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HEALTH AND WELFARE IN ORGANIC DAIRY GOAT FARMS

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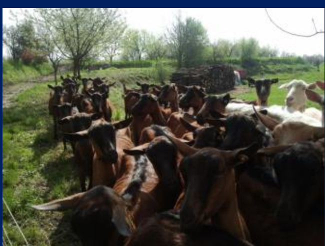

Anno Accademico 2013/2014

A path is only a path, and there is no affront, to oneself or to others, in dropping it if that is what your heart tells you.
Look at every path closely and deliberately.
Try it as many times as you think necessary.
Then ask yourself alone, one question ...
Does this path have a heart?
If it does, the path is good; if it doesn't it is of no use.

Carlos Castaneda

Per Adele,
perché possa trovare il Suo cammino senza accontentarsi. Mai.

Per te, Roberto, un grande uomo e un compagno speciale,
perché *"facciamo finta di esser sani"* insieme.

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THESIS ABSTRACT

European and Italian dairy goat farming has increasingly turned towards organic method. This production system aims at ensuring high levels of animal health and welfare, by reducing the use of allophatic medicine and creating well-balanced agro-ecosystems. Despite the growing importance of organic farming, researches on this field are still relatively limited, in particular with respect to the evaluation of animal welfare in organic goats. New research would contribute to improve organic goat husbandry as well as to fulfil consumers' demand.

This thesis investigates two fundamental health and welfare issues related to the use of pasture in organic dairy goat farming: 1) gastrointestinal nematodes (GIN) and their control strategies (Chapters 1 and 2), and 2) positive emotional state and its assessment in goats (Chapter 3). It relies on a series of experimental studies performed in a commercial organic dairy goat farm in Lombardy between 2012 and 2014.

Following a literature review on sustainable strategies to control GIN, the first study evaluates the efficacy of a commercial herbal product in controlling GIN compared to conventional allopathic anthelmintic.

The results show significant differences between treatments (conventional < phytotherapic) ($P < 0.05$) in terms of fecal egg count per gram (EPG), throughout the experimental period. Both the anthelmintic products (conventional and phytoterapic) showed low efficacy for GIN's control: the allophatic product was effective only at 60 days post-treatment (fecal egg count reduction > 90%), while the phytotherapic product did not reach the threshold values during the whole study period. Furthermore the herbal anthelmintic showed great differences in individual responses within the group.

The second study aims at evaluating the efficacy of pumpkin seeds used as

anthelmintics in traditional veterinary medicine, to reduce fecal egg count. No significant differences in EPG were found in the pumpkin seed-treated group compared to a negative control throughout the study period. In both these trials, the goats showed a great tolerance to GIN, suggesting the goats' ability to cope with infections, and that preventive strategies, including grazing management, are paramount for GIN control.

The third study tests the validity and repeatability of Qualitative Behaviour Assessment (QBA), as a tool to assess positive emotional state and thus the "overall" welfare of dairy goats. Principal Component Analysis on QBA scores point out that goats' demeanour on intensive and organic farms is different, showing that access to pasture has a positive effect on goats' emotional state. Moreover, the results show a good inter-observer reliability across three dimensions of goat demeanour (PC1: $r = 0.75$, $P = 0.001$; PC2: $r = 0.67$, $P = 0.006$; PC3: $r = 0.69$, $P = 0.004$). These results highlight the promising role of QBA as part of welfare assessment protocols for goats, especially in organic farming.

As a broader conclusion, this thesis raises further questions on the extent to which GIN actually represent a serious problem for organic goats' health and welfare. Answering this question would have practical implications for determining the most adequate treatment strategy for goats, both with phytotherapy as well as with traditional methods. In light of these results, further controlled studies are encouraged to assess the health and welfare of organic grazing goats from a multidimensional perspective and to develop standardized methods for their evaluation.

GENERAL INTRODUCTION

SIGNIFICANCE AND MANAGEMENT OF DAIRY GOAT PRODUCTION

Goats were among the first production animals to be domesticated (8,000 BC, *Ganj Darech*, today known as Iran) and soon after were reared for meat and milk production (Boyazoglu *et al*., 2005; Salah 2005; Dubeuf & Boyazoglu, 2009). The great adaptability of goats to varying environmental conditions and the different nutritional regimes made possible their dissemination around the world and contributed to the increasing growth of goat production. Nowadays, goats are the fourth most numerous livestock groups at world level, and they are widespread among all the ecosystems, in particular in dry tropical and subtropical areas of poor agricultural potential. Of approximately 845 million of goats in the world, more than 90% are reared in the developing countries (especially in Asia and Africa) (FAOSTAT, 2012). In these countries goats are raised for meat production in extensive systems (Devendra, 2010) and they contribute largely to the livelihoods of small farmers. Indeed, they represent the only protein supply as the same as the only means of income for marginal people (Boyazoglu *et al*., 2005; Devendra, 2007; Azis, 2010; Escareño *et al*., 2013).

On the other hand, goat farming could be oriented also towards milk production as it happens in Europe that is the only continent where goat milk has considerable economic importance and organization. Europe holds only 5.1% of the world’s goat population, but it produces 15.6% of the world’s goat milk, which is mainly used for cheese production (Escareño *et al*., 2013).

Dairy goat breeding is really common around the Mediterranean basin and represents a significant economic, environmental and sociological aspect for Spain, France, Italy and Greece (Pirisi *et al*., 2007; Pirisi *et al*., 2011; Escareño *et al*., 2013).

In Mediterranean areas, goat farming systems have some peculiar characteristics such as the use of marginal land, the prevalence of grazing system, low level of mechanization as well as the production of typical cheeses (Ronchi & Nardone, 2003; Santos-Silva & Carolino, 2008; Abecia, 2008).

For instance, in Italy, data from the ISTAT (Italian National Institute of Statistics, 2010) show that 861,942 goats are reared. Dairy goat breeding is really common in the South Central part of Italy and in the islands (Sardinia and Sicily). Sardinia is also the most important Italian region for dairy goat breeding (about 25% of the total animals raised) and this record is a result of a long historical tradition.

As it happens in Sardinia, also in many other Italian regions dairy goat products, are part of the cultural heritage. Goats cheeses could represent a perfect example of this cultural connection between breeding, production and typical products. Due to their unique traditional, often artisanal, production technologies, it is created a link with their area of production, which might represent an essential part of sustainable rural development (Pirisi *et al*., 2011).

In Italy, the traditional goat breeding system is extensive, on hill or mountains areas and it uses seasonal natural pastures (Corti, 2007; Battaglini, 2007; Garippa *et al*., 2008). Flocks, are mostly of small and medium size, mainly constituted of local breed and their feeding is greatly dependent on grazing. In such systems, the level of intensification is highly variable because it varies from systems with small infrastructures, low management and productive levels to semi-extensive system where goats are kept on grazing during the day and on housing at night, and they receive feed supplementation according to their production level (Usai *et al*., 2006). In the last years, European and Italian goat farming was experiencing remarkable changes. Consumer’s demand is increasingly rapidly for dairy goats products and milk consumption due to their widely appreciated characteristics (e.g., high digestibility, high organoleptic quality, some therapeutic values) (Park *et al*., 2007; Schirru *et al*., 2012) and to the increased incidence of allergies and intolerances to cow’s milk protein (Businco & Bellanti, 1993). This fact has led part of the traditional goat farms to turn into intensive systems with a high degree of mechanization, breeding of specialized breeds (Alpine and Saanen) and a less use of pasture. Moreover, feeding is based on conserved fodders (hay, silage) and concentrates.

On the other hand, several goats farms turn their status from conventional and traditional to organic due the growing relevance of organic farming. Compared to other livestock productions, this conversion process is probably easier for the small ruminants: their breeding systems remain based on grazing, without the application of agrochemicals and agricultural practices on pasture (Ronchi & Nardone, 2003; Nardone *et al*., 2004; Hoste & Cartier, 2006; Pauselli, 2009).

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ORGANIC GOAT FARMING

"Organic Agriculture is a production system that sustains the health of soils, ecosystems and people. It relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects. Organic Agriculture combines tradition, innovation and science to benefit the shared environment and promote fair relationships and a good quality of life for all involved"

(IFOAM, 2008)

Over the last years increased public awareness on environmental protection, food safety and animal welfare issues has contributed to the grown of organic farming, which is seen as a sustainable alternative to chemical-based agricultural systems (Escobar & Hue, 2007; Lockeretz, 2007; Bellon & Penvern, 2014).

Consumers consider organic farms as a model of the "intact world" of farming and rural living (Rahmann, 2007), they perceive that organic farming provide healthier products and it is better for animal welfare (Harper & Makatouni, 2002; Edwards, 2005; Crandall *et al*., 2009).

From existing data, it seems that organic agriculture is practiced in almost all countries of the world, and the amount of agricultural land and farms is increasing everywhere.

In the world 37 million ha of agricultural land are organic and with 12.2 million ha of organic land, Oceania ranks in first position, Europe ranks second with 10.6 million ha and Latin America third with 6.9 million ha (FiBL-IFOAM, 2013).

The ten countries with the most organic agricultural land are shown in the following figure (Figure 1).

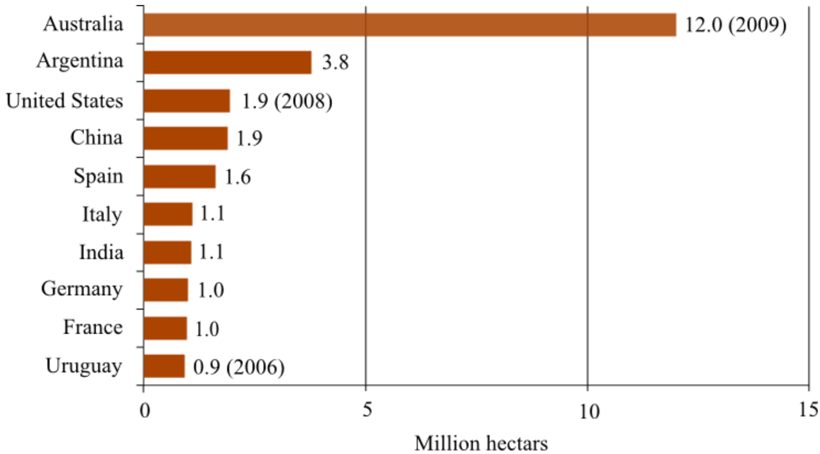

Figure 1. The ten countries with the most agricultural land 2011. Source FiBL-IFOAM, survey 2013, modified.

In spite of the global economy crisis, international sales of organic products continue to increase, especially in North America and Europe that are the two biggest market of this type of food (FiBL-IFOAM, 2013).

In Europe organic agricultural lands increased by more than 7400% within two decades, underlying the growing consumer demands (Lu *et al*., 2010); it represented only the 1% of the total usable agricultural area in 1985 (Lampkin, 2000), and nowadays it constitutes approximately the 5% of the agricultural land (European Commission, 2013).

Moreover, a great part of the organic land is used for permanent grasslands, which emphasize the significance of organic livestock production (Lu *et al*., 2010).

However, in Europe the organic livestock production, if compared to the total animal production, is very poor (about 1%) and the available data show a sharp increase in certified numbers of farm animals of all species.

With regards to organic goats, the sector counts almost 0.4 million heads and it is almost concentrated geographically as it is represented principally by Greece with 180,039 heads (4.1% of all goats in Greece). Italy follows with a herd that represents the 7.5% of the overall Italian sector (European Commission, 2013) (Figure 2). Moreover, Italian organic goat industry has fluctuated in the last years but the new data available show an increasing trend (+15.9% of goats in organic production) (SINAB, 2014).

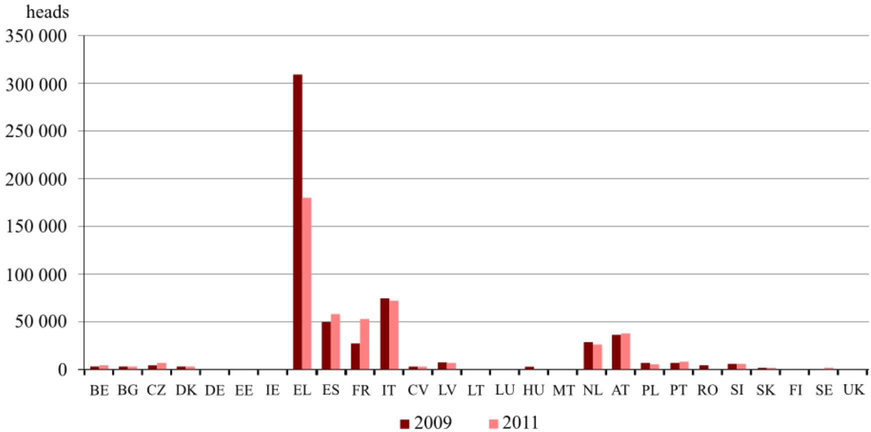

Figure 2. Heads of certified organic goats in 2009 and 2011 in the EU Member States. Source: European Commission, 2013, modified.

In many EU Member States, the organic goat sector is specialized in the production of organic cheese. Martini and Lorenzini (2007) reported varieties of organic products from dairy goat production systems in the Mediterranean basin and highlighted the economic relevance of organic goat farms in Southern Europe.

In these countries, including Italy, organic goat farming can be considered as a feasible system to improve rural development, particularly, in marginal areas (Arsenos *et al*., 2003; Ronchi & Nardone, 2003; Nardone *et al*., 2004; Mena *et al*., 2012).

Thanks to organic system, traditional pasture-based goats farms can been re-evaluated, increasing their ecological sustainability and economic viability. According to several authors (Pearson & Ison, 1987; Cavallero & Ciotti, 1991; Lovreglio *et al*., 2014) a proper grazing for goats has several positive effects on the environment, as it promotes the plants biodiversity, the conservation of a heterogeneous landscape, the prevention of soil erosion and among others the prevention of forest fires.

Furthermore, grazing allows production of valuable goat cheeses, which cannot be produced equally by intensive commercial farming systems, and which meet unique niche and profitable markets (Morand-Fehr *et al*., 2007).

PRINCIPLES OF ORGANIC FARMING

"Organic livestock husbandry is based on the harmonious relationship between land, plants and livestock, respect for the physiological and behavioural needs of livestock and the feeding of good-quality organically grown feedstuffs. All the management techniques should be directed to the good health and welfare of the animals"

(IFOAM, 2014)

Organic systems are designed to achieve a balanced relationship between the components of soil, plants, humans and animals. Animal production plays an important role in these systems because animals are involved in recycling of nutrients as they provide valuable products such as animal wastes, which become organic fertilizer (Thamsborg *et al*., 1999; Vaarst *et al*., 2005).

In contrast to conventional livestock production, organic livestock farming is defined by basic guidelines, which involve a less use of chemotherapy and a holistic approach to the production processes (Sundrum, 2001). Moreover, this set of principles defines also the technical approach to plant and animal production as the same as it draws and accepts the responsibilities for the environmental and social consequences of food production.

The four principles of organic farming, as enunciated by the International Federation of Organic Agriculture Movements (IFOAM)[1], are as follows (IFOAM, 2005):

- *the Principle of Health* - Organic agriculture should sustain and enhance the health of soil, plant, animal, human and planet as one and indivisible;
- *the Principle of Ecology* - Organic agriculture should be based on living ecological systems and cycles, work with them, emulate them and help sustain them;
- *the Principle of Fairness* - Organic agriculture should be built on relationships that ensure fairness about the common environment and life opportunities.

[1] Founded in 1972, IFOAM is the worldwide umbrella organization of the organic agriculture movement, uniting 870 member organizations in 120 countries. Among IFOAM's affiliates are, for example, organic farmers' associations, organizations from the organic food industry, NGOs, government institutions, organic networks, research institutions, as well as certifiers. IFOAM's mission is leading, uniting and assisting the organic movement in its full diversity. The organization's goal is the worldwide adoption of ecologically, socially and economically sounds systems. Democratically organized, it represents the common interest of the organic movement based on the four principles of Organic Agriculture.

- *the Principle of Care* - Organic agriculture should be managed in a precautionary and responsible manner to protect the health and well-being of current and future generations and the environment.

EU STANDARDS FOR ORGANIC GOAT FARMING

History

The EU Regulation on organic crop production was announced in 1991 (2092/91/EEC) but it did not include any standards for livestock. For that reason, it was supplemented by Regulation N° 1804/99/EC on organic production focused on livestock production. This Regulation established rules of production for the main species (bovine, ovine, caprine, equine and poultry) and covered a wide variety of livestock farming conditions, which differed in climate, housing, feeding, management, scale, etc. Some of the rules were valid for all livestock in organic farms, without specification of species and, for instance cattle and small ruminant were not equally considered, while sheep and goats received scant attention (Nardone *et al*., 2004). Actually, after years of debate and discussion, the most important governmental organic farming Regulation of the EU is the Commission Regulation (EC) N° 889/2008 of 5 September 2008, laying down specific rules for the implementation of Council Regulation (EC) N° 834/2007 on organic production.

COMMISSION REGULATION N° 889/2008[2]

Farmland-related animal husbandry

Livestock plays an important role in organic farms, e.g., in nutrient cycling; thus landless animal husbandry is prohibited. The limited livestock density does not exceed 170 kg nitrogen ha/year and is measured in livestock units (1 LU = 500 kg live weight) and about 13.3 adult goats/ha are allowed.

Origin of animals

EU rules require the use of breeds that are vigorous, able to adapt to local conditions and disease-resistant; moreover, strains of animals shall be chosen to avoid specific diseases or health problems associated with some breeds used in intensive production. For these reasons, goat local breeds are preferred.

Organic goats must be born and reared in organic farms. For breeding purposes, non-organically raised goats may be purchased under specific conditions (e.g., the herd establishment, restocking after epidemics and natural calamities - e.g., earthquake -). Such animals and their products may be sold as organic after compliance with a conversion period (6 months). If young stock has to be purchased from conventional farms, the maximum age at time of purchase is 45 days for kids (just after weaning).

[2] In: http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2008.250.01.0001.01.ENG.

Feeding

The feed given to goats should enhance their health and well-being. Feed must not contain any substances that artificially promote growth, synthetic amino acids or genetically modified organisms (GMOs). Organic goats have to be fed with 100% organic feedstuff (up to 30 % of the feed formula of rations on average may comprise in-conversion feedingstuffs). Ruminants have to be fed with a minimum of 60% of roughage (50% only in the first three months of high lactation allowed). At least 50% of the feed shall come from the farm unit itself or in case this is not feasible, be produced in cooperation with other organic farms primarily in the same region and organic feeds can be purchased from other organic farms too.

