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Healthier Food of Animal Origin and Prevention of Campylobacteriosis

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Abstract

Campylobacter species belong to the most important foodborne bacteria which cause gastroenteritis in humans in both developed and developing countries. Applied hygienic measures result only partially efficient, as demonstrated by the high number of reported cases in the human population. Alternative strategies to prevent the disease though a direct action at primary production level have been explored. Preliminary data showed that cattle and pigs fed with bamboo and olive by-products, respectively, were free from *Campylobacter*, suggesting potential sustainable prevention strategy. In addition, biochemical analysis showed high levels of unsaturated fatty acids in red meat and meat products, adequate for the application of health claims in line with EU food law.

Keywords

Campylobacteriosis, Cattle, Food Safety, Health Claims, Pigs, Prevention, Public Health, Zoonosis

Highlights

- Campylobacteriosis is a One Health issue, widely distributed, adversely affecting food safety and public health, with particular pediatric impact.
- Alternative strategies to prevent the disease though a direct action at primary production level have been explored. Preliminary data showed that cattle and pigs fed with bamboo and olive by-products, respectively, were free from *Campylobacter*, suggesting potential sustainable prevention strategy.

- Red meat and meat products were characterized by high levels of unsaturated fatty acids, allowing the application of health claims, foreseen by the EU legislation.

1. Introduction

In the 21st century, zootechnics expanded production capacity reaching high quality levels, facing old and new challenges. Production increased especially in North America, Europe and Asia, determining high domestic animal population concentration levels [1]. Improved breeding, with genetic selection efforts, achieved high quality standards of meat and products of animal origin. Excellences have been reached in different parts of the world, often linked to the valorization of traditional breeds or the development of particular novel farming methods. For example, in Japan, the particular fattening technique has been applied to obtain the highly appreciated wagyu beef from the Japanese black bovine breed [2]. In Europe, pig black breeds, initially neglected due to the slow growth rates, revealed very high-quality meat [3]. Animal health and welfare have been improved, primarily by the control of transboundary diseases, against devastating diseases such as rinderpest (declared eradicated in 2011, according to OIE) [4] and the internalization of health standards. Concerning public health and food safety, particular efforts have been undertaken for the control and eradication of zoonotic pathogens.

Despite unequivocable positive evolution, the zootechnics sector is currently object of various criticisms which require effective responses. In 2015, the red meat was classified as Group 2A, probably carcinogenic to humans, by the World Health Organization [5]. Processed meat was classified as Group 1, carcinogenic to humans, based on sufficient evidence from epidemiological studies that eating processed meat causes colorectal cancer [5]. There were also evidence of links with pancreatic cancer and prostate cancer. Not less relevant, environment and climatic changes claim more and more strongly the reduction of animal farming production, especially bovines, to reduce pollution and methane emissions against greenhouse effect [6] [7] [8]. Furthermore, contamination of food of animal origin by zoonotic pathogens is an important One Health issue. Among food safety threats, *Campylobacter* spp. represent an important public health risk.

The infection caused by thermotolerant *Campylobacter* spp. in man is in constant increase. In Europe, since 2005, the disease represents the most commonly reported food borne infectious gastrointestinal bacterial pathology in humans, with more cases than those caused by *Salmonella* spp., with over 200,000 confirmed cases, notified yearly [9]. In the EU, according to the latest European Union One Health 2018 zoonoses report by EFSA and the European Centre for Disease Prevention and Control (ECDC), in 2018 Member States reported more than 246,000 cases [10]. In 2020, the number of confirmed cases was reduced to about 121,000, while remaining the most commonly reported zoonotic disease [11]. In USA, the Center for Disease Control and Prevention (CDC) estimated that the infection affects yearly more than 1.3 million patients, since many cases are supposed to go undiagnosed or unreported to public health authorities [12]. Despite increasing importance for public heath, in veterinary medicine, monitoring of *Campylobacter* is often incomplete. In Italy, epidemiological studies were mainly focused on poultry meat contamination [13], showing high prevalence in Veneto and Marche regions (about 70%). Calabria region was not included in the study.

Both *C. jejuni* and *C. coli* may cause diarrhea in any category of age. The disease affects especially infants under 4 years of age, causing gastroenteric symptoms, but also responsible of different extra intestinal pathologies [9]. Furthermore, recent studies indicated an association between *Campylobacter* infection and malnutrition, caused by an induced intestinal and systemic chronic inflammation, with a subsequent perturbation of the growth, in low resourced communities, particularly evident also in Peru [14].

The transmission to humans is foodborne or it occurs through contact with animals and their products, such as raw milk and meat. The most frequent way of infection is considered to be related to raw or undercooked (not sufficiently cooked) contaminated poultry meat consumption. Cross contamination can occur at home for improper food handling and hygiene. Contaminations often occur indirectly in home kitchens through kitchen wares used for raw meat and after for other food.

