienol and retinol was assessed by HPLC according to Hewavitharana et al. (2004). Specifically, 2 g of meat sample with 5 mL of distilled water and 4 mL of ethanol were vortexed for 10 s. Then, 4 mL of hexane containing BHT (200 mg/L) was added and the mixture was carefully shaken and centrifuged. 3 mL of supernatant was dried under a stream of nitrogen and then redissolved in 300 µL of acetonitrile. 50 µL were injected into the HPLC system (Jasco, pump model PU-1580, equipped with an autosampler system (model AS 950-10, Tokyo, Japan) on a Ultrasphere ODS column (250 × 4.6 mm internal diameter, 5 µm particles size; CPS analytic, Milan, Italy). A FD detector (model Jasco, FP-1520,

Tokyo, Japan) set at excitation and emission wavelength of 295 nm and 328 nm, was used to identify tocopherols and tocotrienols respectively. These compounds were quantified using external calibration curves prepared with increasing amounts of pure standards in ethanol. Retinol was detected by UV (Jasco UV 2075 Plus, Tokyo, Japan) at 325 nm, and quantified using an external calibration curve as described for tocopherols. In the present work the content of  $\alpha$ -tocotrienol and retinol are expressed as total antioxidants ( $\Sigma$ ).

## 2.5 Oxidative status

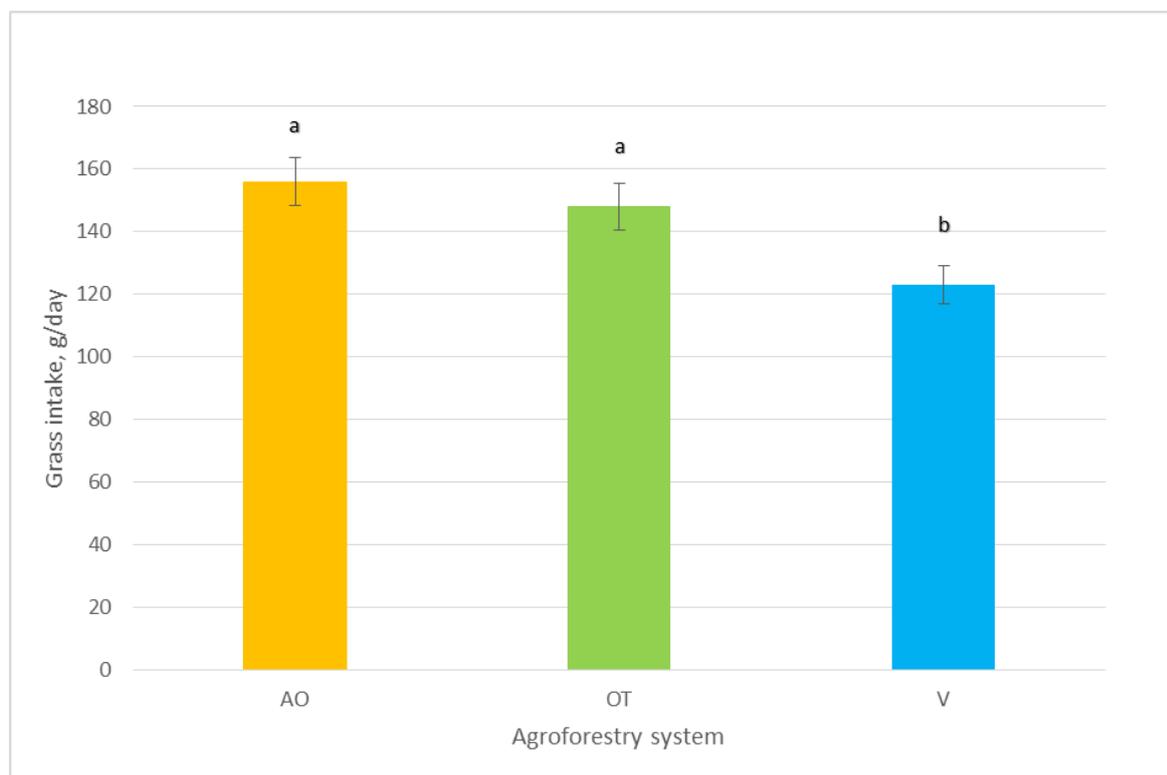
The lipid oxidation of meat was evaluated following the method of Ke et al. (1984). Through a spectrophotometer set at 532 nm (Shimadzu Corporation UV-2550, Kyoto, Japan), the absorbance of TBARS was detected. Oxidation products were quantified as  $\mu\text{g}$  of malondialdehyde per g of muscle.

## 2.6 Statistical analysis

The grass intake and all the meat quality data were analysed through one-way ANOVA using a linear model that accounted for the fixed effect of rearing system (StataCorp, 2015). Multiple comparison of means was performed using Bonferroni's *post-hoc* test. Significance was declared at  $P \leq 0.05$ .