An extended discussion in the design of the Regulation was the feeding of young stock. On many organic farms, kids received only colostrum milk and then powdered milk. The young stock did not suckle or receive natural milk because the organic milk is precious (especially milk from small ruminant) and therefore expensive as young stock feeds. Nevertheless, it was agreed that animal welfare is more important than economic considerations. In 889/08/EC Art. 20 it is defined the feeding of young stock: lambs and kids have to be fed for 45 days, with "natural milk, preferably maternal milk". However, it was not defined what "on the basis of natural milk" means. In practice, it is interpreted that even skimmed powdered milk can be used - as long as it has an organic label (Rahmann, 2009).

It is recommended that ruminants should graze on pastures ("free-range") and not be fed in stables as long as the animal, weather and pasture conditions are suitable. Every animal has the possibility of permanent access to feedstuff and water and this means that a minimum of one feeding place per goat has to be available.

Husbandry management practices

Natural mating should do the breeding of ruminants. Artificial insemination is permitted, but not embryo transfer; oestrus synchronization by hormones is prohibited. Male breeding stock has to be kept on the farm, requiring extra farm resources (space, labour and feeds). Any kind of animal cruelty is forbidden. Dehorning of goats may only be performed under special circumstances, regulated by the certification authorities (e.g., hygiene, animal welfare or bio-security aspects). Castration of male stock is allowed to maintain traditional animal husbandry practices. The castration should be done at a very young age (< 1 month), or under anaesthesia by a veterinarian. Ruminants have to be kept in groups to meet their social needs but how social needs can be fulfilled at farm conditions has not been defined (Rahamann, 2009).

Housing and stocking rates

Ruminants shall have access to pasture whenever conditions allow. If grazing is not feasible, and goats have to be kept indoors, a permanently open-air run is required. An outdoor run may not be present only with permanent summer pasture grazing. The tethering of goats is prohibited. The number of animals kept in the pens must be appropriate to guarantee their comfort. The minimum indoor space of an adult goat is 1.5 m^2 and

0.35 m² for a kid. The out-door run should have a minimum of 2.5 m² for adult goats and 0.5 m² for kids (889/2008 EC Annex III, art. 10/4). A maximum of 50% of the stable surface can be slatted or grid floor, the rest has to be a flat and non-slippery surface. The boxes have to be strawed-in with organic materials (e.g., straw or wood chips).

Health management and veterinary treatments

The guiding principle of animal health is to prevent disease rather than to cure or treat it. Disease prevention in organic livestock production is based on the assumption that feeding, housing and care of the animals is such that they have an optimal natural resistance to control disease (Kijlstra & Eijck, 2006). Thus, preventive treatments with allopathic veterinary medicinal products are prohibited. For therapeutic purpose the use of conventional medicine is further discouraged. EU Regulation requires that goats treated more than three times in a year with allopathic medicine lose their organic status. Moreover, when conventional medicines are used, withdrawal periods for organic products are twice the legal withdrawal periods. Otherwise, EU Regulation recommend the use of alternative veterinary medicines, such as phytotherapy and homeopathy.

Anthelmintics are not considered properly as allopathic products so, for therapeutic purpose, can be administered without any restrictions (except for the double legal withdrawal periods), being a critical point in the Regulation (see review, Grosso *et al*., submitted).

HEALTH AND WELFARE IN ORGANIC GOAT FARMING

Ensure high levels of animal health and welfare represents the main goal for organic livestock production (Alrøe *et al*., 2001; Lund & Röcklinsberg, 2001; Hovi *et al*., 2003; Nicholas *et al*., 2004; Lund, 2006; Valle *et al*., 2007; Marley *et al*., 2010; Vaarst *et al*., 2011) and the concept of "positive health and welfare" has been included in the IFOAM principle of health (Hansen & Sjouwerman, 2007) and in the current EU Regulation. This aim can be accomplished by providing the best husbandry practices that fulfill their behavioural needs and increase the animal ability to cope with diseases. If despite all preventive measures an animal becomes sick, treatments with homeopathy or phytotherapy medicine are preferred in order to reduce dependence on chemotherapy and its adverse effects (e.g., environmental impacts, residues in food, toxicity on animals; Boxall *et al*., 2004; Beynon, 2012).

Nevertheless, these goals of high animal health and welfare represent a challenge for organic livestock farming (Athanasiadou *et al*., 2002; Vaarst *et al*., 2008; Rahmann & Godinho, 2012). According to several articles and workshop papers, the two recent EU network projects: "Network for Animal Health and Welfare in Organic Agriculture (NAHWOA) and "Sustaining Animal Health and Welfare in Organic Farming" (SAFO) concluded that high animal health and welfare levels are not assured simply by farming to organic standards (Vaarst *et al*., 2011). Thus, research efforts to improve and develop organic animal husbandry are essential. Until now, literature reviews have been referred mainly on the health problems associated with organic farming (e.g., Lund & Algers, 2003; Kijlstra & Eijck, 2006; Simoneit *et al*., 2012). Subjective data (on-farm surveys or expert opinions) are available for most farm species, but their reliability depends on the survey and on the type of disease concerned, and good records are available only for the most easily diagnosed diseases (Cabaret, 2003). Objective information is available mainly for dairy-cattle and lameness, infertility, mastitis and internal parasites mentioned as a major health issue (Vaarst *et al*., 2008). Health in organic small ruminant farming is rarely described in the literature. For instance, only 25 out of 569 publications reviewed were related to small ruminant (Sibonet *et al*., 2012), and are mainly focused on sheep, which are affected mostly by lameness, mastitis, fly strike and fasciolosis and other helminthosis as reported by an early survey of organic farmers in UK (Roderick & Hovi, 1999).

And, in the same way, Lindqvist *et al*. (2001) investigated the health problems in organically raised sheep in Sweden and concluded that endoparasites represent the most important problem.

Regarding goats, only limited data are available and are all focused on parasitic diseases (Lindqvist, 2001; Cabaret *et al*., 2002; Silva *et al*., 2011).

On the topic of "animal health in organic farming" very few comparative studies (organic *vs* conventional) have been carried out and they reported conflicting results (Sundrum, 2001; Hovi *et al*., 2003; Vaarst *et al*., 2007). Frequently, disease patterns in conventional and organic farms do not seem to be very different and, indeed, it has been pointed out that the difference between herds within each production system are bigger than a systematic difference between organic and conventional (Thamsborg *et al*., 2004;

Cabaret *et al.*, 2012), stressing the importance of management and husbandry choices on a farm level. In the case of goats farms, parasitic diseases are reported as the major health issue either in conventional and organic farming, mainly because both rearing systems are traditionally based on grazing.

To conclude, organic livestock, including goats, cannot be claimed to be generally healthier than conventional livestock throughout Europe (Vaarst *et al.*, 2008, Nicourt & Cabaret, 2014).

It is clear that "good health" is an essential component of animal welfare but, on the other hand, "welfare" does not mean only absence of diseases. Therefore, the theme "animal welfare" is something controversial.

Already in 1946, the World Health Organization defined health as "*a state of complete physical, mental and social well-being and not merely the absence of disease"* (WHO, 1946). Later, the Brambell Report[3](1965) recognized the role of mental processes in health and welfare. Its definition was the following: *"welfare is a wide term that embraces both the physical and mental well-being of the animal. Any attempt to evaluate welfare,*

[3] In 1965, the United Kingdom (UK) government commissioned an investigation, led by Professor Roger Brambell, into the welfare of intensively farmed animals, partly in response to concerns raised in Ruth Harrison's 1964 book, Animal Machines. On the basis of Professor Brambell's report, the UK government set up the Farm Animal Welfare Advisory Committee in 1967, which became the Farm Animal Welfare Council in 1979. The committee's first guidelines recommended that animals require the freedoms to "stand up, lie down, turn around, groom themselves and stretch their limbs". The guidelines have since been elaborated to become known as the Five Freedoms: (1) Freedom from thirst and hunger - by ready access to fresh water and a diet to maintain full health and vigour. (2) Freedom from discomfort - by providing an appropriate environment including shelter and a comfortable resting area. (3) Freedom from pain, injury, and disease - by prevention or rapid diagnosis and treatment. (4) Freedom to express normal behaviour - by providing sufficient space, proper facilities and company of the animal's own kind. (5) Freedom from fear and distress - by ensuring conditions and treatment which avoid mental suffering.

therefore, must take into account the scientific evidence available concerning the feelings of animals that can be derived from their structure and functions and also from their behaviour".

Therefore, investigating only the health issues in organic farms is a semplicistic way to address the animal welfare question.

At this point, the question about the definition of animal welfare, especially in organic farming arises.

The organic understanding of the animal welfare concept can be compared with some common approaches to the concept:

- *the affective states approach*, arguing that animal welfare concerns are, in fact, concerns about the animals' subjective experiences, hence only animal feelings, such as suffering, pain or pleasure, should include when welfare status is evaluated (e.g., Dawkins, 1988; Duncan, 1993);
- *the biological functioning approach*, arguing that good quality of life is when the animal's biological systems are working in a normal or satisfactory manner or when the animal can cope with its situation (e.g., Broom, 1986; Wiepkema & Koolhaas, 1993);
- *the natural living approach*, proposing that animal's welfare depends on the possibility of expressing its natural behaviour (Webster *et al.*, 1986) and living a natural life according to its genetically encoded nature (Rollin, 1990, 1993).

The reported views can partly overlap depending on the specific and personal interpretation of each position (Fraser *et al.*, 1997). In organic farming the animal welfare concept is interpreted close to the last of these three positions (Alrøe *et al*, 2001; Verhoog *et al*., 2004; Lund, 2006; Vaarst *et al*., 2011; Vaarst & Alrøe, 2012). Therefore, naturalness represents one of the key values in the understanding of animal welfare in organic goat farming (Vaarst *et al*., 2004). In addition, Veroogh *et al*. (2003) underline the importance of natural behaviour within the context of agro-ecosystem, arguing that the naturalness can be an important guiding value only when a wide concept of the "natural" is taken, including the no-chemical and the agroecological approach. With regards to animal welfare, animals should be able to express their natural behaviour in a balanced agro-ecosystem (where plants, animals and human being that live in it are in harmony). Moreover, in these systems the role of the stockperson is also essential since it can affect the welfare concept (e.g., Boivin *et al*., 2003; Hemsworth, 2007). This means that the farmers besides creating the best conditions for the animals (allow them to live as natural a life as likely within the human), should control the environment and intervene in situations of risks, considered as the moment in which the balance breaks (Vaarst *et al*., 2004; Vaarst & Alrøe, 2012).

PASTURE AS A KEY ELEMENT

There is a feature of goat organic farming system that may jeopardize the aims of health and welfare: the pasture.

The current EU Regulation requires grazing for small ruminant and the organic principles aim for organic goat to be managed in a "natural system" (Marley *et al*., 2010; Vaarst *et al*., 2011). Goats are grazing ruminant hence pasture represent their "natural system". Grazing fresh pasture or browse is the essence of a small ruminant (Wolff, 2009) because during grazing the animals recreate a direct relationship with the environment where they live, they perform their feeding habits, being free to choose the essences, which are most good for them both in qualitative and quantitative terms (Martini *et al*., 2009) as well as they have the opportunity to express a greater part of their natural behaviour (e.g., play and social behaviour) (Sevi *et al*., 2009; Dwyer, 2009; Martini *et al*., 2009; Braghieri *et al*., 2011).

Allowing natural behaviours, besides being an effective way to satisfy animal needs, can contribute significantly to the goats' welfare (Miranda-de la Lama & Mattiello, 2010). Extensive rearing conditions, such as access to pasture, allow or even stimulate the richness of natural behaviour and leads to a harmonious development of the various skills in the animals, especially the social ones (Washburn *et al*., 2002; Špinka, 2006).

In extensive systems the levels of aggression, for instance, are lower than those in intensive farming systems (Orgeur *et al*., 1990) where goats are kept indoor under high stocking densities.

Moreover, on pasture, the opportunity to express the natural behaviour has positive effects on the goats' health because it promotes the "psychological" well-being, which generally, results in a stress reduction that enhances the immune function (e.g., Henry & Stephens, 1977; Kiley-Worthington, 1977).

Nevertheless, goats in extensive environments may face a range of compromises to their welfare (e.g., extreme climatic conditions, diseases, inadequate feed and water supply) (Goddard, 2006; Sevi *et al*, 2009; Dwyer, 2009). Therefore, in such conditions, humans have a precise moral obligation to prevent suffering in accordance with the concept of animal welfare (Vaarst & Alrøe, 2012). Pasture-based systems require additional labour input to ensure that grazing areas are managed effectively and animal health and welfare are not compromised (Marley *et al*., 2010). Several factors require consideration such as the provision of sufficient feed on the pastures at all times and the control of parasitic diseases, which are the most relevant and to some extent inter-related.

Feed availability is identified as one of the major constraints for small ruminant systems in the Mediterranean area. In many areas pasture growth is limited by the irregular distribution of rainfall during the year and between years, coupled with high temperatures during summer (Ronchi & Nardone, 2003). Grazing animals are usually subjected to a temporary nutritional stress due to the seasonal fluctuations of herbage amount and quality (Negrave, 1996). If the nutritional stress occurs during mating season, it can reduce small ruminant fertility (Rassu *et al*., 2004). It is necessary to secure sufficient feed on the pastures at all times: stocking rates above or even very close to the carrying capacity of plant production should be avoided. Maximization of pasture feed supply could be achieved through agronomic practices, such as irrigated sown pastures using a combination of vigorous pasture grass and clover species (Arsenos *et al*., 2004).

Another important aspect of grassland management is the handling of endoparasites, especially the gastrointestinal nematodes (GIN)[4]. GIN are the most prevalent parasitic diseases that affect goats, representing a major threat for health and welfare under outdoor grazing rearing systems (Keatinge, 1996; Perry *et al*., 2002; Kaplan, 2004; Nardone *et al*., 2004; Rahamann & Seip, 2006; Hoste *et al*., 2010, 2012, 2014).

In general, GIN have a direct biological life cycle (Figure 3), parasite-infested animal harbours adult worms in the digestive tract and eggs are excreted in the faeces. In the environment, the eggs mature and hatch releasing first stage larvae (L_1), which turn into larvae L_2 and subsequently in the infective form (L_3). These L_3 are highly resistant to external factors, either chemical or physical; their survival rate on pasture is influenced by climatic conditions, varying from 6 to 12 months in temperate regions (Torres-Acosta & Hoste, 2008).

The infestation of goats occurs principally with the ingestion of the third stage larvae (L_3) hence grazing may increases the risk of infection, through prolonged periods of contact with the infectious larvae of GIN on pastures (Waller, 1993; Thamsborg *et al*., 2004; Waller, 2006; Rahmann & Seip,

[4] Nowadays GIN of small ruminants, with economic importance, described in the literature are: *Haemonchus contortus*, *Trichostrongylus* spp., *Teladorsagia* or *Ostertagia circumcincta* (abomasum); *Oesophagostomum* spp., *Chabertia ovina* (large intestine), *Nematodirus* spp. and *Cooperia* (small intestine) (Mederos *et al*., 2012).

2006; Torres-Acosta & Hoste, 2008; Manfredi *et al*., 2010; Di Cerbo *et al*., 2010).

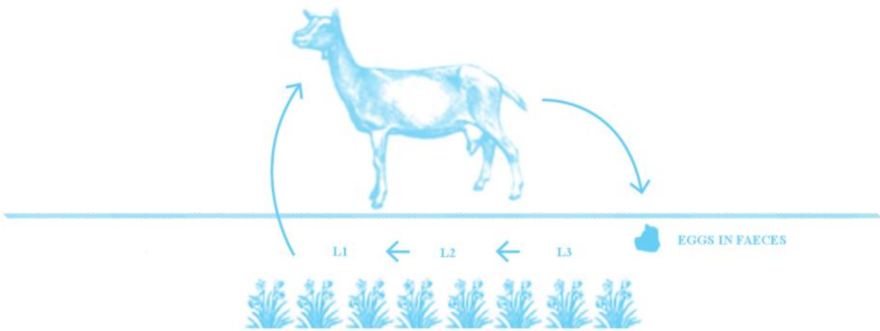

Figure 3. General life cycle of gastrointestinal parasites. Source: Manfredi, 2010, modified.

In many regions grazing small ruminant are infected almost all the time with GIN and ingestion from pasture is almost continuous (Younie *et al*., 2004). The presence of GIN in the digestive tract is generally associated with mild to severe pathophysiological consequences: (i) a reduced appetite; (ii) a malabsorption/mal digestion syndrome; and (iii) a reorientation of nutrient metabolisms in the infected hosts in order first to maintain the homeostasis (Hoste *et al*., 1997; Roy *et al*., 2003). This combination of pathological changes can result in a health distress and can interact negatively with the productive performance of dairy goats (Hoste *et al*., 2005; Waller, 2006; Mederos *et al*., 2012; Hoste *et al*., 2014).

Although, the organic EU Regulation authorize the use of chemical deworming products (anthelmintics - AH), the sustainable GIN control should rely on alternative strategies (Grosso *et al*., submitted). Basically, organic goats farmers should enhance and exploit the goat's own immune status and resistance/resilience[5] to parasitic infection (e.g., appropriate feeding, suitable breed) and should apply non-chemical tools in order to moderate the challenge from pasture (Thambsorg *et al*., 2005).

Grazing management strategies are an important means for GIN control and their use have been described since the end of 1960s. The general goal of these methods is to provide pastures with a minimal infectivity to susceptible animals. This general objective could be achieved by different ways (e.g., reducing the stocking rate on pasture, mixing or alternating grazing between different susceptible species – goats-cattle –, moving animals from contaminated to clean pastures) (Michel, 1976; Thamsborg *et al*., 1999; Cabaret *et al*., 2002; Eysker *et al*., 2005; Rahmann & Seip, 2006; Waller, 2006; Torres-Acosta & Hoste, 2008; Jackson *et al*., 2009; Hoste & Torres-Acosta, 2011; Hoste *et al*., 2014).

The successful implementation of grazing management on the farm requires an increased labour cost and a detailed understanding of the GIN's epidemiology (Hoste & Torres-Acosta, 2011). This difficulty acts as a powerful deterrent, leading to the less exploitation of grazing management in comparison to other control tools (Jackson *et al*., 2009).