Many domestic and wild animal species, primarily avian species, are natural reservoirs. Generally, animals are asymptomatic carriers of *Campylobacter* spp. Poultry show high positivity rates, with mainly *C. jejuni* strains. In pigs, the positivity may be also high (up to 67%), with almost the totality of the isolated strains *C. coli* [15]. Campylobacteriosis (*C. jejuni* and *C. coli*) is not a World Organization for Animal Health (Office International des Epizooties—OIE) listed disease. Cattle is recognized susceptible and campylobacteriosis is internationally notifiable only in this species, in relation to genital infection (Bovine genital campylobacteriosis) [16]. *Campylobacter* is often detected in poultry meat [17], showing also very high levels of contamination, up to >10,000 unit forming colonies (UFC)/g. Prevalence of *Campylobacter* spp. in raw meat from cattle, pigs and small ruminants have been frequently reported in many countries ([18] [19] [20] and contamination of internal organs was also reported. Contamination may be observed in internal organs. Liver is contaminated through biliary ducts, and this is reported also in bovine, ovine and pig. *C. jejuni* was found in lamb liver and *C. coli* predominated in pigs' liver [21]. Meat from pigs and ruminant are considered to present a relative low risk to the consumers in regard to campylobacteriosis, but undercooked offal from these food animals is likely a considerable risk [22]. In Japan, the spread of the infection induced the health authorities to forbid consumption of raw poultry meat, but *Campylobacter* spp. associated fatal cases have been reported in relation to consumption of raw pig liver.

Contamination source of campylobacteriosis of domestic animals is mainly due to contacts with natural reservoirs such as crows (*Corvidae*) and gulls (*Laridae*), in which prevalence may reach from 23% - 25% in USA [23], up to 38.8%, as reported in Italy [24]. Concerning weather influence on campylobacteriosis, a distinctive feature of the disease is the seasonality, with a peak in human during summer in countries with temperate climate [25]. Such weather correlation was also observed in broilers in tropical conditions [26]. Meat becomes contaminated during slaughtering and evisceration process. Good hygienic slaughtering practices reduce the contamination of carcasses by feces, but will not guarantee the absence of *Campylobacter* from meat and meat products. Currently, applied prophylactic measures result only partially efficient, as demonstrated by the high number of reported cases in the human population. Despite experimental studies are carried out to develop pre-harvest immunization in poultry [27], no *Campylobacter* vaccines are currently available. The high prevalence of campylobacteriosis in domestic animals and the generally asymptomatic clinical course in these species do not induce farmers to use antibiotic chemicals for treating the disease, also taking into account the risk of bacterial resistance, especially when wide application is required. Therefore, due to the wide distribution of this microorganism and importance of human campylobacteriosis, it is necessary to explore alternative strategies to prevent the disease through a direct action at primary production level.

The high level of contamination of food of animal origin is the primary issue. It has been estimated that the reduction of the 90% of the cases of human campylobacteriosis could be achieved by limiting contamination levels under 500 UFC per gram in raw poultry meat [15]. It is therefore of outmost importance the reduction of the bacterial contamination burden of raw meat and organs to ensure a radical decrease of clinical forms in humans.

A number of vegetal species have been recognized to possess anti-bacterial activity. Bamboo, such as *Phyllostachys heterocycla*, the most dominant among a variety of bamboo species, showed therapeutical and preventive potential due to anti-bacterial activity. Researches on bamboo extractives have mostly focused on shoots, roots, and leaves for the bioactive components with antioxidant activity and antimicrobial activity [28] [29] [30] [31]. Antimicrobial activity of the extract of the fresh leaves of bamboo, evaluated against both Gram positive and negative bacterial strains, revealed effective inhibitory action against *S. aureus [32]*. The ethanolic extract of Petung bamboo (*Dendrocalamus asper*) leaves was the most effective to inhibit all tested *E. coli* strains [33]. Similarly, the olive tree (*Olea europaea*) contains oleuropein, an important phenolic compound with antibacterial actions against a variety of pathogenic bacteria [34] [35]. In addition to antimicrobial activity, oleuropein is characterized by different other beneficial pharmacological features including anti-oxidant, anti-inflammatory, anti-atherogenic, anti-cancer activities, antiviral activity and hypolipidemic and hypoglycemic effect [36].

In the framework of a first collaboration in veterinary medicine between Japanese and Italian institutions, a study was undertaken with the overall goal of improving consumer health through the reduction of bacterial contamination of food to ensure a decrease of human campylobacteriosis and obtain healthier food of animal origin. The present study investigated preliminarily, from 2014 to 2020, the use of agricultural by-products from plants with bactericidal potential (bamboo and olive) as animal feed supplement for the reduction of *Campylobacter* burden among intestinal bacterial flora in domestic animals, subsequently reducing meat and organs contamination levels for a sustainable prevention strategy.