## 3 Results and discussion

The estimation of grass intake showed that the geese belonging to the AO and OT systems ingested a higher quantity of grass respect to the V group (Figure 1).



a,b Means with different letters differ significantly ( $P < 0.05$ )

**Figure 1** Estimation of grass intake in the geese reared in the apple orchard (AO), olive trees (OT) and vineyard (V) agroforestry systems ( $n = 20$  animals/group)

The fatty acid profile of breast and drumstick of geese reared in the three different agroforestry systems is reported in Table 2. The PUFA profile showed the same trend in both muscles: the n-6 PUFA did not show any significant differences among the groups, while the n-3 PUFA were higher in AO and OT, followed by V and C. Specifically, in the breast of AO geese, the concentration of n-3 PUFA (% of total fatty acids) was six times higher than that in the breast of C geese (3.12% vs. 0.51%,  $P \leq 0.05$ ), whereas in the drumstick the highest value was in the OT group being six times higher compared to the C group (3.92% vs. 0.66%,  $P \leq 0.05$ ). The n-6/n-3 ratio was lower in all the

agroforestry groups respect to the C group for both muscles. In the breast of AO and OT it was six and three times lower, respectively, than the C group (6.07 and 9.76 vs. 37.78,  $P \leq 0.05$ ). The AO group had the lowest n-6/n-3 ratio of the drumstick; indeed, it was five-fold lower than the C group (5.02 vs 27.68,  $P \leq 0.05$ ).

Concerning the oxidative status and the total antioxidant content of breast (Table 3), the AO and the OT groups showed the highest concentration of tocopherols and total antioxidants as compared to V and C, which, on the contrary, showed lower TBARS values. The drumstick had a lower content of tocopherols as compared to the breast. Regarding the TBARS, the highest value was reported in AO geese.

**Table 2** Fatty acid (FA) profile of breast and drumstick muscles of Romagnola geese in control (C) and the three agroforestry systems: apple orchard (AO), olive trees (OT) and vineyard (V) ( $n = 20$  animals/group)

		Agroforestry system				SEM	Significance
		C	AO	OT	V		
<i>Pectoralis major</i>							
$\Sigma$ MUFA	% total FA	45.21	43.93	41.61	46.37	3.45	n.s.
$\Sigma$ n-6 PUFA	% total FA	19.27	18.94	20.90	18.89	1.61	n.s.
$\Sigma$ n-3 PUFA	% total FA	0.51 <sup>c</sup>	3.12 <sup>a</sup>	2.14 <sup>a</sup>	1.36 <sup>b</sup>	0.62	*
n-6/n-3		37.78 <sup>a</sup>	6.07 <sup>c</sup>	9.76 <sup>c</sup>	13.88 <sup>b</sup>	4.06	*
<i>Biceps femoris</i>							
$\Sigma$ MUFA	% total FA	47.34 <sup>a</sup>	45.10 <sup>ab</sup>	41.68 <sup>b</sup>	45.12 <sup>ab</sup>	2.35	*
$\Sigma$ n-6 PUFA	% total FA	18.27	19.58	20.42	20.34	1.76	n.s.
$\Sigma$ n-3 PUFA	% total FA	0.66 <sup>b</sup>	3.92 <sup>a</sup>	2.92 <sup>a</sup>	1.29 <sup>ab</sup>	0.33	***
n-6/n-3		27.68 <sup>a</sup>	5.02 <sup>c</sup>	6.99 <sup>c</sup>	15.76 <sup>b</sup>	4.72	*

MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids; SEM – standard error of the mean  
 Significance: n.s.  $P > 0.05$ ; \*  $P \leq 0.05$ ; \*\*\*  $P \leq 0.001$ )

<sup>a,b,c</sup> Means with different letters within the same row differ significantly ( $P \leq 0.05$ )

**Table 3** Oxidative status and main antioxidants of breast and drumstick muscles of Romagnola geese in control (C) and the three agroforestry systems: apple orchard (AO), olive trees (OT) and vineyard (V) ( $n = 20$  animals/group)

		Agroforestry system				SEM	Significance
		C	AO	OT	V		
<i>Pectoralis major</i>							
$\Sigma$ Tocopherols	$\mu\text{g/g}$	2.20 <sup>b</sup>	4.11 <sup>a</sup>	5.96 <sup>a</sup>	1.17 <sup>b</sup>	1.34	*
$\Sigma$ Antioxidants	$\mu\text{g/g}$	10.26 <sup>c</sup>	18.17 <sup>b</sup>	27.28 <sup>a</sup>	14.70 <sup>a</sup>	4.34	*
TBARS	$\mu\text{g MDA/g}$	0.20 <sup>b</sup>	0.43 <sup>a</sup>	0.37 <sup>a</sup>	0.24 <sup>b</sup>	0.13	*
<i>Biceps femoris</i>							
$\Sigma$ Tocopherols	$\mu\text{g/g}$	0.92 <sup>d</sup>	3.26 <sup>a</sup>	2.56 <sup>b</sup>	2.02 <sup>c</sup>	0.35	**
$\Sigma$ Antioxidants	$\mu\text{g/g}$	19.28	25.13	23.47	23.40	3.51	n.s.
TBARS	$\mu\text{g MDA/g}$	0.24 <sup>b</sup>	0.55 <sup>a</sup>	0.45 <sup>a</sup>	0.22 <sup>b</sup>	0.12	*