In Mediterranean areas the lack of available grazing lands represents an additional constrain and organic farmers give generally higher priority to

[5] *Host resistance*: one component of the host response against nematodes. This is the ability of the host to affect nematode biology either by decreasing the establishment of L3, by delaying the worm growth, reducing female worm fertility and egg excretion or by expelling existing adult worm populations. Immune mechanisms mainly govern host resistance. *Host resilience*: a second component of host response to parasitism. This is the ability of a host to withstand the negative pathological effects of worms in the digestive tract (Torres-Acosta & Hoste, 2008).

grass production than to GIN prevention through their management strategies (Vaarst *et al.*, 2008). This approach, however, has indirect positive effects on GIN control as it ensures adequate feeding supply results into a greater resilience against parasites and it could reduces fecal egg output and subsequent contamination of the pasture (Younie *et al.*, 2004). In any case, the only good nutrition with pasture management is not sufficient to prevent the outburst of GIN infections in all situations (Cabaret *et al.*, 2002; Waller, 2006) and other means for GIN control might be required.

ASSESSING WELFARE

The multidimensional concept of animal welfare in organic farms is still open to debate and it is representing a starting point to study valid welfare indicators that, consequently, will allow the development of methodologies for the welfare assessment of farms. Indeed, in order to provide an advisory tool for farmers, to cover certification-control purposes and, above all, fulfill consumers ethical needs, the level of goats welfare in organic farms should be checked.

Although, organic farming systems require reliable tools for monitoring the welfare state of flock (Knierim *et al.*, 2004) and, nowadays, to date it only few valid and feasible indicators are available for goats (Battini *et al.*, 2014) as well as there are no formal welfare assessment protocols for this species (Muri *et al.*, 2013). Only in recent years the EU-funded project Animal Welfare Indicators (AWIN) has aimed to fill this gap by developing a welfare assessment protocol also for small ruminant, even if it is not focusing specifically for organic animal welfare goals.

In general, both conventional and organic studies on the welfare of small ruminant have developed slowly, mainly due to prevalent extensive production system (Caroprese *et al*., 2008; Sevi *et al*., 2009; Dwyer, 2009). Therefore, the welfare of extensively managed animals has largely been ignored and the widespread perception that welfare in these systems is good could be connected to the fact that sheep and goats are known to have a high degree of adaptability even if these statements are not based on scientific assessments (Goddard, 2006; Turner & Dwyer, 2007).

For the other farm animals species (i.e., cattle, pigs and poultry) welfare assessment protocols have been developed and are currently applied on both organic and conventional farms.

Taking into consideration the multifactorial nature of animal welfare, the welfare assessment schemes evaluate different aspects of welfare by scores, which may be later combined into a final welfare score. Generally these protocols rely either on resource-based indicators, which include structural and technical elements (e.g., space allowance, feeding facilities), the quality of human-animal relationship and management-related factors (e.g., hygienic and climatic conditions and routine farming practices) (Animal Needs Index (ANI) 35L, TGI 200; Bartussek, 1999; Sundrum *et al*., 1994), either animal-based indicators dealing with behaviour, health and physiology of the animals (e.g., Bristol Welfare Assurance; Main *et al*., 2004), or a combination of resource and animal-based measures (e.g., Welfare Quality®; Botreau *et al*., 2009) in order to obtain a valid assessment of animal welfare (Johnsen *et al*., 2001).

Due to the lack of welfare assessment protocols for small ruminant the "Animal Needs Index 35 L", scientifically validated for cattle, was applied to sheep in two recent studies (Napolitano *et al*., 2009; Grosso *et al*., 2012) with the aim, among other things, to compare the welfare state of the animals in organic and conventional farms. No significant differences in terms of animal welfare were observed between organic and conventional farms, indicating that both systems provide adequate welfare. With regards to goats, Martini *et al.* (2014) have conducted a survey in 21 dairy goats farms in Italy, using a questionnaire derived from ANI, including resource and animal-based welfare indicators. Although records on health indicators were better (higher scores) in organic farms, no statistical differences were found related to the production system (conventional, organic or biodynamic).

These results are not unexpected and might be explained by the negligible difference in dairy small ruminant production: despite farming methods, all rearing systems were extensive.

The ANI system used in the studies reported above relays mainly on environmental measures but not on the state of the animals. The validity of resource-based assessment schemes, in terms of reporting the real impact of housing systems on the animals, is questionable. Preconditions for animal health and welfare are contained rather than the real impact of housing systems on the animals (Hörning, 2001; Whay, 2007). More recent developments follow the basic assumption that proper assessment systems should be based on animal-related measures (e.g., social behaviour, body condition) because these directly reflect how the animals are affected by their environment providing a more direct measure of the current animal's welfare state (Smulders & Algers, 2009, Blokhuis, *et al.*, 2010; Appleby *et al.*, 2011). The recent EU-funded project (AWIN) is also focused primarily on simple and accessible animal-based indicators.

Due to the importance of animal welfare in organic dairy goat production, tools for measuring how well that goal is being fulfilled are required.

Moreover, on the other hand, consumers are prepared to pay higher prices for organic animal products if animals actually experience good welfare (Rahmann & Godino, 2012).

In organic farming framework the welfare assessment approach should be developed taking into account the organic concepts of animal health and welfare within the four principles for organic production (Vaarst & Alrøe, 2012).

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AIM & OBJECTIVES

The aim of this PhD was to investigate health and welfare concerns in organic goats farms related to the use of pasture.

Pasture represents an increased threat for health-related issues, namely gastrointestinal nematodes. In organic goats farms GIN are endemic and perceived as the most important health problem of grazing goats causing chronic infections and production losses.

Although the general goal promoted in organic farming is to achieve a sustainable and integrated GIN control, reliance on chemotherapy is still high even in organic farms.

Due to the need of improving the research studies in alternative strategies, literature has been reviewed to highlight promising tools for GIN control in small ruminant organic farms. The outcomes of this literature review are presented and discussed in Chapter 1.

Among the alternative to synthetic anthelmintics, phytotherapy stands out and, connected to this theme, the aim of this thesis focuses primarily on the evaluation of the efficacy of herbal medicine treatments for GIN control. To do so, two field trials were performed in an organic dairy goats farm with the objective to evaluate the antiparasitic effect of herbal remedies.

First, the efficacy of a commercial herbal product was evaluated compared to a conventional allopathic anthelmintic and then the efficacy of pumpkin seeds, traditionally used as a vermicide in popular veterinary medicine, was tested.

The results of these trials are presented and discussed in Chapter 2.

Pastures are not only a threat for GIN infections, but allow goats to perform their natural behaviours and to live positive experiences, that are considered as an essential component of animal welfare. The second objective of this thesis was to evaluate whether pasture really provide a welfare benefit for organic goats. The QBA approach was used to assess the "overall" goat's welfare in organic farms compared to intensive ones, where goats have no access to pasture.

The results are presented in Chapter 3.

CHAPTER 1

SUSTAINABLE CONTROL OF GASTROINTESTINAL NEMATODES IN ORGANIC SMALL RUMINANT FARMING: A REVIEW

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ABSTRACT

Gastrointestinal nematodes are relevant diseases in grazing small ruminants. The use of chemical deworming is authorized in organic farming, but the occurrence of resistant parasites and the environmental impact are increasing rapidly. The use of alternative control measures has improved recently in order to maintain an adequate level of animal welfare, reduce the risk of residues in food and fulfil the organic principles. This paper aims to give an overview on the current literature regarding the sustainable strategies to control gastrointestinal nematodes in organic sheep and goats. Phytotherapy is described in detail, as it may represent a viable therapeutic option.

Keywords: gastrointestinal nematodes; organic farming; parasite control management; phytotherapy; goat; sheep

INTRODUCTION

Organic farming encompasses the concept of a sustainable approach to agriculture production and aims to create an agroecological system based on a balanced interdependence between people, animal and environment (Thompson & Nardone, 1999).

In organic farming, livestock plays an important role in providing manure and good quality products based on nonchemical prevention of diseases (Thamsborg *et al*., 1999) and organic livestock production is defined by basic standards, based on a decreased use of chemotherapy and a holistic approach to the production processes (Sundrum, 2001).

To enhance a high level of animal health and welfare is the major goal for organic farming (Marley *et al.*, 2010; Vaarst *et al*., 2011), achieved by keeping animals as close as possible to their natural habitat (e.g., access to grass and rangelands for maximum periods), reducing stocking rate and dependence on chemotherapy (Thamsborg & Roepstorff, 2003).

A sharp increase in certified animals and farms for all species (FiBL-IFOAM, 2013) is reported worldwide, including sheep and goats that are traditionally reared under extensive systems. Considering the number of certified animals in the EU, United Kingdom plays the leading role for sheep and Greece for goats, both followed by Italy (European Commission, 2013).

In organic farming the health status of herd represents a key factor to ensure a viable economic outcomes and parasite-related diseases are the major concern affecting organic livestock more than conventional one (Thamsborg *et al*., 1999; Lund & Alger, 2003), mainly because animals are managed in outdoor system.

Gastrointestinal nematodes (GIN) still remain one of the most prevalent parasitic diseases in grazing small ruminant, representing a threat for livestock production, health and welfare (Hoste *et al*., 2010, 2012).

GIN infections are responsible for major economical losses in the sheep and goat industries due to either direct (e.g., nutritional penalties, decreases in production) or indirect (e.g., treatments, labour costs) consequences (Roy *et al*., 2003; Rinaldi *et al*., 2007; Molento, 2009; Mederos *et al*., 2010).

The European legislation (Reg. 889/2008/EC) on organic farming requires grazing for small ruminant, but there is still a debate for maintaining the derogation on pasture due to the risk of infection. On the other hand, grazing allows animals to express natural behaviour and reflects the organic principles. Grazing certainly increases the animal welfare but also the risk of infection, through extended periods of contact with the infectious larvae of internal parasites (Rahmann & Seip, 2006).

The scientific interest about the spread of GIN, especially in order to define the incidence of the problem, is highlighted by several authors, who analyse the impact of parasites in organic small ruminant farms in different countries (Thamsborg *et al.*, 1999; Cabaret *et al*., 2002; Rahmann & Seip, 2006; Mederos *et al*., 2010; Silva *et al*., 2011).

CONVENTIONAL ANTHELMINTIC TREATMENTS IN ORGANIC FARMS: A CONTROVERSIAL ISSUE

For many decades anthelmintic (AH) drugs were the preferred tools to control the GIN infection, even without any diagnostic findings (Liu *et al*., 2005). Farmers use allopathic AH treatments not only to cure parasitic infection but also for prevention in order to maintain the a high level of productivity. Despite the high initial efficacy of AH against GIN, the indiscriminate use of these chemicals (e.g., excessive administration, under-dose, no rotation of products) led to widespread parasite resistance (Hoste *el al*., 2002; Kaplan, 2004; Waller, 2006; Leathwick, 2014); in some farms GIN developed resistance to all available commercial dewormers (Terrill *et al*., 2012).

AH resistance represents one of the negative consequences of the massive use of AH. Several studies reported the ecotoxicity of AH drugs on the terrestrial and aquatic environments, as the action is not specific against parasitic nematodes these products may affect non-target organisms (McKellar, 1997; Beynon, 2012; Beynon *et al*., 2012; Horvat *et al*., 2012). Treated animals excrete AH on soil impacting dung fauna and decreasing rates of dung decomposition, and so affecting soil fertility. In addition drug residues can pollute watercourse causing potentially harmful effects on public health, especially in countries where no environmental protection policies are applied (Beynon, 2012). Many studies have been performed on the ecotoxicity effects of the macrocyclic lactones (e.g., ivermectin), one of the most used AH. After oral administration to sheep, ivermectin is excreted in faeces and remains active for a long time in the soil, with both lethal and sub-lethal effect on coprophagous flies and dung beetles (Beynon, 2012).

Beyond to the environmental impact, some authors pointed out other side effect of improper AH use such as the public concerns about drug residues in animal products. A recent study investigated the presence of AH residues (benzimidazoles and their metabolites) in 143 raw milk samples for human consumption collected from dairy farms (goats, sheep and cattle) in Greece. A high percentage of samples was positive for benzimidazoles, a common class of AH. Although the levels of compounds did not exceed the acceptable daily intake, it is recognized that benzimidazoles may cause teratogenicity and embryotoxicity in many animal species, hence a stricter Regulation for dairy product is required (Tziboukis *et al*., 2013).

Finally, the indiscriminate use of AH products for preventive treatments do not allow animals to develop their natural immunity against GIN (Ketzis *et al*., 2006) resulting in additional treatment rate.

The EU Regulation on organic farming modified the health management approach introducing restriction in the use of anthelmintics to prevent parasitic diseases. Moreover when allopathic veterinary medicinal products are used, withdrawal period for organic products are twice the legal withdrawal period.

Organic farming aims to avoid chemicals and farmers are encouraged to rely on prevention (e.g., grazing management, appropriate breeding, good nutrition). Nevertheless most of organic sheep and goat farms still depend on the therapeutic use of anthelmintic drugs (Cabaret *et al*., 2002; Rahmann & Seip, 2006).

Organic standards in Europe allow deworming of small ruminant with AH, recognizing that GIN control is difficult to achieve through natural strategies and nematodes may compromises animal welfare (Lu *et al*., 2010). The control of GIN in small ruminant remains a controversial and debated issue: therapeutic deworming is allowed by EU Regulation, but other international standards (e.g., the NOP National Organic Standards in the U.S.) do not permit this practice.

In organic small ruminant farming GIN are endemic and treatments with large spectrum AH – in some cases on a six-week bases – are common, with several negative effects on environment and animal welfare (Rahmann, 2007). Although AH are used only for therapeutic purpose in organic farms, the routine use represents a strong critical point because it leads to the dependence by allopathic medicine in GIN control, as in conventional farms (Thamsborg *et al*., 2004).

The control of GIN is considered one of the most important animal health and welfare issue to assure a sustainable development for organic small ruminant farming (Cabaret *et al*., 2002; Rahmann & Seip, 2006; Pisseri *et al*., 2013). This review aims to describe the current alternative strategies to control GIN in sheep and goat farming according to the organic principles.

ALTERNATIVE GIN CONTROL MEASURES IN ORGANIC SHEEP AND GOAT FARMING

Nowadays, it is universally recognized that only an integrated approach, based on combination of monitoring tools, will lead to a more sustainable control of GIN (Torres-Acosta & Hoste, 2008; Jackson *et al*., 2009; Molento, 2009; Amarante, 2014; Bath, 2014; Larsen, 2014; Leathweak, 2014; Hoste *et al*., 2014).

In organic farming, parasite control should be based on alternative management strategies not aiming at a total elimination of parasites – impracticable, in most cases, and generally not necessary to achieve an acceptable control – but maintaining the latter at adequate levels of animal welfare. It should be kept in mind that a minimum presence of parasites allows animals to develop and maintain a certain degree of immuneresponse (Molento, 2009). Parasites are living organisms naturally present on the gastrointestinal tract of small ruminants and sometime they represent a potential problem to address in order to improve the breeding system (Bath, 2006). A common misinterpretation amongst veterinarians and farmers is that the presence of GIN is by itself negative and implies disease. Within a flock, gastrointestinal nematodes are not equally distributed amongst animals either in sheep (Sreter *et al*., 1994) or goats (Hoste *et al*., 2001) and evidence indicates that a small number of animals are heavily infected whereas most individuals of the flock show a moderate worm burden.

The crucial goal in control strategies is to develop livestock production systems where parasites are present in small number without affecting health or performance of herds. Deworming treatments, whether administered using natural products or not, should therefore be used in emergency situation or in particular conditions, as targeted selective treatments (Duval, 1994; Kenyon *et al*., 2009; Terrill *et al*., 2012).

In the ethical and legal framework of the organic farms, alternative preventive and curative measures to control GIN undertake a crucial role and they will be described in the following chapters.

1. Routine monitoring and clinical investigation

An integrated GIN control plan should begin with the identification of the type of parasites present and its infestation burden (Fecal Egg Count). Diagnostic tools are available but not commonly used, although they are necessary (Cabaret, 2004). Parasitological examination of faecal samples aims to evaluate the GIN epidemiology (Sargison, 2013) and represents the first step in building up a sustainable control program. The obtained results have to be correlated with the health status and performance of the herd in order to minimized sanitary risk. Clinical signs typically related to parasitism (i.e., anaemia, diarrhoea and weight loss) must be reported. Moreover, the evaluation of management and environment of farms plays an important role allowing to identify any possible factors linked to diet, soil, climate or intermediate biological hosts, that could increase the risk of parasitic infection (Pisseri *et al*., 2013; Sargison 2013; Sargison, 2014).

An appropriate monitoring allows to evaluate the level of parasitic infections and to take consequent control measures.

2. Biological control

The principle of this control method is based on “biological rule”: all species are regulated by the existence of other organisms, which are able to prevent an uncontrolled growth of those populations (Grønvold *et al*., 1996).

Many studies have been done to investigate the alternative use of *Duddingtonia flagrans*, as a biological control agent against sheep (Silva *et al*., 2009; Santurio *et al*., 2011) and goat (Epe *et al*., 2009; Vilela *et al*., 2012) GIN. This fungus has the potential to break the GIN life cycle by capturing larval stages in the faeces before they migrate to pasture. In order to achieve optimal results, the spores need to be continuously shed in the faeces concurrently with output of parasite eggs. Therefore, daily supplementation of spore material should be defined according to the epidemiological background in order to produce satisfactory effects (Waller, 2003). To date, the path and the necessary period of administration (at least two months in temperate countries) clearly represent serious drawback in the practicability of this method. Moreover, the development of controlled release devices or feed-blocks has not yet reached an adequate level of effectiveness and practicability (Waller & Thamsborg, 2004).

3. Bioactive forage

Forages containing condensed tannins, such as chinese bushclover (*Sericea cuneata*), bird’s-foot trefoil (*Lotus corniculatus*), chicory (*Cichorium intybus*), sainfoin (*Onobrychis viciifolia*) and sulla (*Hedysarum coronarium*), have shown anthelmintic activity against gastrointestinal nematodes of sheep and goats (Hoste *et al*., 2012; Juhnke *et al*., 2012; Werne *et al*., 2013). They may ideally play a role in a rotation grazing system and may be included in integrated control plan (Waller & Thamsborg, 2004). These specialized crops, which are bioactive forages, are either grazed or fed after conservation with the main purpose of preventing or curing disease (Waller *et al*., 2001).

Preliminary studies showed that certain leguminous plants in the pasture with high tannin content allowed a reduction of gastro-intestinal nematode infection in sheep (Niezen *et al*., 1998).