2. Material and Methods

2.1. Animals

Cattle and pigs: experiments have been conducted on two zootechnics excellences: the Japanese black (*Bos taurus*) and the Calabrian Black (*Sus scrofa domesticus*). The cattle breed, used for the production of marbled beef, characterized by high quality organoleptic traits, was raised at the Miyazaki Prefectural Livestock Experimental Station, Japan. The pig breed, producing capocollo ham, accredited with the European Union product certification quality label for protected designation of origin (PDO) [37], was raised at the Madeo Industria Alimentare srl breeding farm, San Demetrio Corone, Cosenza province, Italy. Pig fattening farms located in the studied area could not be considered as control group with animals receiving standard feeding since they were subject to antibiotic medicated feed administration, legally prescribed, therefore expected to be negative for *Campylobacter* spp. In order to evaluate the circulation of the pathogen in the studied area, poultry was considered, as recognized highly susceptible species. Two broiler chicken groups, older than 7 weeks of age, were selected from a medium size poultry farm, with ground rearing, and a large size (70,000 animals) intensive production system farm, respectively. Details of farms sampled for testing of *Campylobacter* spp. in cattle from Miyazaki Prefecture, Japan, and pigs and poultry from Calabria region, Italy, are summarized in **Table 1**.

Table 1. Details of farms sampled for testing of *Campylobacter* spp. in cattle from Miyazaki Prefecture, Japan, and pigs and poultry from Calabria region, Italy. (*) Eight pigs tested only for biochemical analysis.

Farm	Animals Species/breed	Number of animals Total/Tested	Location	Production type
Cattle farm Miyazaki livestock experiment Institute	Cattle/Japanese black	100/6	Prefecture of Miyazaki, Japan	Teaching Experimental
Pig farm	Pigs/Calabrian black	1831/58*	Calabria region, Italy	Semi-free ranging
Poultry farm (large size)	Poultry/broilers	70,000/60	Calabria region, Italy	Intensive battery rearing
Poultry farm (medium size)	Poultry/broilers	5500/40	Calabria region, Italy	Indoor ground rearing

2.2. Animal Feeding

In a first phase, 6 bovines (Japanese Black), 20- to 24-week-old (**Figure 1**), from which *Campylobacter* were isolated, were randomly selected within the same herd for the execution of a three months feeding trial. One group (n = 3) was fed with bamboo silage or straw *ad libitum* in the trial period (**Figure 2**) and a second one (n = 3), placed in equivalent conditions, received standard feeding, constituting a control group. In a second phase, based on observations on cattle fed with bamboo, field survey was conducted in the Italian southern region of Calabria. Pigs (Calabrian black) (n = 40: 4 adult males, 36 adult females), sows and piglets, were reared in paddocks with olive trees (**Figure 3** and **Figure 4**), with free access to olives and olive leaves as supplementary feeding. Olive trees were not harvested and olives, naturally falling to ground at maturity during winter months, and spring sprouts were made available for the exclusive consumption of pigs. A control group of fattening piglets (>30 kg of live weight with about 3 months of age) (n = 10), originating from the same farm, received standard feeding. Broiler chickens (n = 100) received standard feeding.

2.3. Sampling

All cattle and pigs were sampled *in vivo*. Rectal swabs, collected from each animal/animal group, were placed in plastic tubes with 1 ml of sterile saline or transport medium. Poultry samples were collected at slaughterhouse. Per each slaughter batch, caecal samples for the detection of *Campylobacter* were taken at the time of evisceration and placed in separate sterile plastic bags. All samples, selected at random throughout each animal group or slaughtering batches, were kept at below +8˚C for a few hours and sent to the laboratory for testing. A single sample from processed pork meat (spicy meat paste) was also collected.

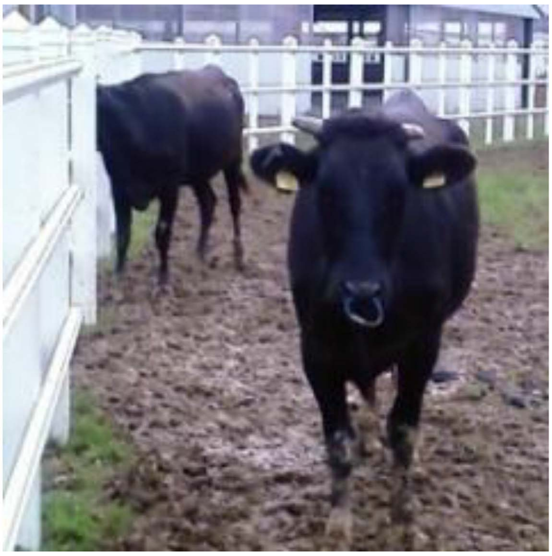

Figure 1. Japanese black. Marbled beef, highly appreciated and widely distributed, is produced on the base of rigorous standards.