$\Sigma$  Antioxidants - tocopherols ( $\alpha$ -tocopherol and its isoform  $\beta + \gamma$  and  $\delta$ ) +  $\alpha$ -tocotrienol + retinol; TBARS – thiobarbituric acid reactive substances; MDA – malondialdehyde; SEM – standard error of the mean

Significance: n.s.  $P > 0.05$ ; \*  $P \leq 0.05$ ; \*\*  $P \leq 0.01$

<sup>a,b,c,d</sup> Means with different letters within the same row differ significantly ( $P \leq 0.05$ )

Geese are naturally grazing animals and this behaviour is confirmed by the estimation of grass intake. Only few studies evaluated the grass intake of poultry. Hughes and Dun (1983) estimated that the daily intake of herbage in layer hens was about 30-40 g of dry matter. This value was confirmed by Dal Bosco et al. (2014) who reported an ingestion of 46 g/day in meat-type chickens. Other experimental studies showed that the use of pasture is strictly related to the chicken strains and a large variability among commercial poultry genotypes exists (Castellini et al., 2016, Cartoni Mancinelli et al., 2020). Some authors (Gatellier et al., 2004, Sossidou et al., 2015) demonstrated that the intake of the principal dietary components, like proteins and energy, was not affected by the ingested pasture which only enriched the products (meat and eggs) with bioactive compounds such as antioxidants, vitamins, and n-3 precursors. Our results confirmed that the grass intake improves the tocopherols and the antioxidant content of the meat in outdoor reared geese.

The presence of pasture also improved the concentration of n-3 PUFA in the meat of poultry reared outdoor respect to those reared indoor (Ponte et al., 2008, Dal Bosco et al., 2016). This finding is supported by our results that showed a greater concentration of n-3 PUFA both in breast and drumstick of geese having access to pasture respect to the C group that was reared indoor. Due to the higher concentration of n-3 PUFA in the agroforestry groups, the n-6/n-3 ratio was lower especially in the breast of AO and OT groups and in the drumstick of OT respect to the C group. The recommendation of FAO (2010) suggested that in the human diet the optimal value of the n-6/n-3 should be 4; in our trial the n-6/n-3 ratio of the drumstick in the OT group and in the breast of AO group was very close to this value.

The geese of the V group had a lower grass intake than the other agroforestry groups; accordingly, they showed a lower concentration of n-3 PUFA, tocopherols and antioxidants compared to geese bred in the AO and OT groups. This could be explained by the lower presence of grass and the presence of trees (in AO and OT) that make animals feel safe regarding to predators and provide more shade in the hottest hours of the day. With this environmental enrichment, animals are more stimulated to explore pasture and consequently to ingest grass (Dal Bosco et al., 2014).

Many studies showed a positive correlation between antioxidants content and TBARS in the meat (Dal Bosco et al., 2019, Jouki et al., 2020) but in the present study we did not find any relation. This discrepancy is probably due to the greater locomotor activities of the AO and OT groups, which have triggered the oxidative reaction of the muscle fibres. Therefore, the content of antioxidants ingested through the grass has not been able to counteract the oxidative mechanism induced by kinetic activity (Mattioli et al., 2017). In a previous study we found a similar trend (Cartoni Mancinelli et al., 2019): geese reared outdoor showed a worst oxidative status as compared with those bred indoor. Therefore, the balance between the kinetic activity and the antioxidant intake is very unstable and could depend on many factors like genetic strains, botanic essence of pasture, season, and diet. Due to the highest content of PUFA, geese meat is more susceptible to lipid oxidation; in agreement, our results showed a higher value of TBARS in the meat of geese with higher content of n-3 PUFA, i.e. geese reared in the AO and OT systems.

#### **4 Conclusions**

It has been widely demonstrated that agroforestry represents a sustainable food production system. In particular, agroforestry, by mean of suitable crops-animals interaction, could provide several economic, environmental and quality benefits. Our study highlighted that the geese, being animals with a high grazing aptitude and little environmental necessities, are suitable to be reared in this system. The presence of pasture increased the n-3 PUFA, tocopherols and antioxidant contents in the meat of geese, especially in those reared in agroforestry systems with trees. In particular, the n-6/n-3 ratio was more balanced and very close to the recommendation for human diet. Concerning the oxidative status of meat, further studies are needed to better understand how to counteract the oxidative mechanisms triggered by the locomotory activity connected with the grazing activity. Probably, it would be necessary to implement the diet of grazing animals with further complex antioxidants such as vitamin E, vitamin C, and polyphenols.

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