There are several studies that analyse the effect of tannins on ruminants: voluntary intake, digestibility of the diet, ruminal fermentation, production, toxicity, and parasite control (Méndez-Ortíz *et al*., 2012). The positive effect on the host can be direct, decreasing the viability of the larval stages, or indirect, increasing the availability of protein and thereby the resilience (Min & Hart, 2003).

Regardless of the mechanism of action, several *in vitro* (Athanasiadou *et al*., 2001; Molan *et al*., 2003; Bahuaud *et al*., 2006) and *in vivo* assays (Athanasiadou *et al*., 2001; Heckendorn *et al*., 2007; Terrill *et al*., 2009; Valderrábano *et al*., 2010) demonstrated the efficacy of these substances, although not all plants that contain tannins have the same effects against parasites (Häring *et al*., 2008). The inconsistent results observed in different studies are probably due to some variables, such as soil type, climate, season, cultivar, cutting, grazing, affecting the concentration of secondary metabolites in these plants.

More knowledge on these aspects and on the interaction with animal nutrition are required before the use of bioactive forage in organic farming becomes routine; furthermore, the agronomic practices for the cultivation need to be deeply investigated in order to verify if they fit to the organic farming (Rahmann & Seip, 2006).

4. Grazing management

Grazing management strategies are an important tool for the control of GIN. The use of grazing management principles has been the subject of several review papers (Thamsborg *et al*., 1999; Cabaret *et al*., 2002; Eysker *et al*., 2005; Rahmann & Seip, 2006; Waller, 2006; Torres-Acosta & Hoste, 2008). The objective of grazing management is to limit the contact between susceptible host and the parasite infective stage. According to Michel (1976), these include preventive (putting worm-free animals onto a clean pasture), evasive (worm challenge is evaded by moving animals from contaminated to clean pastures) and diluting (worm challenge is relieved by diluting pasture infectivity) strategies.

Several studies described and postulated various management practices: to alternate use of land for grazing and crops; to avoid animals of different ages to graze together (young animals should grazing ahead of the older ones); to avoid animals from different farms to share the same pasture; to plan a rotation of pastures according to the seasonal development of parasites; to introduce the mixed grazing (alternating different species on the same pasture); finally, to keep a low stocking rate, as required in organic farming (Mahieu & Aumont, 2009).

Organic sheep and goat farmers have to find a compromise between the suitable grazing management procedures and the availability of herbage during the time. Factors such as farm layout, size of field and timing of forage conservation limit the practice of clean grazing as control strategy. Moreover, the effectiveness of clean grazing without anthelmintic treatments is still to be documented (Waller, 2006).

Grazing management strategies are usually inexpensive but they can be associated with an increased labour cost. In addition, at farm levels, these control plans will be accepted only if matching to animal feeding strategies (Hoste & Torres-Acosta, 2011). Moreover, despite of the huge amount of studies published on the topic, it is impossible to set a unique strategy for parasites control by grazing management. The majority of studies refers to typical climatic conditions of northern Europe or Australia, which differ from the Mediterranean areas, where lack of available grazing land represents an additional constrain.

5. Supplementary feeding

It has been well documented that dietary supplementation results in both the increase in productivity and in resistance to parasites (Houdijk, 2012; Méndez-Ortíz *et al*., 2012); proteins (Steel, 2003) and mineral supplements (Sumbria & Sanyal, 2009) seem to have an important role in protection against infestation by nematodes, improving the host resilience and resistance.

A balanced grazing system provides an adequate source of nutrients and maintains an acceptable GIN infestation, allowing an optimum level of productivity, and a breakdown in such balance may induce severe parasite infections (Torres-Acosta *et al*., 2012).

Supplementations to young animals and/or periparturient ewes or goats have been proven to be effective (Kahn, 2003), but further researches are still needed to measure more precisely the deficiencies due to parasite infection in order to adapt the supplementation and cover the additional requirement in infected animals (Torres-Acosta & Hoste, 2008; Torres-Acosta *et al*., 2012).

6. Breeding parasite-resistant animal

The genetic selection of host represents another option to limit the nematode populations - selection for resistance - or their pathological consequences - selection for resilience (Bishop, 2012). In small ruminants variation in the level of GIN infection between breeds has been reported, although goats have been studied less than sheep (De la Chevrotiere *et al*., 2011). In addition, the improvement of resistance against nematodes through genetic selection within a breed has been the aim of research programs in some countries (De la Chevrotiere *et al*., 2011 Saddiqi *et al*., 2012; Amarante, 2014; Bath, 2014). Such programs for some highly productive breeds achieved good progress (Bishop, 2012); this approach fits closely with the organic principles and the selection of the most suitable breeds should always be kept in mind (Hoste *et al*., 2010).

7. Phytotherapy

The current standards for organic animal production recommend that phytotherapic or homoeopathic products should be preferably used instead of allopathic medicines. Homeopathy will not be examined in this review, as the different approach compared to traditional occidental medicine requires a separate discussion. However, homeopathy has been recognised as a therapeutic measure against GIN on organic farms, but it is currently considered unsuitable for short-term treatments (Cabaret *et al*., 2002).

Phytotherapy seems to be a feasible option for GIN control and may represents a promising alternative to synthetics AH (Molento, 2009). The research in phytotherapy has been widely promoted in the last years and the scientific validation through *in vitro* and *in vivo* testing for assessing the anthelmintic properties and safety of plants is encouraged (Rates, 2001; Rahmann & Seip, 2006; McGaw *et al*., 2008).

Several plants with AH property have been tested under controlled studies in different parts of the world achieving different results which are summarized in Table 1. Most of these studies aimed to evaluate the anthelmintic activity of plants preparations against *Haemoncus contortus*, the most common gastric blood-sucking nematode of small ruminant. Results about seventeen plants and a mixture of two plants (onion and coconut) are reported.

The majority of studies focused on GIN in small ruminant were performed on sheep; however, the differences between sheep and goats regarding to GIN infections are well documented and the results from one species are not necessarily valid for the other (Hoste *et al*., 2010).

The anthelmintic activity against GIN, both *in vivo* and *in vitro* studies, was proved in sheep for seven plants: *Hedera helix* (Eguale *et al*., 2007a), *Artemisia brevifolia* (Iqbal *et al*., 2004), *Achillea millefolium* (Tariq *et al*., 2008), *Artemisia absinthium* (Tariq *et al*., 2009), *Nigella sativa* (Al-Shaibani *et al*., 2008), *Khaya senegalensis* (Ademola *et al*., 2004) and *Nicotiana tabacum* (Hamad *et al*., 2012). The *in vivo* efficacy was found for *Allium sativum* (Sunada *et al*., 2011), *Zingiber officinale* (Iqbal *et al*., 2006), *Coriandrum sativum* (Eguale *et al*., 2007b), *Cucurbita* sp. (Strickland *et al*., 2009), and *Fumaria parviflora* (Akhtar *et al*., 2000; Hördegen *et al*., 2003). Moreover the *in vivo* study on the antelmintic activity of *F. parviflora* reported the same efficiency than conventional AH product (Hördegen *et al*., 2003).

No effect against GIN was found about the *in vivo* AH activity of papaya (*Carica papaya)*, probably in relation to the administration of single dose (Burke *et al*., 2009).

Different results were found about the anthelmintc activity of neem tree (*Azadirachta indica*): two studies by administration of dried leaves reported no effect (Costa *et al*., 2006; Chagas *et al*., 2008), while other trials using seeds (Iqbal *et al*., 2010) and bark extract (Swarnkar *et al*., 2008) found a high and moderate AH efficacy, respectively. Variation in the AH activity of neem may be due to different concentrations of the active component (azadirachtin) in different parts of the plant (Iqbal *et al*., 2010): no studies reported significant EPG reduction suggesting that the leaves did not have AH activity.

A mixture of onion *(Allium cepa)* and coconut (*Cocos nucifera)* powder, integrated in a dry milk powder to increase palatability and distributed on fed, were tested by Mehlhorn *et al*. (2011): the combination of these plants was effective against cestodes and nematodes, suggesting a synergistic effect.

In goats *Eucalyptus staigeriana* showed *in vitro* and *in vivo* efficacy against GIN (Macedo *et al*., 2010), while powder fruit of *Melia azedarach* (Akhtar & Riffat, 1984) and papaya seed (*Carica papaya*) (Vieira *et al*., 1999) exhibited *in vivo* AH effect.

A juice of *Agave sisaliana* showed *in vitro* AH activity but its efficacy was reduced when administered to animals (Domingues *et al*., 2010), probably due to administration of a low dose. The difference between *in vivo* e *in vitro* results was attributed to many factors, such as the concentration of active components in the plant preparation and bioavailability and biotransformation of these compounds in animals, especially considering the ruminant physiology (Domingues *et al*., 2010).

In vitro tests on pumpkin (*Cucurbita* spp.) reported high efficacy of seed extract against *Haemoncus contortus* (Iqbal et el., 2001; Marie-Magdeleine *et al*., 2009) while *in vivo* studies reported contradictory results. No effect was pointed out using seed extract in a single dose (Nogueira *et al*., 2006); grinded pumpkin seed mixed with fed showed high anthelmintic activity in a study carried out by Almeida *et al*. (2007), while Grosso (2014) reported their inefficacy even at a high dose. Contradictory results reported on the AH activity of *Cucurbita* spp., as well as of other plants, may be related to several factors (e.g., the variety of plants, the part of the plant/type of extract and the dose used) that can affect the antiparasitic activity.

Neem did not exhibit AH activity in goats treated with two different doses of fruit extract, suggesting to test different posology (Nogueira *et al*., 2006).

Table 1. The most relevant potential AH plants for which controlled studies have been performed on sheep and goats. [a]: G: goats; S: sheep; - only *in vitro* testing.

Botanical name	**Part used or preparation**	**Dose**	**Host[a]**	**Scientific validation**	**References**
Achillea millefolium	Aqueous extracts and ethanolic extracts	2 g/kg BW	S	*In vitro* and *in vivo* studies revealed significant anthelmintic effects on live *H. contortus* worms	Tariq *et al*., 2008
Agave sisaliana	Juice	0.92 g/kg BW for 4 and 8 days	G	Effective against GIN *in vitro* tests; efficacy was markedly reduced *in vivo*	Domingues *et al*., 2010
Allium cepa and *Cocos nucifera*	Dried bulbs and fruit flesh	60 g *Allium cepa*, 60 g *Cocos nucifera* (dried powder), 10 g of milk powder/animal for 8 days	S	The worm stages disappeared from the faeces and were not found 9 and 20 days after the treatment	Mehlhorn *et al*., 2011
Allium Sativum	Dried powder mix with feed	6 g/animal/day	S	*In vivo* activity *vs* GIN	Sunada *et al*., 2011
	Juice, commercial product	Not specified	G	No anthelmintic effect was pointed out	Burke *et al*., 2009

Artemisia absinthium	Crude aqueous extracts and crude ethanolic extracts of the aerial parts	1–2 g/kg BW, single dose	S	*In vivo* and *in vitro* study show significant anthelmintic effects on *H. contortus* worms	Tariq *et al*., 2009
Artemisia brevifolia	Crude aqueous extract	3 g/kg BW	S	*In vivo* and *in vitro* anthelmintic activity *vs H. Contortus*, with significant FEC reduction	Iqbal *et al*., 2004
Azadirachta indica	Crude aqueous extract of eeds	3 g/kg BW	S	*In vivo* anthelmintic activity *vs H. Contortus* and *Trichostrongylus*, with significant FEC reduction	Iqbal *et al*., 2010
	Dried leaves	0.1-0.2 g/kg BW	S	No anthelmintic effect was pointed out	Costa *et al*., 2006
	Dried leaves	12.5, 25.0 and 37.5 g/animal/day, administered for alternating 15-day periods during 18 months	S	No anthelmintic effect was pointed out	Chagas *et al*., 2008
	Alcoholic extract of bark	50 mg/kg BW	S	*In vivo* moderate effect *vs* GIN	Swarnkar *et al*., 2008
	Fruit extract	1g and 3g/kg BW, administered for 3 time for alternating 13-day periods	G	No anthelmintic effect was pointed out	Nogueira *et al*., 2006
Carica papaya	Seeds, in a mixture with other plants	Not specified	G	*In vivo* trials have confirmed anthelmintic activity	Vieira *et al*., 1999

Carica papaya	Seeds	80 gr/lamb/diluted with 110 ml of water, single dose	S	No anthelmintic effect was pointed out	Burke *et al*., 2009
Coriandrum sativum	Seeds, aqueous extract	0.45-0.9 g/kg BW	S	*In vivo* anthelmintic activity *vs H. Contortus*	Eguale *et al*., 2007b
Cucurbita spp.	Aqueous, methanolic and dichloromethane extracts of *C. moschata* seeds		-	Effective against *H. contortus in vitro* tests; the extracts exhibited larval development inhibition at all concentrations and extraction methods examined.	Marie-Magdeleine *et al*., 2009
	Methanolic extracts of *C. mexicana* seeds		-	83.4% effective against *H. contortus in vitro* tests	Iqbal *et al*., 2001
	Ground seeds mix with feed	Not specified; 2 weeks of treatment	S	*In vivo* anthelmintic activity against *H. Contort*, with 65% FEC reduction	Strickland *et al*., 2009
	Ground seeds mix with feed	2.6 g/kg BW for 3 consecutive days; then, 30 g/animal of linseeds meal mixed with pelleted ration, for other 3 days	G	No anthelmintic effect was pointed out	Grosso, 2014
	Grinded seeds mix with feed	1.9 g/kg BW, for 3 consecutive days	G	*In vivo* anthelmintic activity, with significant FEC reduction	Almeida *et al*., 2007
	Seed extract	3 g/kg BW, single dose	G	No anthelmintic effect was pointed out	Nogueira *et al*., 2006

Eucalyptus staigeriana	Essential oil (leaf extract)	500 mg/kg BW	G	*In vitro* and *in vivo* trial showed efficacy against GIN	Macedo *et al.*, 2010
Fumaria Parviflora	Whole plant powder	2 g/kg BW	S	Efficacy against *Trichostrongylus*, *Haemonchus* and *Trichuris*	Akhtar *et al.*, 2000
	Aqueous ethanol extract of the whole plant	183 mg/kg BW	S	The study reported the same efficiency as conventional anthelmintic product	Hördegen *et al.*, 2003
Hedera Helix	Fruit extract	1.13 and 2.25 g/kg BW, single dose	S	*In vivo* and *in vitro* anthelmintic activity *vs H. Contortus*, with significant FEC reduction	Eguale *et al.*, 2007a
Khaya senegalensis	Ethanol extract of the powdered bark	500 mg/kg BW	S	*In vitro* and *in vivo* tests confirmed anthelmintic potential	Ademola *et al.*, 2004
Melia azedarach	Powder fruit	30 mg/kg BW	G	*In vivo* Activity against *H. Contortus* and other GIN	Akhtar & Riffat, 1984
Nicotiana tabacum	Crude aqueous methanol extract of leaf	2 g/kg BW	S	*In vitro* and *in vivo* antinematicidal activity against *H. contortus*	Hamad *et al.*, 2012
Nigella sativa	Aqueous and ethanolic extract of seeds	200 mg/kg BW	S	Trial *in vitro* and *in vivo* revealed higher activity *vs* GIN	Al-Shaibani *et al.*, 2008
Zingiber officinale	Crude powder and crude aqueous extract of dried rhizome	1–3 g/kg BW	S	*In vivo* the crude powder of ginger exhibited moderate anthelmintic activity	Iqbal *et al.*, 2006

Results obtained in sheep and goats suggest that phytotherapy may be a good alternative to allopathic AH treatments although further investigations are required and collaborative studies with the contribution of chemists, botanists and animal scientists should be planned. The scientific validation is complicated by the variety of bioactive compounds in a single plant remedy and by the large number of plants traditionally used in veterinary medicine against GIN. Moreover, there is not a standardized methodology to test the AH activity of medicinal plants (Hoste *et al*., 2008).

CONCLUSIONS

Gastrointestinal nematodes remain a major risk to small ruminant health and welfare worldwide and the demand for alternative control measures has constantly increased during the last years, particularly in relation to the rising relevance of organic farming. The use of chemical deworming products, which are authorized under organic EU Regulation, is an important criticism, since synthetic AH are routinely used increasing AH resistance and leading to high environmental impact and public health-related issues. According to organic principles, farmers should apply preventive measures and breeding strategies to avoid massive parasitic infections.

Alternative AH control strategies are essential to create sustainable, environmental- and consumer-friendly livestock systems, reducing the risks of residues in food and pollutants in the environment. Particularly organic farmers are demanding valid scientific information about advantages and limitations of alternative tools for GIN control, especially with regard to phytotherapic treatment.

In conclusion to promote scientific research on sustainable and alternative control strategies against GIN, in compliance to organic farming principles, becomes of primary relevance.

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CHAPTER 2

PHYTOTHERAPY AS A TOOL FOR GASTROINTESTINAL NEMATODES CONTROL

CHAPTER 2.1

COMPARISON BETWEEN THE EFFICACY OF A CONVENTIONAL AND A PHYTOTHERAPIC ANTHELMINTIC IN ORGANIC DAIRY GOATS

CHAPTER 2.2

IN VIVO EFFICACY OF PUMPKIN SEEDS (*Cucurbita maxima* DUCH) TO CONTROL GASTROINTESTINAL NEMATODES IN ORGANIC DAIRY GOATS

INTRODUCTION

Anthelmintic (AH) treatment remains an important tool for controlling GIN, even in organic farms (Cabaret *et al*., 2002; Cabaret *et al*., 2009). When preventive measures (e.g., grazing management) fails, recourse to treatment could be necessary in order to maintain animal health and welfare (Cabaret *et al*., 2009). Synthetic AH for therapeutic purpose may be used without any restrictions according to the current organic Regulation, but their indiscriminate use cause several side effects (see Grosso *et al*., submitted) hence farmers should turn to complementary medicines (phytotherapy and homeopathy). Phytotherapy (or herbal medicine) is among the most documented both in men and animals (Cabaret *et al*., 2002; Wynn & Fougere, 2007).

Herbal medicine is an ancient science, *Aristotele* (384-322 BC) wrote that the use of dittany (*Dictamus albus*) to cure the wounds was indicated by the goat to man and *Plutarco* (46-127 AD) said that the bear, after hibernation, free the intestine by eating wild arum (*Arum maculatum*) (Severino, 2009). *Claudio Galeno* (130-200 AD), a Greek physician of Pergamon, received notoriety for applying medicines prepared from vegetable substances by infusion, or decoction. These became known generically as galenical drugs, or preparations, and established the foundation for modern pharmacology. Even at global level, the modern pharmacopeia still contains about 25% of drugs derived from plants and many others are synthetic analogues based on compounds isolated from plants (Waller *et al*., 2001; Pignattelli, 2007; Sahoo *et al*., 2010).