Figure 2. Bamboo processing for cattle feeding. Subsequent to harvesting, bamboo plants were cut and conditioned in rolls for transport to stables where, after unassembling, feed was made available *ad libitum* for the entire trial period (three months).

Figure 3. Calabrian black farmed at Madeo srl organic black pig farm, Calabria region, Italy. Sows and piglets are reared in paddocks with olive trees, with free access to olives and olive leaves as supplementary feeding. Preliminary samples resulted negative for *Salmonella* spp. and *Campylobacter* spp. in pigs fed with olive by-products.

Figure 4. Calabrian black farmed at Madeo srl organic black pig farm, Calabria region, Italy. Detail of pig housing dedicated to sows and piglets under olive trees.

2.4. Pathogen Detection, Characterization and Quantification

The number of *Campylobacter* spp. in the feces was enumerated by tests performed in accordance to the OIE Manual and relevant international standards. Isolation and confirmation of *Campylobacter* from food chain samples was undertaken as described in ISO 10272-1:2006(E). *Campylobacter* species were identified using phenotypic methods as described in ISO 10272-1:2006(E). The quantitative detection of *Campylobacter* spp. was carried out according to ISO/TS 10272-2:2006. The processed meat samples were also tested for the presence of *Salmonella* spp., *Listeria* spp., *E. coli*, *Staphylococcus aureus*, *Enterobacteriaceae* and *Bacillus cereus*.

2.5. Meat and Meat Products Biochemical Analysis

Eight pigs (pig finishers, 4 males and 4 females, over 70 kg of live weight with about 6 months of age), from the same farm where olive by-products supplement feeding was applied, were selected for serum lipid profile determination. Pig meat was tested for fat percentage and quality. Fatty acid composition in four meat products (spicy sausage, fresh capocollo ham, crude and backed ham) was identified using biochemical analytical methods, as described in ISO 16958 2015. Bovine meat samples were tested also for biochemical characteristics.

2.6. Statistical Analysis

Samples were compared for *Campylobacter* spp. infection rate proportions, according to their association with farming system, by computing Fisher's exact test statistics, using Easy Fisher Exact Test Calculator, Social Science Statistics [38].

2.7. Ethical Approval

The protocol and procedures employed did not require ethical approval taking into account that feeding with agricultural by-products was applied by the farmers before the scope of the study and sampling and testing were performed in the framework of routine official controls at farm and slaughterhouse levels.

3. Results

In this preliminary survey on *Campylobacter* spp., all bovines fed with bamboo supplement become negative for *Campylobacter* spp. during the 3 months of bacteriological monitoring. The three cattle in the straw-fed group remained positive during three months.

All pigs, farmed with olive by-products supplementary feeding, showed negative results for *Campylobacter* spp. (**Table 2**). Similarly, the pig control group was also negative. The sample from processed meat (spicy pork meat paste) was negative for *Campylobacter* spp. and also for *Salmonella* spp., *Listeria* spp., *E. coli*, *Staphylococcus aureus*, *Enterobacteriaceae* and *Bacillus cereus*.

Chickens tested from a large size poultry farm showed relatively high levels of bacterial contamination (up to 6200 CFU/gr), indicating the circulation of the pathogen in the considered region, while those from small size farm resulted free from *Campylobacter* (**Figure 5**).

The serum collected from Calabrian black pigs showed high concentration of HDL cholesterol and the lipid composition of pig meat resulted to contain high quantity of monounsaturated fatty acids. Furthermore, backed ham showed also to be a "Source of omega-3 fatty acids", containing at least 0.3 g alpha-linolenic acid per 100 g and per 100 kcal. In **Table 3**, details of the Calabrian black pig biochemical lipidic profile in serum and the fat composition in muscle and four meat products are presented. In addition, bamboo fed cattle meat contained higher levels of oleic acid when compared to the control group (data not shown).

The statistical tests applied to compare the groups associated with presence of *Campylobacter* spp. with other groups demonstrated that the difference between the groups was statistically significant. The results of the Fisher's exact test ($p = < 0.00001$) indicated a significant association between poultry intensive breeding system and positivity to *Campylobacter* spp.

4. Discussion

Eating less meat is considered by some researchers a climatic priority [6] [7] [8]. If the impact of meat on climatic global warming is often call into question, to reduce the consumption might contribute for some extent to the decrease of greenhouse effect gas emissions. On the other hand, many national health recommendations advise people to limit intake of processed meat and red meat, which are linked to increased risks of death from heart disease, diabetes, and other illnesses. Some dietary guidelines also recommend limiting consumption of red meat or processed meat, but these are focused mainly on reducing the intake of

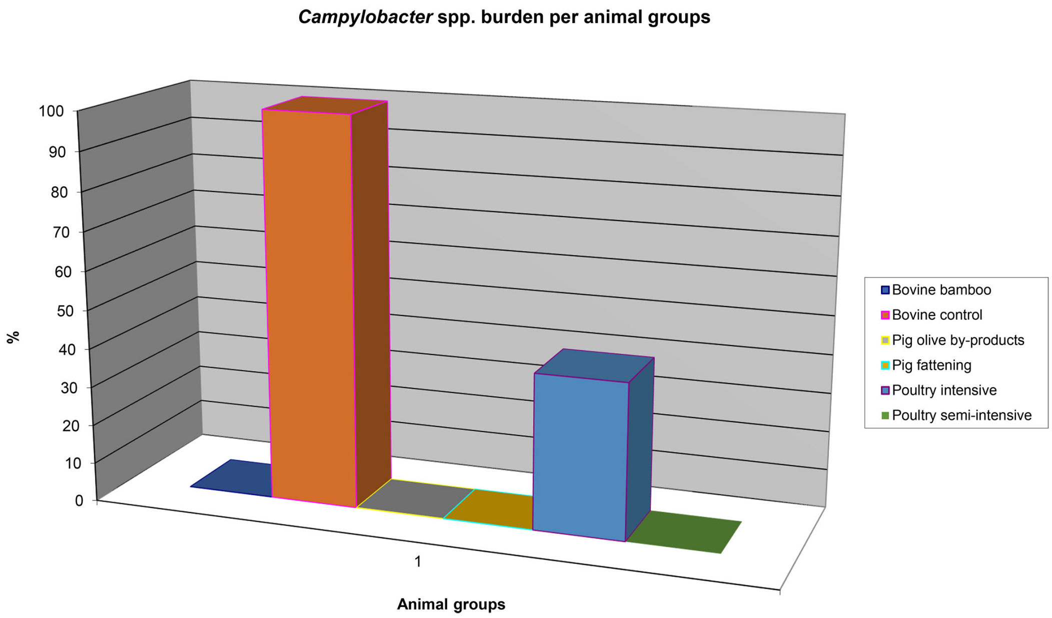

Figure 5. Summary results of screening for *Campylobacter* spp. in 6 animal groups. Bacterial count in positive animals ranged from 1500 to 6200 colony forming units (CFU) per gram.

Table 2. Summary results of screening for *Campylobacter* spp. CFU: Colony forming unit; (*) standard feeding—control group.

Farm	Intestinal samples (rectal swab/caecum)	Animals slaughtered	Research for *Campylobacter* n positive/%	Count for *Campylobacter* (CFU/gr)	Biochemical identification
Cattle farm Miyazaki, Japan	3	0	Negative		Negative
	3*	0	Positive 3 (100%)	ND	*Campylobacter* spp.
Pig farm Calabria, Italy	40	0	Negative		Negative
	10*	0	Negative		Negative
Poultry farm (large size) Calabria, Italy	10	400	Positive 5 (50%)		ND
				6000	
				4800	
				6200	
				3600	
				2800	
	10	600	Positive 5 (50%)		ND
				2600	
				3600	
				5100	
				3600	
				4500	
	10	500	Positive 4 (40%)	ND	3 *Campylobacter coli* 1 *Campylobacter jejuni*
	10	600	Positive 7 (70%)		
				3200	*Campylobacter coli*
				2400	*Campylobacter coli*
				1500	*Campylobacter coli*
				2800	*Campylobacter coli*
				1800	*Campylobacter coli*
				3100	*Campylobacter coli*
				4300	*Campylobacter coli*
	11	500	Positive 3 (27%)		
				5200	*Campylobacter coli*
				4400	*Campylobacter coli*
				ND	*Campylobacter coli*
	9	400	Negative	<10	Negative
Poultry farm (medium size) Calabria, Italy	10	1000	Negative	<10	Negative
	20	1120	Negative	<10	Negative
	10	1200	Negative	<10	Negative

Table 3. Calabrian black pig biochemical analyses. (a) Lipidic profile in serum and fat composition in muscle (pig finishers, 4 males and 4 females, over 70 kg of live weight with about 6 months of age). In Calabrian pigs, serum lipidic profile showed a four folds higher HDL/LDL balance.HDL: High-density lipoproteins; LDL: Low-density lipoproteins; SD: standard deviation; CV: coefficient of variation; (b) Analytical results of acid composition in four meat products from Calabrian black pig. Values per 100 g (Limit of Quantification—LoQ 0.0010 g) and percentage on total fatty acids (LoQ 0.0050 g)/±: Uncertainty of measure; (c) Details of analytical results of acid composition (Mono- and polyunsaturated fatty acids) in backed ham produced from Calabrian black pig. According to EU Regulation 116/2010, backed ham showed to be a "source of omega-3 fatty acids", containing more than 0.3 g alpha-linolenic acid per 100 g. Values per 100 g (LoQ 0.0010 g) and percentage on total fatty acids (LoQ 0.0050 g)/±: Uncertainty of measure.