Herbal medicine has been widely used in the past for the treatment of many diseases in livestock (Bullitta *et al*., 2007) and is still an indispensable tool in the management of extensive farming in developing countries (Nanyingi *et al*., 2008), due to the affordability combined with the local availability of plant resources (Athanasiadou *et al*., 2007).

The use of medicinal plants for the treatment of gastrointestinal parasitism is a relevant part of ethnoveterinary medicine (Athanasiadou *et al*., 2007), that is being defined as a mode of identifying, use and integration of many local knowledge, related skills and custom procedures created by people for purpose of preserving animal health and welfare (McCorkle, 1986). This knowledge is generally transmitted orally from generation to generation and, as other traditional beliefs, is currently threatened by technological development, socio cultural and environmental changes (McCorkle & Martin, 1998; Tabuti, 2003).

In ethnoveterinary medicine, for instance, seeds of garlic, onion and mint have been used to treat animals against GIN (Guarrera, 1999). A mixture of 5 leaves of wormwood (*Artemisia absinthium)*, a handful of sunflower (*Helianthus annuus*), a couple of cloves of garlic (*Allium sativum*), remains of onion and honey as a sweetener, administered once a week, have been reported as a preventive measure to control endoparassitosis in ruminants (Lans *et al*., 2007). Leaves, dried flowers and oil from *Chenopodium ambrosioides*, a shrub originated from Central America and now distributed around the world, have all been used as anthelmintics since the early 1900s (Guarrera, 1999). Moreover, in a recent survey on smallholder organic farmers in Uruguay, plant preparations of lavender (*Lavandula officinalis*) and wormwood have been reported as a therapeutic remedies against goats’

GIN (Grosso *et al*., 2010).

As it is clearly demonstrated by these examples, traditional veterinary pharmacopeia based on plant remedies remains the principal resource to treat animals against GIN in a large part of the world (Torres-Acosta & Hoste, 2008) and this trend is enhanced as a promising alternative to synthetics for parasitic control (Burke *et al*., 2009a; Molento, 2009).

Due to the huge variety of plants and to the interest in obtaining new active compounds, research in phytotherapy for the control of GIN in small ruminants has been extensively promoted in the last years (Molento *et al*., 2011).

Research efforts aim to fulfil the efficacy criteria that are the first to be evaluate for any AH, either chemical or natural. Nowadays there are no specific guidelines to evaluate the antiparasitic activity of plants. Therefore, the recommendations and methodologies described in the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines (Wood *et al*., 1995) to assess the efficacy of chemical AH, remain the most common approach to determine the efficacy of plants remedies (Hoste *et al*, 2008).

Scientists, in different parts of the world, are testing several plants with very different results, which are confirming or denying their traditional antiparasitic properties on small ruminant (Grosso *et al*., submitted).

If many studies are performed on individual plants, occasional studies have been carried out using herbal remedies commercially available (Podstatzky-Lichtenstein, 2009). In Italy only a commercial herbal product (Fitover/o Plus) has been tested, but the diffusion of the obtained results was spread exclusively into a local level. Moreover, the obtained results were contradictory: two studies showed no efficacy of the product against GIN (Virdis, 2000; Minghetti *et al*., 2010), whereas one study reported efficacy despite it did not achieve the same result than the allopathic drug (Roncoroni *et al*., 2008).

In U.S., Burke *et al.* (2009b) conducted a field study to evaluate the efficacy of a commercial herbal dewormer containing a mix of plants: the obtained results showed no benefits on the control of parasites.

In any case validation of a herbal preparation, whether commercial or not, is an on-going process, that requires a multidisciplinary approach but it is essential to extensively promote the use of phytotherapy in organic goat farms, as it is explained later. So far, not many organic farmers use this medicine mainly because of lack of scientific evidences of effectiveness (Kijlstra & Eijck, 2006; Peixoto *et al*., 2013).

As suggested by Hoste and colleagues (2008), it seems essential to increase the number of field studies in various epidemiological conditions, to assess the AH potential of plant resources and then to provide useful advices to organic goats farmers. Phytotherapy could lead to a more sustainable control of GIN with a reduced reliance on chemical AH and a higher reliance on natural local resources, being a medicine approach in accordance with organic principles.

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CHAPTER 2.1

COMPARISON BETWEEN THE EFFICACY OF A CONVENTIONAL AND A PHYTOTHERAPIC ANTHELMINTIC IN ORGANIC DAIRY GOATS*

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ABSTRACT

Organic dairy goat farming is a rising sector in Italy. Gastrointestinal nematodes (GIN) can have a significant impact in organic farming systems, mainly related to pasture use. The EU Organic Regulation limits the use of allopathic medicines, recommending preventive strategies or the use of phytotherapy. In this study on an organic farm, thirty lactating goats were divided into two treatment groups (n = 15/treatment): Group C (8 primiparous, 7 secondiparous) was treated with netobimin and Group P (8 primiparous, 7 secondiparous) with an herbal anthelmintic in order to compare the *in vivo* efficacy of the two products. Fecal samples were collected before treatment and monthly, over 3 months, in order to evaluate worm burden (in eggs per gram). The results showed significant differences between categories (primiparous < secondiparous) and between treatments (netobimin < phytotherapic) throughout the experimental period (both at $P < 0.05$). The allopathic product only showed efficacy at 60 days post treatment (fecal egg count reduction > 90%), while the phytotherapic one was not effective during the whole study period. Considering that GIN control should be based mainly on preventive strategies, further controlled studies and appropriate policies are required to assess and develop effective herbal remedies.

Keywords: gastrointestinal nematodes; anthelmintic; herbal remedies; dairy goats; organic farming

INTRODUCTION

Organic dairy goat farming has been increasing in Italy over the last several years. While the goat sector has been fluctuating over the years due to the high production cost, new data show an increasing trend (Table 1; SINAB, 2014), mainly related to the public's increasing concern about the sustainability of animal production. At present, Italy is the second largest producer of certified goats in Europe – after Greece (European Commission, 2013). The maintenance of a high standard of animal health and welfare is the primary aim of organic farming (Lund & Röklinsberg, 2001; Alrøe *et al.*, 2003; Lund, 2006; Valle *et al.*, 2007; Vaarst *et al.*, 2011); to achieve it, organic farming involves 'best practices' suitable for each species (such as grazing), reduced stocking rate, and reduced dependence on chemical inputs (Thamsborg & Roepstorff, 2003). The EU Regulation 889/2008 on organic production introduced a new approach in the health management of flocks: animals' health should be based on the prevention of disease, i.e. increasing their welfare and diseases resistance. Antibiotics and allopathic synthetic products are not allowed for prevention, while phytotherapy and homeopathy are recommended when needed.

	2008	2009	2010	2011	2012	2013	**Var.%'12-'13**
Number of certified goats	83,411	74,500	71,363	72,344	79,683	92,330	**15.9**

Table 1. Organic goats in Italy. Source: SINAB, 2014, modified.

In small ruminants raised organically, gastrointestinal nematodes (GIN) are considered to be one of the main hindrances to reaching high levels of health and welfare (Keatinge, 1996; Cabaret *et al*., 2002; Nardone *et al*., 2004; Hoste *et al*., 2010, 2014). The EU Regulation 889/2008 on organic production recommends grazing, which leads to a higher incidence of gastrointestinal parasitosis on organic breeding farms to conventional ones (Lund & Algers, 2003). Even if grazing has a positive effect on animals' welfare – as it allows them to express their natural behaviour – it represents a risk factor, due to the continuous exposition of goats to the infectious larvae of GIN (Waller, 2006; Rahmann & Seip, 2006; Di Cerbo *et al*., 2010).

Gastrointestinal parasites are a difficult to control, highly significant health problem in goats, and remain a serious threat to long term economic production (Cabaret *et al*., 2002, Hoste *et al*., 2005, Hoste *et al*., 2012, Mederos *et al*., 2012, Hoste *et al*., 2014). Moreover, they can cause poorer milk quality (Rinaldi *et al*., 2007), with a lower fat, protein and lactose content, such that milk is less suitable for processing.

In Italy, GIN control in small ruminants is based mainly on synthetic anthelmintics (Cringoli *et al*., 2009; Zanzani *et al*., 2014); furthermore treatments are often applied without any previous parasitological exam (Manfredi *et al*., 2010). While organic standards have introduced some restrictions on the use of chemotherapy, many organic farmers still rely on chemical anthelmintics for GIN control (Thamsborg *et al*., 2004, Rahmann & Seip, 2006; Cabaret *et al*., 2009). In fact, their therapeutic use is allowed by the EU Regulation. The chemical treatment of GIN implies some potential risks: immediate side effects on animals, drug resistance phenomena, possible drug residues in food animal products and, last but not least, environmental contamination (Roncoroni *et al*., 2008).

In organic goat farming, parasite control should be based on alternative management strategies, which aim to reduce anthelmintic use and to increase animal resistance to parasites (Molento, 2009). These interventions do not aim for a total elimination of parasites, but to maintain them at adequate levels for animal welfare (Silva *et al*., 2011).

Several no-chemical strategies for GIN control have been suggested: bioactive forage use, dietary supplementation, selective breeding for resistance, and the use of phytotherapic treatments. These represent interesting opportunities, and in fact, phytotherapy has been used – also with anthelmintic aims – for thousands of years. Notably, many of the active ingredients in current pharmaceutical drugs come from plants. Research into phytotherapy for the control of GIN in small ruminants has been extensively advocated in the last decade (Molento, 2009), and a scientific approach is necessary to ascertain the anthelmintic potential of plants and to evaluate whether they are affordable in terms of safety (Rates, 2011). While many studies are available on single plants (e.g., Iqbal *et al*., 2006; Tariq *et al*., 2009; Domingues *et al*., 2010), only a few have been carried out on phytotherapic anthelmintics commercially available (Burke et al., 2009). These herbal products – regulated in EU by Dir. 24/2004/EC and Reg. 726/2004/EC and its subsequent amendments – are often sold as complementary food, based on vegetable raw materials and labelled as dietary supplements, not as veterinary medicine. In addition they contain extracts from different plants, in unspecified proportions: this complexity may characterize their efficacy, but it makes their validation more difficult (Hoste *et al*., 2008). The aim of this study was to evaluate the *in vivo* anthelmintic efficacy of a phytotherapic anthelmintic compared to a conventional one in an organic dairy goat farm setting.

MATERIALS AND METHODS

Farm and animals

The experiment was performed at Azienda Agricola Cascina Bagaggera, a private organic dairy goat farm, in the Montevecchia and Curone Valley Regional Park (Lombardy, Italy).

The farm's main activity is the breeding of Alpine goats, and has been certified organic since 2009. The milk production (total in 2012: 23,820 L; mean per head: 385 L) is entirely transformed into dairy products: fresh and mature cheese and yogurt. At the time of this study, there were 77 lactating goats (2 groups of primiparous: 16 + 18; 1 group of secondiparous: 20; and 1 group of multiparous: 23), 48 young goats and 5 bucks. The farm encompasses 19 ha: 4 ha for grazing and 15 ha for forage production. Adult goats have access to pasture from 9:00 to 12:00 and young stock from 9:00 to 17:00. The pasture is divided into enclosed plots of land with electric fences, which are moved every 3 days. Each group grazes upon different plots.

Lactating goats are supplemented with mixed grass hay and alfalfa, which is mainly produced on the farm. At the milking parlor, animals are fed ≥ 800 g/goat/day of a whole organic concentrate (Bioforce Starch, 9.6% CP;

corn, barley, soybean cake, sodium bicarbonate, calcium carbonate, magnesium oxide; PROGEO, Reggio Emilia, Italy), with the amount depending on the level of milk production. This organic concentrate is mixed with yeast at a dose of 5g/goat/day.

Parasitological monitoring using a qualitative and quantitative fecal examination procedures (Soulsby, 1982) was carried out for 2 years (2011 and 2012), which served to confirm that the goats were naturally infected with mixed species of GIN prior to the start of this experiment.

Experimental design and anthelmintic treatment

Thirty lactating goats were divided in two treatment groups (n = 15/treatment), according to EPG means ± SD at T_0 that were compared using a two-tailed Student's *t*-test. Both groups were splitted by category: group C consisted of 8 primiparous and 7 secondiparous; Group P consisted of 8 primiparous and 7 secondiparous. Multiparous were excluded in order to avoid a variability factor due to the number of parturitions.

Group C = Conventional, received a 15 mL netobimin dose, which was repeated after 21 days.

Group P = Phytotherapic, was treated with a non-toxic herbal anthelmintic, that contained *Carduus marianus*, *Gentiana lutea*, *Urtica fissa*, *Mallotus* spp., *Dryopteris* spp., *Eucalyptus* spp., which did not require the withdrawal period. Goats received a 30 mL dose according to manufacturer recommendations, which was repeated after 21 days.

Both treatments were orally administered in the morning using a syringe.

The groups shared shelters and pasture for the whole experimental period, and they continued with the same semi-extensive grazing regimen, receiving mixed fodder, as well as concentrate supplements twice a day.

Procedures

Individual fecal samples were collected from the rectums on day 0, pre-treatment (T_0: may 2012), and then monthly, from June to August 2012. Faeces were examined with a copromicroscopic quantitative exam (McMaster technique - Gordon & Whitlock 1939, Whitlock 1948), in order to evaluate eggs per gram (EPG). A fecal egg count reduction (FECR) test was performed, following the recommendations of the World Association for the Advancement of Veterinary Parasitology (Wood *et al*., 1995). The arithmetic means of EPG were calculated for the four groups at T_0, T_1, T_2, T_3, and the FECR percentage was subsequently calculated using the following formula (Chapman, 1991; Kochapakdee *et al*., 1995), in which each group's mean EPG at T_0 serves as a control:

$$\text{FECR\%} = 100 \text{ X } [1 - (\text{EPG post treatment} / \text{EPG pre treatment})]$$

Results were interpreted following Kaplan's suggestions (2004):

- FECR > 90%=anthelmintic effective;
- 80% ≤ FECR ≤ 90% = anthelmintic of uncertain efficacy;
- FECR < 80% = anthelmintic ineffective.

A clinical monitoring was also performed monthly during the study period in order to detect clinical signs of infestation (e.g., anemia, diarrhea).

Statistical analysis

Variation in mean epg for each group was analyzed using repeated-measures analyses of variance (ANOVA). Data were statistically analyzed using SPSS software version 19.0, with category (primiparous *vs* secondiparous), treatment (conventional *vs* phytotherapic) and their interaction as fixed effects.

RESULTS

The results showed statistically significant differences for category (primiparous < secondiparous) (*P* < 0.05) (Figure 1) and treatment (netobimin < phytoterapic product) (*P* < 0.05) (Figure 2), over the whole study period. Notably, parasite infection was high in all of groups.

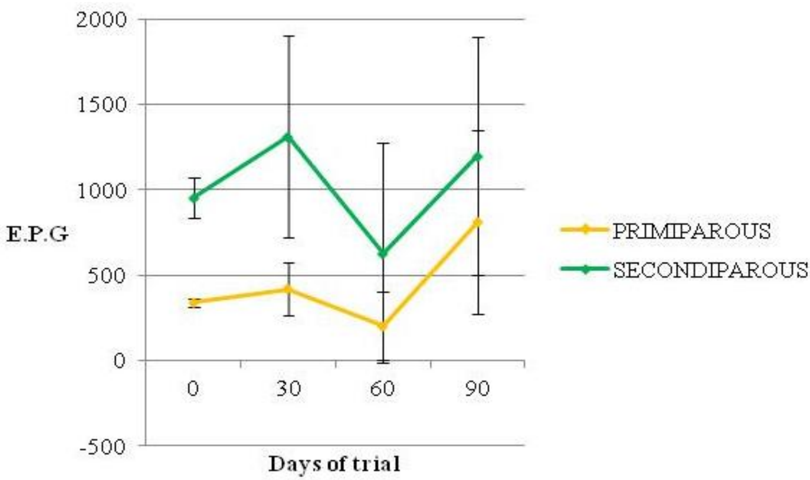

Figure 1. Mean Fecal Egg Count (EPG ± SD) in primiparous and secondiparous during the experimental period.

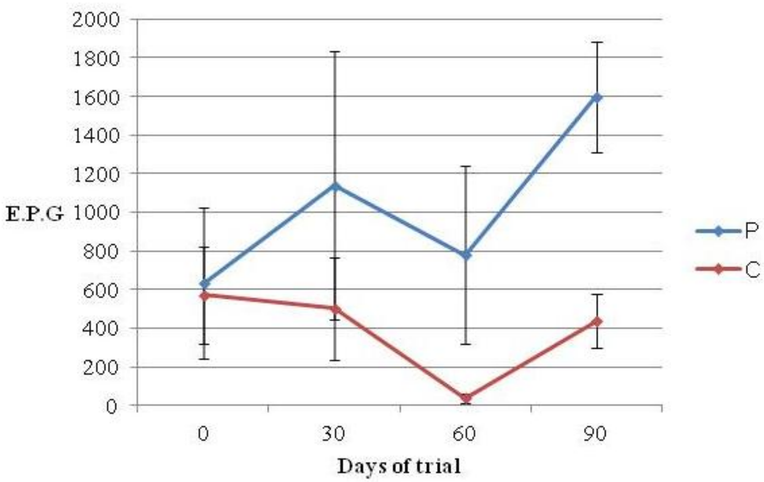

Figure 2. Mean fecal egg count (EPG ± SD) in the treatment groups (P = Phytotherapic; C = Conventional) during the experimental period.

The conventional product was only effective at 60 days post-treatment, showing a FECR of 94.83% and 93.49% in primiparous and secondiparous individuals, respectively. The phytoterapic treatment was not effective and no FECR was detected during the whole study period.

No clinical signs of infestation were recorded during the trial period.

DISCUSSION

Despite a high worm burden, no goat showed clinical signs of parasitic gastroenteritis, suggesting that these goat have an ability to cope with gastrointestinal nematodes (GIN). This notable result requires further investigation through controlled studies. Different levels of infection between primiparous and secondiparous are not surprising, even if a recent study showed that goats at first lactation can eliminate more parasite eggs than older goats (Manfredi *et al*., 2010). Farm management can definitively affect this outcome: on some farms, young goats are kept indoors, having no contact with the infectious larvae of GIN, and they are more likely due to be infected in adult life. On the farm in this study, young goats have access to pasture, so they have already been in contact with parasites.

In addition, the goats' immune response to GIN is peculiar: similar levels of infection have been reported in both young and adult animals; on the contrary, among sheep, adult animals are usually far less infected than young ones. In goats, there is scarce acquisition and expression of immune responses, with a trend of continuous re-infections linked to higher egg excretion during the whole grazing period (Hoste *et al*., 2010).

Both the anthelmintic products (conventional and phytoterapic) showed low efficacy for controlling GIN: the allopathic product showed a certain efficacy, but only at T_2 (FECR > 90%), while the phytotherapic product did not reach threshold values during the whole study period, confirming results from other studies (Ghitiori, 2004; Luginbuhl *et al*., 2006, Burke *et al*., 2009).

Although phytotherapic product used in this trial did not show suitable efficacy, a critical issue for the validation of phytotherapic anthelmintic is the standards used. In general, the anthelmintic activity of plants has been found to be lower than that reported for synthetic anthelmintics (Macedo *et al*., 2010). An acceptable limit of efficacy must be established, but it should be different from that applied for chemical products (Athasiandou *et al*., 2007), with the aim to evaluate whether the plant's anthelmintic activity is suitable to ensure animals welfare, health and productivity.

Similar responses across the whole group of goats treated with netobimin were observed, while important differences in individual reaction were noted in goats treated with the phytotherapic product. This variability is difficult to interpret considering the lack of information about the phytotherapic's mechanism of action. These botanical remedies are not required to meet any labelling standards: known active compounds are not quantitatively and qualitatively identified on the label (Wynn & Fougere, 2007); hence their effectiveness can only be assessed empirically.

On the other hand, the inadequate efficacy of the conventional product may be related to its mal-absorption in the hosts. The anthelmintic used was a pro-benzimidazole registered for use in goats and it was properly dosed, but this product is a so-called pro-drug and it has to be metabolized by the host to become pharmacologically active. As suggested by Zajac & Gibson (2000), splitting the total dose into equal doses given 12 hours apart might be more effective than a single dose, extending the anthelmintic's contact time. Lastly, anthelmintic resistance phenomena can be excluded, as the allopathic product selected had never been used before.

CONCLUSIONS

As this study was a field trial, some variables could not be controlled. Nevertheless, the obtained results do lead to some conclusions: first of all, gastrointestinal parasitosis control should be based mainly on integrated preventive strategies (e.g., grazing management, reducing stocking rate), and not only on the use of anthelmintic products. Competent pasture management may reduce the risk of GIN infections and hence the rate of pharmacological treatments, even if management costs and the lack of suitable areas make this practice difficult to accomplish at times. It has been ascertained that prolonged grazing of the same area makes new infections more likely because the ingestion of larvae is an almost continuous process. Also, a too-short rest period for the pasture can easily lead to new infections, as there is not sufficient time to devitalize larvae. Another facilitating element can be the lack of grazing alternation during seasons. Rotational grazing systems, organized following goats' nutritional needs, has shown some effectiveness in controlling gastrointestinal parasites, even when practiced without considering the epidemiology of the infectious larvae (Garippa, 2006).

In the farm considered in this study, GIN infections have never been previously controlled, while rotational grazing was implemented for the first time during the last season; in any case, parasite control by grazing management is difficult to achieve on this farm due to the limited extension of pasture area in relation to the stocking rate. When grazing management fails, phytotherapy could represent a valid therapeutic option, especially on organic farms which aim to reduce allopathic medicine treatments. Appropriate policies on quality, safety and effectiveness of herbal commercial products should be developed, and further controlled studies are needed to better assess the anthelmintic potential of herbal remedies. Organic goat farmers are demanding recommendations regarding the limitations and the opportunities of phytotherapy for GIN control.

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CHAPTER 2.2

IN VIVO EFFICACY OF PUMPKIN SEEDS (*Cucurbita maxima* Duch) TO CONTROL GASTROINTESTINAL NEMATODES IN ORGANIC DAIRY GOATS

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ABSTRACT

Ethnopharmacology relevance: Pumpkin seeds (*Cucurbita* spp. - Cucurbitaceae) have been reported in ethnoveterinary literature and they are extensively used in traditional medicine for their anthelmintic activity. This field study was performed to determine the validity of such claims.

Aim of the study: The aim of this study was to evaluate the *in vivo* anthelmintic effect of feeding pumpkins seeds in organic dairy goats, naturally infected with gastrointestinal nematodes.

Materials and methods: Twenty-four lactating Alpine goats were used for the *in vivo* 2-month trial, carried out in a commercial organic dairy goat farm. The animals were divided into two homogeneous groups of twelve goats each, and assigned to different treatments: Group 1 - goats treated with ground pumpkin seeds mixed into feed at dose of 130 g/head for three consecutive days; afterward the goats were given 30 g/head of linseeds meal mixed into feed too, for following three days. Group 2 – control, animals did not receive any anthelmintic treatment.

Individual fecal samples were collected directly from the rectum on day 0 pre-treatment and every 15 days post-treatment, to perform fecal egg count (FEC) and fecal egg count reduction (FECR) test. Clinical monitoring was also performed to detect signs of clinical infestation.

Results: Pumpkin seeds were not effective in reducing FEC in the treated goat, eggs per gram (EPG) increased in both groups throughout the study period. No clinical signs of infestation were recorded.

Conclusions: These preliminary results showed that the pumpkin seeds used in the treatment of goats had no anthelmintic activity in terms of

reducing FEC. Controlled experimental studies are required to better assess the anthelmintic potential of *Cucurbita maxima* in goats. Furthermore, a more comprehensive framework is needed to test the anthelmintic efficacy of this medicinal plant, rather than relying only on fecal egg counts.

Keywords: pumpkin seed; *Cucurbita maxima*; goat; organic farming; gastrointestinal nematodes; anthelmintic

INTRODUCTION

Gastrointestinal nematodes (GIN) represent the most significant threat to the health and welfare of goats raised on pasture as seen in organic farming (Rahmann & Seip, 2006; Hoste *et al*., 2014).

The presence of GIN in parasitized host results in a health distress causing chronic infections that determine long-term production losses (Cabaret *et al*., 2002; Hoste *et al*., 2005; Waller, 2006; Mederos *et al*., 2012; Hoste *et al*., 2014).

Allopathic anthelmintics (AH) remain an important tool for controlling GIN in organic farms (Thambsborg *et al*., 2004; Cabaret *et al*., 2009) and the current EU Regulation (Reg. 889/2008/EC) allows the therapeutic use of AH without any restriction, although organic principles aim to reduce the reliance on allopathic medicinal products.

The massive use of allopathic AH implies severe consequences on the environment (Beynon, 2012; Horvat *et al*., 2012) and contributes to the increasing trend of anthelmintic resistance in goat farms worldwide (Hoste & Torres-Acosta, 2011; Roeber *et al*., 2013), including Italy (Zanzani *et al*., 2014). The use of these products has also raised public concerns about food safety and animal welfare issues, which are particularly relevant in the organic farming framework.

In this context, research needs to provide alternative treatments to control GIN and medicinal plants traditionally used in ethnoveterinary medicine represent a promising option to synthetic AH (Burke *et al*., 2009; Molento, 2009, Molento *et al*., 2011).

Plant remedies are the principal resource to treat livestock against GIN in a large part of the world (Akhtar *et al*., 2000; Iqbal *et al*., 2003; Githiori *et al*., 2006; Grosso *et al*., 2010; Babar *et al*., 2011; Okombe Embeya *et al*., 2014) due to their affordability combined with local availability (Athanasiadou *et al*., 2007; Torres-Acosta & Hoste, 2008). Also in Western countries, the use of traditional antiparasitic medicinal plants is gathering significance (Ortiz Suarez, 2010; Benítez *et al*., 2012), due to the rising interest towards "natural medicine" (Viegi *et al*., 2003) and to the increasing numbers of organic farms (EU, 2013; SINAB, 2014). The Italian folk veterinary phytotherapy was emphasized by Viegi *et al* (2003), who created the first national databank of ethnoveterinary knowledge; this exhaustive review reports the use of several medicinal plants including those against gastrointestinal disorders.

Pumpkin seeds (*Cucurbita* spp. - Cucurbitaceae) have been reported in ethnoveterinary literature and they are extensively used in traditional medicine for the anthelmintic activity. Traditionally the seeds are used as a vermicide both in humans (Capasso & Grandolini, 1996; Guarrera, 1999; Uncini Manganelli *et al*., 2002; Kulkarni *et al*., 2012) and in farm animals (Balazar & McCorkle, 1989; Duval 1994; Giove, 1996; Waller *et al*., 2001; Altung & Captan 2002; Viegi *et al*., 2003; Mederos *et al*., 2012). Regarding small ruminants, Altung & Captan (2002) reported the ethnoveterinary efficacy of pumpkin seeds against *Haemonchus contortus*, a blood-sucking nematode localized in abomasum that causes anemia, reduces animal health, welfare and productivity. Duval (1994) and Giove (1996) suggest an antiparasitic treatment for sheep and goats consists of 60 gr/animal in three doses administered with mineral oil to expel worms.

The popular medicinal use of pumpkin seeds promoted early researches on their phytochemistry. A rare amino acid, named cucurbitine (3-amino-3-carboxypyrrolidine), was characterized by Chinese researchers in *Cucurbita* spp. (Sun *et al*., 1961). According to several authors, cucurbitine is the active constituent responsible for the anthelmintic effects of the seeds (Colorado-Iris *et al*., 1950; Fang, 1961; Plotnikov *et al*., 1972; Bombardelli & Morazzoni, 1997; the properties of *Cucurbita pepo* are reviewed by EMA, 2011). The chemical structure is similar to the kainik acid (Bruneton, 1999), which is a nematicidal compound with a neurodegenerative action on nematodes by substituting for glutamate. Cucurbitine, as well as kainik acid, seems to paralyze worms and causes a worm-expelling effect by detaching the parasite from the intestinal wall of the host (Capasso & Grandolini, 1996).

These findings confirm the traditional use of pumpkin seeds against GIN even if the scientific *in vivo* and *in vitro* validation through controlled experiment should be promoted. Scientific evidence to support the antiparasitic activity in small ruminant is lacking and few studies are reported in literature about the use of pumpkin seeds in goats.

The aim of this study was to evaluate the *in vivo* anthelmintic effect of pumpkins seeds in organic dairy goats, naturally infected with GIN.

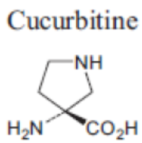

Figure 1. Structure of cucurbitine (WHO, 2009; modified).

MATERIALS AND METHODS

Farm, animals and treatment

The study was carried out at Azienda Agricola Cascina Bagaggera, a commercial organic dairy goat farm, in Montevecchia and Curone Valley's regional park (Rovagnate, Lombardy, Italy).

The farm's main activity is the breeding of Alpine goats, reared in a semi-extensive system. Goats have access to pasture in the morning and are kept indoor during the rest of the day. In the pen, goats are supplemented with mixed grass hay and alfalfa twice a day. At milking parlor animals are fed with a whole organic concentrate (Bioforce Starch, 9.6% CP; corn, barley, soybean cake, sodium bicarbonate, calcium carbonate, magnesium oxide; PROGEO, Reggio Emilia, Italy), at least 600 g/goat/day depending on level of milk production, mixed with yeast at dose of 5g/goat/day.

Twenty-four lactating Alpine goats (multiparous between the third and tenth lactation) were used for an *in vivo* 2-month trial from April to June 2014.

Before the start of experiment, the goats were confirmed to be naturally infected with mixed species of GIN by a previous parasitological monitoring, carried out for 2 years (2012 and 2013), using the qualitative and quantitative fecal examination procedures (Soulsby, 1982).

The animals were divided into two homogeneous groups of twelve goats each according to the results of multivariate analysis of variance (treatments = eggs per gram (EPG) pre-treatment, milk production - 2013 data -, lactation number) and randomly assigned to different treatments:

Group 1 - animals treated with ground pumpkin seeds mixed into feed at dose of 130 g/head (2.6 g/kg BW) for three consecutive days; afterward the goats were given 30 g/head of linseeds meal mixed with pelleted ration, for following three days, as a natural laxative. The treatment was administered in the milking parlor at the first milking time.

Group 2 – control, animals did not receive any AH treatment.

The seeds were purchased and were ground in the farm's mill. The experimental dose were adapted according to the local ethnoveterinary practices (Pisseri, 2013).

During all the study goats were housed in two adjacent pens and grazed together; the same semi-extensive management was applied.

Procedures and statistical analysis

Individual fecal samples were collected directly from the rectum on day 0 pre-treatment and every 15 days post-treatment, to perform fecal egg counts (FEC). According to Vercruysse *et al*. (2001), this parasitological exam is the recommended method to evaluate the effectiveness in the field studies. EPG was determined by the McMaster technique according to Gordon & Whitlock (1939).

A clinical monitoring was also performed during all the study period to detect clinical signs of infestation (e.g., anemia, diarrhea).

Variation in mean EPG, for each group over time was analyzed using General Linear Model by repeated measures analysis of variance. The data were statistically analyzed using SPSS software, version 19.0. In all the analyses, the confidence level was held at 95% and $p < 0.05$ was set for significance. Efficacy test using fecal egg count reduction (FECR) was determined according to the method described by Coles *et al*. (1992).

FECR was calculated using the following formula:

$$FECR\ (\%) = 100 \times (1 - [T2/C2])$$

where T2 = arithmetic means of EPG post treatment on treated group; C2 = arithmetic means of EPG post treatment on control group.

RESULTS

No significant differences in EPG were found in the group treated with pumpkin seeds compared to control throughout the period (Figure 2).

Pumpkin seeds were not effective in reducing FEC in the treated goat, EPG continued to increase in both groups and FECR was not detected.

No clinical signs of infestation were recorded.

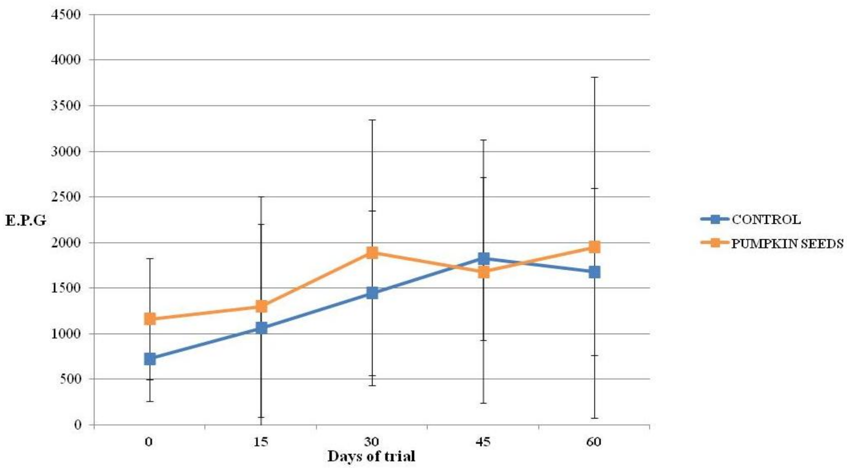

Figure 2. Mean fecal egg count (EPG ± SD) of goats fed with and without pumpkin seeds during the experimental period.

DISCUSSION

The lack of efficacy reported in this study is in contrast to the data described in literature concerning ethnoveterinary use of pumpkin seeds against GIN in goats (Duval, 1994; Giove, 1996; Altung & Kaptan, 2002; Viegi *et al*., 2003). Scarce experimental studies have been performed on the antiparasitic activity of pumpkin seeds in small ruminant, both in sheep and goats. A recent *in vitro* experiment (Marie-Magdeleine *et al*., 2009) confirmed the anthelmintic properties of pumpkin seeds extracts against *H. contortus*. However, it is important to emphasize that *in vitro* conditions and concentrations used are not always comparable to those *in vivo*, and thus often the results can differ in the two assays (Athanasiadou *et al*., 2007). It is clear that *in vivo* studies are more relevant to common farming practices and thus could be considered more reliable than *in vitro* studies (Githiori *et al*., 2006).

Despite the lacking of *in vivo* studies in small ruminant, discussion will focus only on goats, as the differences between sheep and goats concerning GIN infections (e.g., metabolic and detoxification capacity, immune response) are well documented and the results from one species are not necessarily valid for the other (Hoste *et al*., 2010).

The two in vivo studies on the anthelmintic property of pumpkin seeds in goats have presented contradictory results, confirming (Almeida *et al*., 2007) or denying (Nogueria *et al*., 2006,) their effectiveness.

The unsatisfactory activity of pumpkin seeds in reducing FEC reported in this study might be related to several factors including the method of administration and the dose used. The grinded pumpkin seeds might not have been the most effective treatment method because the goats failed to consume all the seeds fed and sorted quite a bit. As an alternative, the efficacy of pumpkin seeds should be tested in different forms (drench and oil) that could allow more control on posology.

In addition, it is crucial to highlight the importance of the dose used. The inefficacy observed in the current study of administering pumpkin seeds in doses of 2.6 g/kg BW could be due also to the destruction of the active substances by the ruminal flora and ruminal pH and this may have contributed to the inefficacy of pumpkin seed. This might probably be improved by increasing the dose or by repeated dosing for more than 3 days. The common use of pumpkin seeds as food proves not to be harmful so their administration can be considered safe (EMA, 2011).

Furthermore, a longer treatment period could increase the seed/parasite contact time and presumably improve elimination of the parasites.

Considering the dose, Nogueria *et al*. (2006) examined the efficacy of *Cucurbita* spp. extract in a single dose of 3 g/kg orally administered to an experimental group of six goats. The pumpkin seed drench was prepared from a traditional method for the treatment of worms by adding 70 gr of ground pumpkin seeds in 500 ml liters of water. The effectiveness of the herbal preparation was evaluated based on FEC on the day of treatment and seven days after. In spite of the reduction of EPG (41%) achieved by pumpkin seeds, the authors concluded that the dose used were not effective for the control of GIN in goats.

A critical point in the study conducted by Nogueria *et al*. (2006), was the sample size: although the number of animals recommended by the

W.A.A.V.P (World Association for the Advancement of Veterinary Parasitology) guidelines for testing anthelmintic drug efficacy is at least six per group (Wood *et al*., 1995; Vercruysse *et al*., 2001;), to evaluate plant efficacy this number should probably be higher due to smaller differences between treated and controls, and usually higher levels of variance (Githiori *et al*., 2006). Moreover, the parasitological analysis was performed only 7 days after the treatment. Better results should have probably been obtained with a longer monitoring period.