(a)

Serum (Values expressed in mg/dl).

	Animals							
Parameter	**C.1/F**	**C.2/M**	**C.3/F**	**C.4/M**	**C.5/F**	**C.6/M**	**C.7/F**	**C.8/M**
Total cholesterol	89	91	90	95	83	90	89	92
Triglycerides	66	65	44	25	56	32	28	30
LDL cholesterol	<12	<12	<12	<12	<12	<12	<12	<12
HDL cholesterol	<40	<40	<40	<40	<40	<40	<40	<40

	Mean values					
	Mean	**SD**	**Median**	**Min**	**Max**	**CV%**
Total cholesterol	90	3	90	83	95	3.8
Triglycerides	43	17	38	25	66	39

Red meat (*vastus lateralis*) (Values expressed in percentage)

Parameter	**Lipid Numbers**	**Characteristic**	**Mean %**
Myristic acid	C14:0	Saturated fatty acid	1.28
Palmitic acid	C16:0	Saturated fatty acid	24.3
Palmitoleic acid	C16:1	*ω*-7 Monounsaturated fatty acid	2.8
Stearic acid	C18:0	Saturated fatty acid	12.7
Oleic acid	C18:1	*ω*-9 Monounsaturated fatty acid	40.1
Linoleic acid	C18:2	*ω*-6 Polyunsaturated fatty acid	12.2
f-Linoleic acid	C18:3	*ω*-6 Polyunsaturated fatty acid	0.14
***α*-Linolenic acid**	C18:3	*ω*-3 Polyunsaturated fatty acid	0.59
Eicosanoid acid	C20:1	*ω*-9 Monounsaturated fatty acid	0.77
Eicosadienoic acid	C20:2	*ω*-6 Polyunsaturated fatty acid	0.42
Eicosatrienoic acid	C20:3	*ω*-3 Polyunsaturated fatty acid	0.15
Arachidonic acid	C20:4	*ω*-6 Polyunsaturated fatty acid	1.29
Nervonic acid	C24:1	*ω*-9 Monounsaturated fatty acid	0.42

(b)

Pig meat product	Saturated fatty acids g/100g (%)	Monounsaturated fatty acids g/100g (%)	Polyunsaturated fatty acids g/100g (%)	Polyunsaturated/ monounsaturated fatty acids proportion	Polyunsaturated/ saturated fatty acids proportion
Spicy sausage	9.12 ± 0.60 (40.6% ± 3.0%)	10.44 ± 0.79 (46.5% ± 4.1%)	2.85 ± 0.28 (12.7% ± 1.5%)	0.273 ± 0.034	0.313 ± 0.037
Backed ham	5.63 ± 0.41 (32.0% ± 2.6%)	9.30 ± 0.71 (52.8% ± 4.7%)	2.68 ± 0.27 (15.2% ± 1.7%)	0.288 ± 0.037	0.476 ± 0.059
Crude ham	7.51 ± 0.52 (36.4% ± 2.9%)	11.27 ± 0.82 (54.5% ± 4.5%)	1.88 ± 0.21 (9.1% ± 1.1%)	0.167 ± 0.022	0.250 ± 0.033
Fresh capocollo ham	4.16 ± 0.32 (41.6% ± 3.6%)	4.51 ± 0.38 (45.0% ± 4.3%)	1.35 ± 0.16 (13.5% ± 1.7%)	0.299 ± 0.044	0.325 ± 0.046

(c)

Lipid Numbers	*ω*-n	Common Name	Chemical name	Monounsaturated fatty acids g/100g (%)	Polyunsaturated fatty acids g/100g (%)
C16:1	*ω*-7	Palmitoleic acid	hexadec-9-enoic acid	0.460 ± 0.064 g (2.61% ± 0.39%)	
C18:1	*ω*-9	Oleic acid	cis-9-octadecenoic acid	8.64 ± 0.71 g (49.0% ± 4.7%)	
C18:2	*ω*-6	Linoleic acid	all-cis-9,12-octadecadienoic acid		2.29 ± 0.26 g (13.0% ± 1.7%)
C18:3	*ω*-3	*α*-Linolenic acid	all-cis-9,12,15-octadecatrienoic acid		0.334 ± 0.052 g (1.90% ± 0.31%)
C20:1	*ω*-9	Eicosanoid acid	cis-11-eicosenoic acid	0.151 ± 0.032 g (0.86% ± 0.19%)	