However, the method of administration of pumpkin seeds and the dose are definitely not the only factors that may affect their anthelmintic efficacy.

As reported by Almeida *et al*. (2007), using a lower dose than the one administered in the present study (1.9 g/ kg for crumb of the pumpkin seeds, for 3 consecutive days), after 30 days of treatment the achieved FEC reduction in the treated goat (n=10) was to the therapeutically required level.

It is important to underline that any field study is subject to various limitations and some variables could not be controlled. Overgrazing was one of the limitations: due to the small size of pasture (four ha), grazing management strategies (e.g., pasture rotations) cannot be applied efficiently. It is well recognized that high stocking rate leads to increasing levels of parasitism in grazing livestock (Garippa, 2006; Mahieu & Aumont, 2009). Immunity in goats is reduced compared to other species so if the environmental load is high the animals are constantly being infected.

In addition, the study period coincided with the commonly known “spring rise”, a periparturient relaxation of goats’ immunity (Michel, 1976; Taylor *et al*., 1990; Barger, 1993). This phenomenon leads to a higher worm burden in the gastrointestinal tract and a higher number of nematode eggs in the faeces of periparturient goats. As a result, goats contaminate the pasture with GIN eggs, which turn to infective larvae in the mild conditions of the spring, increasing the infection risk and causing re-infections (Torres-Acosta *et al*., 2012). No clean pasture was available during the study, therefore the control of parasitic load was more difficult.

Another problem could be attributed to the characteristic of raw material used. Differences in the chemical composition of pumpkin seeds between individual plants vary considerably depending on several factors (e.g., growth, fertilization and the harvest time - Croom, 1983; Al-Khalifa, 1996; Glew *et al*., 2006 -). Before testing the *in vivo* anthelmintic activity of pumpkin seeds macroscopic and microscopic examination and high performance liquid chromatography should be the common testing procedures to determine the concentration of active principle. Cucurbitine content is quite variable (EMA, 2011; Kulkarni *et al*., 2012) and qualitative test should be performed to confirm its concentration in seeds. None of the *in vivo* studies reported in literature performed a previous phytochemical characterization of the pumpkin seeds before their use. Although the high cost of these analyses is the main limitation in field studies, it is essential that phytochemical studies could be performed to provide suggestions to scientific community.

Despite the high level of infestation, none goat showed clinical signs of infection (e.g. diarrhea, bottle jaw, anemia) therefore was not dewormed. This finding suggests a high tolerance of the goats with respect to parasites that should be investigated further. The access to pasture might promoted positive experience in goats, resulting in a stress reduction that enhances the immune function.

It seem essential to assess the anthelmintic potential of pumpkin seeds through scientific validation by standardized methods (Ketzis *et al*., 2006; Githiori *et al*., 2006; Athanasiadou *et al*., 2007) and taking into account not only the FEC (Keatinge *et al*., 2002). A holistic approach to assess the overall effects of medicinal plants on the welfare of parasitized animals should be considered. For instance, monitoring goats' behaviours or performance might be a relevant tool to evaluate whether medicinal plants improve the resistance or resilience of the parasitized goats (Athanasiadou *et al*., 2007).

CONCLUSIONS

In organic goat farming, a sustainable GIN control should rely on preventive measure and breeding strategies (e.g., high-quality feeding, grazing management, low stocking rate), which aim to reduce the use of synthetic AH.

Ethnoveterinary represent an option, being a valuable resource of medicinal plant traditionally used to treat goats against GIN. Pumpkin seeds are claimed to have anthelmintic activity and are commonly used in Italian farms. In this field study, the preliminary results showed that the pumpkin seeds used in the treatment of goats had no anthelmintic activity in terms of reducing FEC.

Controlled experimental studies using pumpkin seeds of proved quality or other specific pumpkin extracts, at different dose levels, are required to better assess the anthelmintic potential of *Cucurbita maxima* in goats. Furthermore, a more comprehensive framework is needed to test the anthelmintic efficacy of medicinal plants, rather than relying only on fecal egg counts.

Further investigations should be pursued to better understand possibilities and limitations of phytotherapy for GIN control in organic dairy goat farms.

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CHAPTER 3

QUALITATIVE BEHAVIOUR ASSESSMENT AS A TOOL TO ASSESS POSITIVE EXPERIENCES

CHAPTER 3.1

QUALITATIVE BEHAVIOURAL ASSESSMENT OF INTENSIVE AND EXTENSIVE DAIRY GOAT FARMS

INTRODUCTION

The possibility to assess positive experiences, and thus the "quality of life" is very important in organic goat farms. Positive experiences are suggested to be a core component of animal welfare (Boissy *et al*., 2007; Yeates & Main, 2008) and some behaviours are useful welfare indicators (e.g., play behaviour) but not feasible on extensive farms (Battini *et al*., 2014).

Furthermore, these welfare indicators are tested as quantitative measures and tend not to consider an animal as an integrated being. Thus they not provide, for themselves, a correct view of the animal's "quality of life"(Wiseman-Orr *et al*., 2006).

Holistic methods, which include also qualitative measures have been proposed as a way to characterize global expressive affects in animals, providing the most information possible on an animal's welfare experience (Wemelsfelder *et al*., 2000; Wemelsfelder & Farish, 2004; Wemelsfelder, 2007; Meagher, 2009).

Qualitative Behaviour Assessment (QBA) is one of these methods. QBA is a whole-animal approach, integrating perceived details of animals' expressive demeanour or body language (Wemelsfelder, 2007). To this aim, human observers have to see more than just "behaviour": e.g., not just "standing immobile", but "how" the animal is standing immobile; this gives us information about the animal's underlying emotional state. In sum, this method relies on the observers' ability to perceived animal behaviour expression, using descriptors such as "tense", "anxious", or "relaxed" (Wemelsfelder *et al*., 2000; Wemelsfelder *et al*., 2001; Wemelsfelder, 2007).

As QBA is a qualitative characterization of animal expression, many behavioural scientists have argued that it is an anthropomorphic evaluation of questionable validity (Rousing & Wemelsfelder, 2006, Wemelsfelder, 2007). For this reason, several researchers investigated the validity, reliability and feasibility of this approach. As for all the indicators to be included in a protocol for on-farm assessment of animal welfare should be valid (meaningful about animal welfare), reliable (reflecting the tendency to give the equivalent results on repeated measurements) and feasible (concerning time and financial requests) (Napolitano *et al*., 2009).

The first studies on QBA were carried out by Wemelsfelder and colleagues (2000, 2001), whom developed the method. The observations were performed on pigs and the terms used for scoring animal's behavioural expressions were developed by a procedure called Free-Choice Profiling (FCP), in which each observer generates their own descriptors (Wemelsfelder *et al*., 2000; Wemelsfelder & Lawrence, 2001; Wemelsfelder *et al*., 2001). This methodology has the benefit to prevent observer's bias due to a pre-fixed descriptors list, but it is not very feasible in practice, so later on the use of a pre-fixed list of descriptors was generally preferred for on-farm assessment (Wemelsfelder *et al*., 2009).

The results of the first studies on QBA showed significant inter-observer agreement in their evaluation of animals' behavioural expression (Wemelsfelder *et al*., 2000, 2001).

The subsequent studies reported good results and validated QBA as a welfare indicator in many production and companion animals (see the following chapter). In these studies observations were video-based showing a wide range of animal's behaviours performed by individuals or group of animals in different experimental or on-farm conditions (e.g., live transport, home-pen or paddock, pre-slaughter, human-animal interaction) (Andreasen *et al*., 2013).

Given these promising results, in recent years QBA have been included and used in the Welfare Quality® protocol for cattle, poultry and pig as the only measure linked to the criterion "positive emotional state" (Wemelsfelder & Millard, 2009).

About goats, only a published paper reported a first portended attempt to apply QBA in intensive goats farms as yet (Muri *et al*., 2013).

In organic goats farms, where animals are reared in extensive conditions, breeders, veterinarians and all people involved in farm management, are demanding a practical tool to assess the goat's well-being, in order to easy-detect any signs of stress, pain or illness, and to enable focused intervention. In such conditions, QBA could offer several advantages: it is a non-invasive method, it does not require the restraint of animals minimizing the stress reaction, it can be applied to the whole herd, and it is not labour-intensive. Moreover, QBA, being a holistic approach, seems to be the most appropriate method to assess the "overall" welfare of organic goats.

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CHAPTER 3.1

QUALITATIVE BEHAVIOURAL ASSESSMENT OF INTENSIVE AND EXTENSIVE DAIRY GOAT FARMS*˟

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˟ *This study was performed in extensive farms managed according to organic principles with or without formal certification.*

ABSTRACT

Qualitative Behaviour Assessment (QBA) is a whole-animal approach, integrating perceived details of animals' expressive demeanour, using terms such as tense, anxious, or relaxed. To evaluate the validity and repeatability of QBA for dairy goats, two observers assessed 16 goat farms at the same time, using a list of 16 QBA terms based on literature study and discussion with an experienced focus group. There were 8 'housed' (H) farms, where animals were observed in free stall pens with permanent straw litter, and 8 'pasture' (P) farms, where animals were observed in open pasture ranges. One H farm was removed from analysis due to procedural error.

QBA scores generated by observers for the 15 farms were analysed together using Principal Component Analysis (correlation matrix, no rotation). Observer agreement for farm scores on PCA Components (PCs) and on separate QBA terms was investigated using Pearson and Spearman correlations respectively. The effects of housing system and observer on PC scores were analysed using analysis of variance (treatments = observer, housing system and their interaction; block = farm).

PCA distinguished three meaningful dimensions of goat expression: PC1 (29%) 'content/calm-frustrated/aggressive'; PC2 (20%) 'curious/attentive-calm/bored; PC3 (12%) 'sociable/playful-alert/agitated'. Farm scores generated by the two observers on the three PCs were significantly correlated (PC1: r = 0.75, P = 0.001; PC2: r = 0.67, P = 0.006; PC3: r = 0.69, P = 0.004). Observers' farm scores on separate QBA terms were significantly correlated for 7 out of 16 terms (P < 0.05), and approached significant correlation for an additional 2 terms (P < 0.1), indicating an integrated PCA approach to QBA to be more robust.

There were significant effects of housing system on PC1 (*P* = 0.05) and PC2 (*P* = 0.02), indicating goats on P farms to be more 'content/calm', and more 'curious/attentive', than goats on H farms. There was a significant observer effect on PC2 (*P* = 0.04), and a significant observer by housing interaction on PC3 (*P* = 0.009).

These results show good inter-observer reliability across three dimensions of goat demeanour. However observers differed in their quantification of several QBA terms, indicating the need for further training and refinement of the descriptor list. QBA found the goats' demeanour on H and P farms to differ along two dimensions, confirming that access to pasture may have a positive effect on goats' emotional state. In sum, these results suggest that, given further refinement, QBA could make a valuable contribution to goat welfare assessment protocols.

Keywords: Qualitative Behaviour Assessment; dairy goat; inter-observer reliability; extensive farms; intensive farms; animal welfare

INTRODUCTION

During the last decades, the assessment of animal welfare at farm level has received increasing attention, in response to consumer demand for assurance schemes of high quality animal products, including aspects regarding animal welfare.

Most of the indicators developed for welfare monitoring focus on negative aspects. However, the inclusion of positive indicators may play a key role in the communication of animal welfare to the stakeholders, and therefore deserves further attention. In a recent review on animal-based welfare indicators for dairy goats, Battini *et al*. (2014) identified Qualitative Behaviour Assessment (QBA) as the most promising approach to evaluate positive emotional state in this species.

Qualitative assessment methods that aim to address positive welfare states of animals are presently gaining interest (FAWC, 2009), as they aim at determining the actual welfare of animals, including both physical and mental state, in agreement with recent EFSA guidelines (EFSA, 2012).

Qualitative Behaviour Assessment (QBA) is a whole-animal approach, integrating perceived details of animals' expressive demeanour or body language, and describing them using descriptors such as 'tense', 'content', or 'relaxed' (Wemelsfelder *et al*., 2000; Wemelsfelder *et al*., 2001; Wemelsfelder, 2007). Descriptors have an emotional implication and can give additional information directly relevant to animal welfare to those achieved by quantitative indicators (Wemelsfelder, 1997; Wemelsfelder *et al*., 2001; Rutherford *et al*., 2012).

To be included in on-farm welfare evaluation protocols, indicators should be valid, repeatable and feasible (Martin & Bateson, 2007; Knierim & Winckler, 2009). In the last decade, the validity and reliability of QBA have been thoroughly investigated. Its validity was confirmed by meaningful correlations with quantitative behavioural measures (e.g., Minero *et al*., 2009, Rutherford *et al*., 2012) and with physiological measurements of stress (Stockmann *et al*., 2011, 2012; Wickmann *et al*., 2012). In addition, two recent studies demonstrated that QBA in pig and veal calves was not distorted by environmental rearing conditions (Wemelsfelder *et al*., 2009a; Brscic *et al*., 2009). Several studies on many species, such as pigs (Wemelsfelder *et al*., 2000, 2001, 2009a, 2012; Temple *et al*., 2011; Rutherford *et al*., 2012), poultry (Wemelsfelder *et al*., 2009b), horses (Napolitano *et al*., 2008; Minero *et al*., 2009), dogs (Walker *et al*., 2010), cattle (Rousing & Wemelsfelder, 2006; Brscic *et al*., 2009; Stockman *et al*., 2011, 2012; Bokkers *et al*., 2012; Andreasen *et al*., 2013), buffalos (Napolitano *et al*., 2012) and sheep (Wickham *et al*., 2012; Phythian *et al*., 2013) reported good levels of inter- and intra-observer reliability. Different observers' backgrounds, experience and view of the species under study did not affect QBA reliability (Wemelsfelder *et al*., 2012).

As to on-farm feasibility, QBA can offers several advantages: it is non invasive, it does not require the restraint of animals, it can be applied to the whole herd, and it is not labour-intensive.

Goats are very expressive animals and therefore QBA seems very well suited for evaluating their welfare. However, to date only one published paper reported a first attempt to apply a simplified QBA on this species

(Muri *et al*., 2013). In this study, QBA was included in a comprehensive welfare assessment protocol for intensively farmed dairy goats. It was applied at group level and consisted of only 5 descriptors (‘resting’, ‘aggressive’, ‘inquisitive/interested’, ‘fearful’ and ‘calm and indifferent’) derived from those used in the Welfare Quality® Protocol for dairy cows. Significant associations were found between QBA and indicators of health and stockmanship, suggesting the validity of the method also for goats. Inter- observer reliability was not evaluated in this study.

The present investigation aims at confirming the validity and repeatability of QBA for dairy goats. The method was tested by two observers in intensive and extensive goats farms in order to verify whether it could discriminate between these two farming systems. This was based on the assumption that, in extensive production systems, goats are allowed to express their natural behaviour, whereas in intensive ones the animals cannot perform part of their behavioural repertoire (Casamassima *et al*., 2001; Sevi *et al*., 2009; Dwyer, 2009; Braghieri *et al*., 2011). According to the natural living approach of animal welfare, it is assumed that pasture can represent an important benefit for animal well-being (Wemesfelder & Birke, 1997; Regula *et al*., 2004; Lund, 2006; Caroporese 2008; Von Keyserlingk *et al*., 2009; Burow *et al*., 2013); therefore, we may expect that grazing goats will show more positive behaviour expressions than intensively managed ones and that both observers will be consistent in evaluating the farms using QBA.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

- **QBA Fixed List Descriptors**

The QBA descriptors used for scoring goats in this study were generated by a specific literature review followed by a focus group carried out in April 2013. A panel of 10 Italian goat experts met at the University of Milan for a discussion on QBA descriptors. The experts were chosen among goat farmers, vets, technicians and researchers. The aim of this focus group was to select descriptors of goat behavioural expressions and state, to be used for QBA assessment on-farm. During the meeting, the QBA method was explained and discussed and a preliminary list of 32 descriptors selected by literature review was presented.

After the discussion, some descriptors were removed, because they were considered too prone to anthropomorphism (e.g., ‘inquisitive’, ‘anxious’, ‘angry’), or because they were too generic (e.g., active), or because they were very similar to other terms already present in the list (e.g., ‘exploratory-curious’, ‘agitated-nervous’, ‘calm-relaxed’); other descriptors were replaced by new terms that best described the behaviour of the goat (e.g., ‘interested’ was replaced with ‘attentive’); others terms were suggested ex-novo by the experts (e.g., ‘bored’). After discussion, an agreement on the use and definition of 15 descriptors was reached.

Later on, the discussion was enlarged at international level, involving 9 goat experts engaged in the European Animal Welfare Indicators (AWIN) project and a new attribute was added (‘suffering’). Our final list of descriptors included 16 fixed terms: aggressive, agitated, alert, bored, apathetic, attentive, content, curious, frustrated, playful, irritated, fearful, sociable, suffering, calm, lively. Each descriptor was accurately described, in order to facilitate its interpretation (Table 1).

Descriptor		***Definition***
AGGRESSIVE	An aggressive goat…	*…bites other goats (especially the ears), voluntarily attacks or threatens other goats with the intention of hurting or disturbing them, butts the belly or the head of other goats. She is intentionally noxious to other goats. The aggressive behaviour can be related to dominance, fear or resource protection*
AGITATED	An agitated goat is…	*…restless, not at ease, highly reactive, she can move her ears, vocalize or nervously move around*
ALERT	An alert goat is…	*…ready to react to a potential danger or to something that frightens her. She can emit acoustic or visual alarm signals (e.g. vocalizations, snorts, stamping, ears in upright position, stiff body)*
APATHETIC	An apathetic goat…	*…shows little or no movements or reactions to stimuli and often remains isolated from the group, depressed*
ATTENTIVE	An attentive goat is…	*…concentrated on something that is happening or is going to happen, waiting for an event, she looks around but often concentrates her gaze towards a specific direction or signal*
BORED	A bored goat is…	*…wearied, dull, she is uninterested in the surrounding environment, feeling tired of something that has continued for too long; lack in stimulation; she may be looking for something to do*
CALM	A calm goat is…	*…quiet, relaxed and she feels at ease*
CONTENT	A content goat is…	*…appeased, gratified, happy, comfortable, at ease, satisfied about the situation, positively engaged in something*
CURIOUS	A curious goat is…	*…explorative, intrigued by something, attracted by the surrounding environment and by novelties (e.g. people, goats in oestrus, objects), engaged in exploratory behaviour*
FEARFUL	A fearful goat is…	*… a scared and shy animal. She may look for shelter or for a way out and crouches down or may tend to hide in the middle of the group. A whole group may run around*

FRUSTRATED	A frustrated goat is…	*…annoyed and impatient because prevented from achieving something (e.g. queuing at feeding rack or water place, passive behavior)*
IRRITATED	An irritated goat is…	*…bothered or annoyed by something (e.g. flies, pruritus, noise) that can disturb or upset or trouble or exasperate her*
LIVELY	A lively goat is…	*…busy in different activities. She is active, animated, full of life and energy*
PLAYFUL	A playful goat…	*…jumps, performs ritualized non aggressive fights (sparrings), plays and makes noise with objects, climbs or tries to climb. They stimulate each other and laterally run together*
SOCIABLE	A sociable goat is…	*…friendly with other goats. She has affiliative (e.g. grooming, sniffing, resting in pairs) and playful contacts with other goats*
SUFFERING	A suffering goat is…	*…feeling pain, often with contracted muscles, possibly in antalgic postures*

Table 1. List and definition of the 16 QBA descriptors.