fat and sodium, which are risk factors for cardiovascular disease and obesity. The recommendation for moderate red meat and processed meat consumption was recently emphasized by WHO, in relation to the consideration of long-term epidemiological studies by the international agency for the research on cancer (IARC) and subsequent inclusion in risk categories Group 1 (carcinogenic to humans) and Group 2A (probably carcinogenic to humans) of processed meat and red meat, respectively [5]. This recommendation was based on epidemiological studies suggesting that small increases in the risk of several cancers, especially colorectal cancer, may be associated with high consumption of red meat or processed meat. The IARC Working Group considered more than 800 different studies on cancer in humans (some studies provided data on both types of meat; in total more than 700 epidemiological studies provided data on red meat and more than 400 epidemiological studies provided data on processed meat). According to the most recent estimates by the Global Burden of Disease Project, an independent academic research organization, about 34,000 - 50,000 cancer deaths per year worldwide are attributable to diets high in processed meat [5]. Processed meat has been classified in the same category as causes of cancer such as tobacco smoking and asbestos. The problem is obviously lower than the about

1 million cancer deaths per year globally due to tobacco smoking, 600,000 per year due to alcohol consumption, and more than 200,000 per year due to air pollution. Taking into account a global increasing of meat and meat consumption, although these risks are small, they could be important for public health. Therefore, the observations on red meat and processed meat cannot be neglected. Meanwhile, it said red meats were "probably carcinogenic" but there was limited evidence, the WHO did stress that meat also had health benefits. Furthermore, the Cancer Research UK said this was a reason to cut down rather than give up red and processed meats.

However, the point is also the availability of healthier meat and meat products for human consumption. Trans fatty acids (e.g., hydrogenated-saturated fatty acids) have been shown to impact cholesterol levels by increasing the share of bad LDL cholesterol and reducing HDL good cholesterol levels. It is therefore necessary to limit as far as possible the consumption of foods rich in saturated fatty acids. Increasing concentrations of high-density lipoproteins (HDL) particles are strongly associated with decreasing accumulation of atherosclerosis within the walls of arteries. Recent studies confirm the fact that HDL has a buffering role in balancing the effects of the hypercoagulable state in type 2 diabetics and decreases the high risk of cardiovascular complications in these patients. Also, the results obtained in this study revealed that there was a significant negative correlation between HDL and activated partial thromboplastin time (APTT) [39]. Recommended optimal condition considered correlated against heart disease foreseen high HDL level (>59 mg/dl) in men and women [40]. In this perspective, it is particularly important the ability of the bovine Japanese black breed to deposit more monounsaturated fatty acids, with increasing fat quality scores: decrease of palmitic acid (increase serum cholesterol in humans) and increase in oleic acid (lowers serum cholesterol). The ratio of monounsaturated to saturated fats exceeded 2 to 1, in marked contrast to the approximately 1 to 1 ratio observed in beef from other cattle breeds [41]. In the present study, this characteristic resulted enhanced in cattle fed with bamboo. In Calabrian pigs, serum lipidic profile showed a large presence of HDL cholesterol (the good cholesterol), with a four folds higher HDL/LDL balance. The analysis of the lipidic profile confirmed a higher quantity of unsaturated fatty acids in meat and meat products, both fresh and processed, made from Apulo-Calabrian black pig. In particular, according to Regulation 116/2010 of the European Union, amending Regulation 1924/2006 on health claims [42], all the four tested products were rich in monounsaturated fatty acids, a claim that a food is high in monounsaturated fat, and any claim likely to have the same meaning for the consumer, may only be made where at least 45% of the fatty acids present in the product derive from monounsaturated fat under the condition that monounsaturated fat provides more than 20% of energy of the product. Furthermore, backed ham resulted also "Source of omega-3 fatty acids". In pigs, muscle fatty acid content and composition are determined by a complex combination of genetic and environmental factors that often interact [43]. The most abundant saturated fatty acids are palmitic (C16:0), stearic (C18:0) and myristic (C14:0), whereas oleic (C18:1) and linoleic (C18:2) are the predominant monounsaturated and polyunsaturated, respectively [43]. Compared to biochemical profiles observed in other commonly reared pig breeds, such as the French Landrace, the British Large white, where the lipid composition and nutritional properties of pig meat intramuscular fat is basically composed by a mixture of saturated (~40%), monounsaturated (~42%) and polyunsaturated (~18%) fatty acids [43], in the tested samples from Calabrian black pig meat and meat products, saturated fatty acids were lower (32% - 41% - mean 37.78%) and monounsaturated fatty acids were higher (45% - 54% - mean 48.58%), while polyunsaturated were lower (9% - 15% - mean 13.02%). Similarly, the observed lipidic profile in serum from Calabrian black pigs was clearly superior when compared to crossbreed pigs as Duroc × Petrain [44], with an HDL/LDL ratio of 3.33 (means HDL 40; LDL 12) in the former and 0.49 (means HDL 23.68; LDL 49.46) in the latter, respectively, or other equivalent ratios in Duroc × Landrace × Yorkshire crossbreed pigs (0.56) [45] and Large White (0.36) [46]. The qualitative positive traits were constant during the study period, from 2015 to 2020, maintaining the same feeding and farming approaches, indicating the sustainable respect of animal welfare and the obtention of high-quality products of animal origin for human consumption.

The observations on use of bamboo and olive by-products in cattle and pigs resulted correlated to *Campylobacter* absence, suggesting that the use of olive by-products or other plants with bactericidal potential as feed supplement might represent an innovative and sustainable alternative prevention strategy for the achievement of the objective of the limitation of food contamination levels by *Campylobacter*. While in Japan and other Italian regions the circulation of *Campylobacter* spp. was reported and indicating the existence of epidemiological pressure of the pathogen on domestic and wild animal populations, in Calabria, since no previous studies have been conducted, campylobacteriosis was investigated in the most susceptible domestic host (poultry). The isolation of *Campylobacter* spp. in broilers confirmed the presence in the region and corroborated the significance of the absence of the bacterium in the black Calabrian pigs. Interestingly, *C. coli* was almost the only species detected (frequently reported in non-avian domestic species as cattle and pigs), in contrast to the *C. jejuni*, which is generally prevalent in avian species. The fact that the control group of pigs was also negative may suggests that fattening animals maintain high welfare traits and continue to benefit of the healthy environment of origin, despite change of feeding and management (concentrate in stabulation vs olive by-products supplement and free ranging).

Furthermore, the use of by-products, completely not armful for the animal or the consumer, currently considered cheap feed, should be considered not only economically interesting to reduce production costs, but also for their therapeutic and preventive nutritional components through a precise application strategy (e.g., higher doses at the end of production cycles), before slaughtering, to reduce bacterial burden, even if natural sterilization may occur. Practically, according to this research, the control of campylobacteriosis in domestic animals might be obtained through improved welfare farm management and feeding with by-products with anti-bacterial effect at the pre-harvest level to reducing foodborne infections. Not less important are the obtained organoleptic characteristic with beneficial nutritional effects due increased quantity of mono and polyunsaturated acid fats of the series omega-3, -6, -7 and -9. This strategy might be applied not only in zootechnical industry but also at rural level directly and easily by farmers. The use of bamboo is possible in tropical and subtropical regions where is widely available. In particular bamboo is one of the fast-growing forest plants. Similarly, the olive tree by-products are largely available in the Mediterranean bacine (90% of the world production). Furthermore, farm size and management appeared important factors influencing pathogen epidemiology.

5. Conclusions

Campylobacteriosis is a One Health issue, representing both a food safety and public health risks, with particular pediatric impact. In addition to the base for preventive approach against foodborne diseases which should focus on improved farm management and animal welfare, slaughterhouse hygiene and science based consumers' risk communication, the application of antibacterial properties of selected agricultural by-products at primary production level suggested a potential sustainable preventive approach against enteric pathogens with the active involvement of veterinarians in the perspective of an interdisciplinary One Health approach and a combined effort of all stakeholders. At present, a complete elimination of *Campylobacter* in the food chain appears highly problematic and probably not feasible [47]. Therefore, to ultimately reduce the burden of campylobacteriosis cases in humans, efforts should be focused to establish control measures and intervention strategies to minimize the occurrence of *Campylobacter* spp. in livestock and to reduce the quantitative *Campylobacter* burden in animals and foods, flanked by consumer advice and education campaigns to raise the awareness towards *Campylobacter* infections [47].

The study was based on innovative principles of preventative approaches to food safety management articulated through strengthened and enhanced food safety prophylactic measures applicable at level of the primary production, including direct potential benefit for rural populations, with special attention to campylobacteriosis. Furthermore, this strategy could allow to decrease campylobacter contamination in food without chemical compounds or drugs use reducing exposure risk for consumers. Antibiotics use in animals is related to antibiotic resistance problem so employ plants with bactericidal potential could help contain this phenomenon.

Despite food safety advances in general, and interesting results in studied animals, eating raw meat remains a habit that should be avoided being not completely safe, and it should be kept in mind that the risk of infection from consumption of raw meat remains. The study was only preliminary, but it deserves attention and need further investigations suggesting a potential preventive approach against enteric pathogens with active involvement of veterinarians in the perspective of One Health. In conclusion, this approach might represent an innovative alternative for a sustainable prevention strategy, in the full respect of the EU food law and coping with the increasing consumers' demand of naturally produced and healthy food without use of chemical compounds or antibiotics.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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