- **Farms and animals**

We performed QBA in 16 farms: 8 intensive ("housed", H) and 8 extensive ("pasture", P) dairy goat farms. Only farms with more than 30 female adult goats were included. Before farm visits, the farmers were contacted and received basic information about the project.

Only female adult animals, both lactating and dry, were assessed. All farms were visited during May 2013, after the birth period and when all animals reared in extensive system had access to pasture.

- **Observers**

Two independent observers conducted the QBA on farm.

Both observers were unfamiliar with the 16 selected farms, so that their judgment could not be biased by any preconception, and they had previous experience with goats and with welfare assessment protocols. Observer A (Obs-A) was a female vet, PhD student, with work experience from goat practice, and Observer B (Obs-B) was a female post-doc researcher, specialized in farm animal welfare and familiar with dairy goats. None of them had previous experience with QBA.

Before data collection on-farm, the two observers followed a specific training led by Dr. Francoise Wemelsfelder. They received an explanation of how to apply QBA to groups of goats and they were instructed on how to use the Visual Analogue Scale (VAS). After a theoretical training, they scored several video clips showing groups of goats, using the 16 QBA descriptors. After watching videos, the assessors were allowed to compare their results and discussed about their evaluation, until when they reached an agreement on the interpretation of each descriptor.

- **Data collection**

On farm, the assessment was performed on the whole herd by direct observations carried out during an activity period of goats. In H farms, goats were observed in free stall pens with permanent straw litter at least 60 min after the feed distribution or 90 min before the milking procedures, whereas in P farms goats were observed in open pasture ranges at least 60 min after the end or 90 min before the milking procedures.

Obs-B was responsible for selecting suitable observation point(s). The number of observation points depended on the complexity of the housing environment and the group size. These points guaranteed that all possible situations were covered during the observation time, i.e. they allowed observing all the different structures of the housing environment (e.g. deep straw barn, outside field; or pens of different sizes in different areas/corners of the farm).

During the choice of the observation points, the observers spent some time moving around the pens, or the grazing area, in order to allow the animals to become familiar with them.

During the observation, the breeder was asked to keep out of goats' sight, in order to avoid biased behaviour.

The assessment was independently performed by both observers at the same time, without distracting each other or blocking each other's view. It was directed on the whole herd and not on individual animals.

The total observation time for each farm ranged from a minimum of 10 to a maximum of 20 minutes, depending on the number of observation points. If only one or two observation points were necessary for having a complete view of the herd, than the total observation time lasted 10 minutes; if more than two observation points were required, then a maximum of 20 minutes of total observation was performed, and the duration from each point (maximum 8 points) was set based on Table 2.

Number of observation points	*Duration of observation from each point (min)*	*Total observation time (min)*
1	10	10
2	5	10
3	6.5	19.5
4	5	20
5	4	20
6	3	18
7	2.5	17.5
8	2.5	20

Table 2. Observation time for each farm depending on the number of observation points.

When the observation from all the selected points was completed, the two observers scored the 16 descriptors using a VAS in a specific application for Android devices, developed by Scottish Rural College (SRUC, Edimburgh). To score each term, observers touched the tablet screen across the VAS at the appropriate point. Each VAS ranged from 0 mm (minimum) to 125 mm (maximum). The measure for each term was the distance in millimeters from 0 to the point where the VAS was touched. Zero meant that the expressive quality indicated by the descriptor was completely absent in all the observed animals. Maximum was scored when the descriptor was dominant and present in the whole herd. All descriptors were scored (even their value was 0).

To ensure the independence of each observer, silence was strictly maintained during assessment procedures.

STATISTICAL ANALYSIS

For statistical analysis, one H farm was removed due to procedural error. This was the only farm where the observers had the opportunity to know the farmer and to discuss with him before the QBA assessment. From preliminary analysis, this farm resulted to be an *outlier*, probably because the impression that each observer received from the farmer introduced a *bias* in the outcomes. The analysis was then performed on 15 (8 extensive and 7 intensive) farms.

Data collected in the QBA app for the 15 farms were exported and analyzed using SPSS version 20 (IBM SPSS Inc, Chicago, USA).

The 16 QBA descriptors' scores generated by the two observers for all farms were analysed together using Principal Component Analysis (PCA) (correlation matrix, no rotation).

PCA is the most common method for data exploration and its use allows to extract the greatest information contained in a multivariate dataset and to synthesise it into two or more dimensions (Principal Components, PC) of the original variables (descriptors), explaining the variance between goats expressions in the different farms; each farm received a score on each of the main principal components. The algebraic solutions obtained by PCA can be graphically represented in two-dimensional plots: a *loading plot*, which shows the relationship among variables (i.e, in our case, the 16 descriptors), and a *score plot*, which shows the relationship among objects (i.e., our 15 farms for each observer) or a *biplot*, containing information on both variables and objects. The loadings of the descriptors quantify the weight that each of them has on the two main axes (Mattiello *et al*., 1997; Rencher, 2002). A loading value of ± 0.24 was used as cut-off, since no rotation was used in PCA (Andreasen et al., 2013). Observer agreement for farm scores on the first three PCA Components (PCs) was investigated using Pearson correlations (r). ANOVA was used to test the effects of housing system and observer on PC scores (treatments=observer, housing system and their interaction; block=farm). Spearman correlation ranks (ρ) between separate QBA terms generated by Obs-A and Obs-B were also calculated.

RESULTS

PCA on Obs-A and Obs-B scores analyzed together

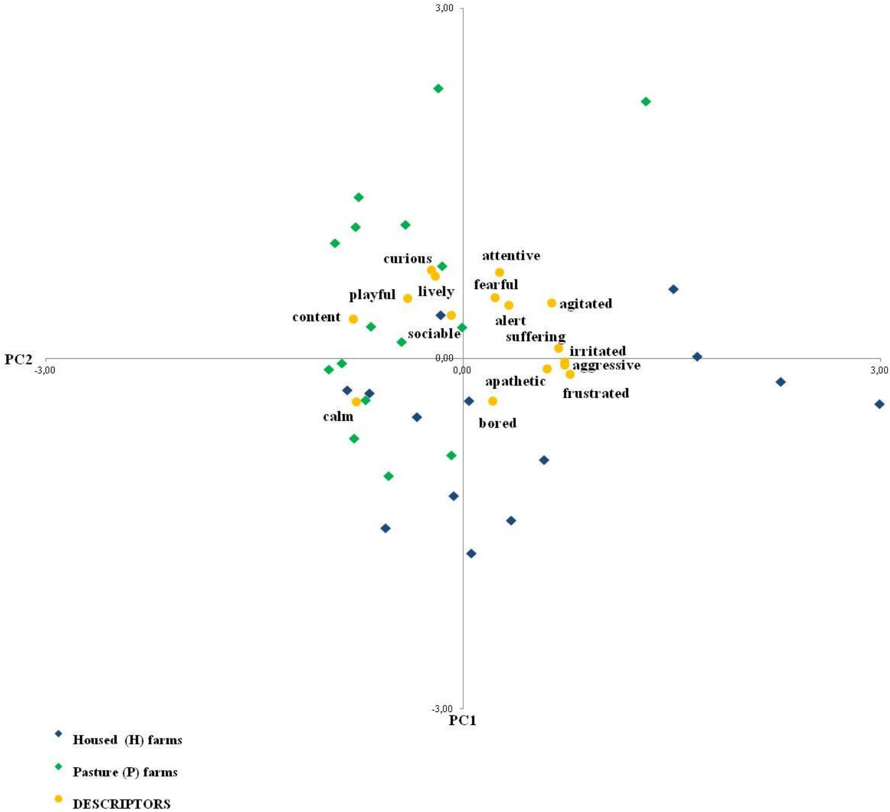

Figure 1. Biplot of PCA using 16 descriptors (PC1 vs PC2).

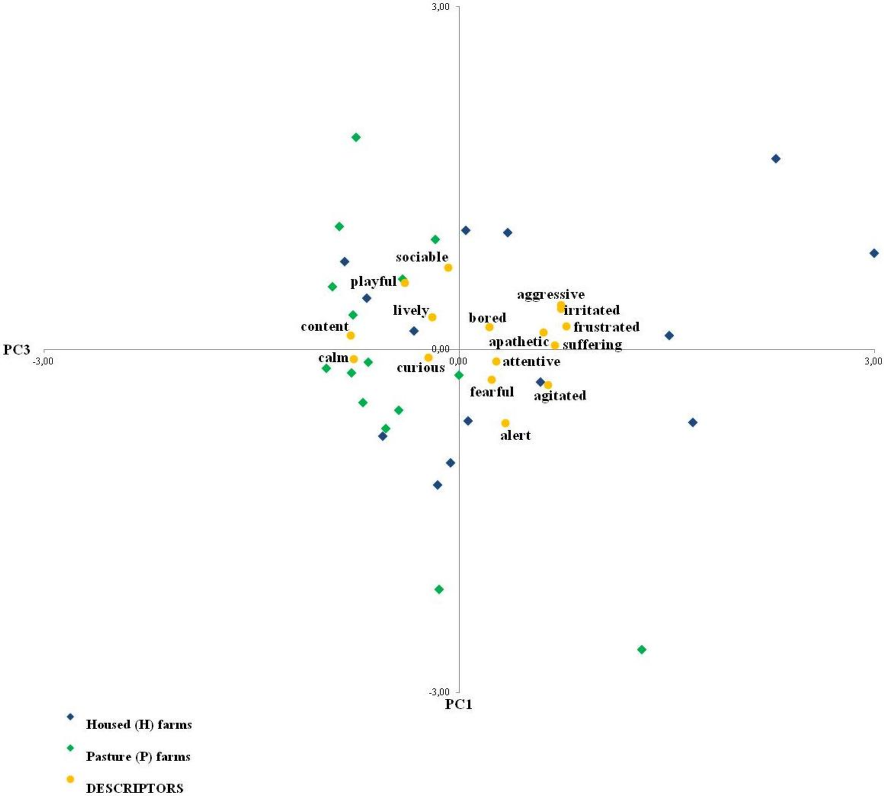

Figure. 2. Biplot of PCA using 16 descriptors (PC1 *vs* PC3).

	PC1	PC2	PC3
Negative loading	Content (-0.79) Playful (-0.40) Calm (-0.77)	Calm (-0.37) Bored (-0.37)	Alert (-0.64) Agitated (-0.31) Fearful (-0.26)
Positive loading	Frustrated (0.77) Aggressive (0.73) Irritated (0.73) Suffering (0.69) Agitated (0.64) Apathetic (0.61) Alert (0.33) Attentive (0.27)	Curious (0.76) Attentive (0.74) Lively (0.71) Fearful (0.52) Playful (0.51) Agitated (0.48) Alert (0.46) Content (0.34) Sociable (0.37)	Sociable (0.72) Playful (0.59) Irritated (0.36) Lively (0.29)

Table 3. Loadings of each descriptor on the first three PCs. Only descriptors which load more than ± 0.24 are shown in the table.

On PC1 (explaining 29% of variation between goats' behaviour) a clear separation can be observed between descriptors showing positive and negative emotional state (positive descriptors on the left, negative ones on the right) (Figure 1; Table 3). On PC2 (20% of explained variance) a clear separation was found between descriptors with positive and negative levels of arousal (positive descriptors on the top, negative ones on the bottom) (Figure 1; Table 3). Farms were well separated on PC1 depending on the housing system: P farms were homogeneously distributed on the left (characterized by positive emotional state), while the majority of H farms were scattered on the right, although this category seemed less homogeneous (Figure 1). The third component (PC3; 12% of explained variance) did not bring any additional relevant information (Figure 2).

Inter-observer reliability

Farm scores generated by the two observers on the three PCs were significantly correlated (Table 4) indicating a good level of inter-observer reliability.

PCs	Pearson Correlation r	Sig. (2-tailed)
PC1 Obs-A and PC1 Obs-B	0.75^{**}	0.001
PC2 Obs-A and PC2 Obs-B	0.67^{**}	0.006
PC3 Obs-A and PC3 Obs-B	0.69^{**}	0.004

**. $P < 0.01$.

Table 4. Pearson correlation ranks between scores generated by the two observers on each PC.

Observers' scores on separate QBA terms were significantly correlated for 7 out of 16 terms ($P < 0.05$), and approached significant correlation for an additional 2 terms: 'aggressive' ($P = 0.102$) and 'attentive' ($P = 0.103$) (Table 5).

Descriptors	**Spearman's correlation coefficients (ρ)**	**Sig. (2-tailed)**
Aggressive	0.44	0.102
Agitated	0.40	0.135
Alert	0.57*	0.027
Bored	0.11	0.689
Apathetic	0.40	0.139
Attentive	0.44	0.103
Content	0.78**	0.001
Curious	0.05	0.849
Frustrated	0.56*	0.019
Playful	0.73**	0.002
Irritated	0.30	0.277
Fearful	0.37	0.176
Sociable	0.69**	0.004
Suffering	0.82**	0.000
Calm	0.09	0.741
Lively	0.83**	0.000

* $P < 0.05$; ** $P < 0.01$.

Table 5. Spearman correlations between observers for the 16 descriptors.

Spearman correlations between descriptors analyzed separately for Obs-A and Obs-B are shown in Tables 6 and 7.

Obs-A		Agitated	Alert	Bored	Apathetic	Attentive	Content	Curious	Frustrated	Playful	Irritated	Fearful	Sociable	Suffering	Calm	Lively
Aggressive	ρ	0.42	0.17	0.36	0.16	0.14	-0.15	0.10	0.06	0.19	0.09	0.07	0.49	0.38	-0.66**	0.25
Agitated	ρ		0.83**	0.39	0.25	0.29	-0.52*	0.01	0.24	-0.35	0.20	0.26	0.09	0.40	-0.85**	-0.14
Alert	ρ			0.30	0.03	0.59*	-0.44	0.31	0.26	-0.47	0.13	0.23	-0.06	0.33	-0.75**	-0.13
Bored	ρ				0.40	-0.30	-0.53*	-0.57*	0.75**	-0.15	0.64*	0.01	0.32	0.37	-0.48	-0.51
Apathetic	ρ					-0.38	-0.12	-0.46	0.26	0.13	0.33	0.06	0.07	0.77**	-0.16	0.11
Attentive	ρ						0.13	0.87**	-0.15	-0.09	-0.14	0.03	0.02	0.05	-0.46	0.31
Content	ρ							0.23	-0.57*	0.62*	-0.50	-0.63*	0.29	-0.36	0.39	0.74**
Curious	ρ								-0.38	-0.03	-0.32	0.17	-0.07	-0.06	-0.15	0.45
Frustrated	ρ									-0.20	0.84**	0.09	0.01	0.31	-0.31	-0.58*
Playful	ρ										-0.12	-0.48	0.60*	-0.22	0.20	0.63*
Irritated	ρ											0.02	0.22	0.36	-0.19	-0.48
Fearful	ρ												-0.42	0.30	-0.17	-0.24
Sociable	ρ													-0.13	-0.23	0.30
Suffering	ρ														-0.45	0.04
Calm	ρ															0.08

**. *P* < 0.01; *. *P* < 0.05.

Table 6. Spearman correlations between descriptors by Obs-A.

Obs-B		Agitated	Alert	Bored	Apathetic	Attentive	Content	Curious	Frustrated	Playful	Irritated	Fearful	Sociable	Suffering	Calm	Lively
Aggressive	ρ	0.43	0.34	0.14	0.42	0.46	-0.58 *	0.02	0.47	-0.26	0.49	0.18	0.12	0.59*	-0.49	-0.18
Agitated	ρ		0.22	-0.01	0.56*	0.40	-0.22	-0.02	0.64*	-0.15	0.77**	0.51	0.13	0.64*	-0.71**	0.10
Alert	ρ			-0.34	0.11	0.53*	-0.12	0.07	0.22	-0.18	0.05	0.75**	-0.33	0.22	0.03	0.12
Bored	ρ				0.42	-0.08	-0.60*	-0.32	0.32	-0.25	0.13	-0.41	-0.08	0.05	-0.28	-0.74**
Apathetic	ρ					0.29	-0.66**	-0.36	0.98**	-0.43	0.74**	-0.02	-0.12	0.62*	-0.49	-0.42
Attentive	ρ						-0.19	0.35	0.29	0.01	0.38	0.58*	0.06	0.45	-0.23	0.14
Content	ρ							0.51	-0.66**	0.49	-0.50	0.21	0.19	-0.40	0.31	0.75**
Curious	ρ								-0.41	0.61*	-0.24	0.34	0.59*	-0.14	0.16	0.57*
Frustrated	ρ									-0.45	0.75**	0.07	-0.17	0.69**	-0.50	-0.40
Playful	ρ										-0.41	0.17	0.80**	-0.35	0.09	0.39
Irritated	ρ											0.21	-0.02	0.53*	-0.65**	-0.09
Fearful	ρ												0.03	0.07	-0.22	0.39
Sociable	ρ													-0.09	-0.28	0.32
Suffering	ρ														-0.33	-0.06
Calm	ρ															0.00

**. *P* < 0.01; *. *P* < 0.05.

Table 7. Spearman correlations between descriptors by Obs-B.

ANOVA

The housing system had a significant effect on the scores of the farms on the first (*P* = 0.05) and second component (*P* = 0.02). Goats reared in extensive conditions obtained significantly higher scores, indicating goats on P farms to be more ‘content/calm’, and more ‘curious/attentive’, than goats on H farms. However, analysis of variance found that there was a significant observer effect on PC2 (*P* = 0.04), and a significant observer by housing interaction on PC3 (*P* = 0.009).

DISCUSSION

The first aim of the present study was to determine the validity of QBA as a welfare indicator in dairy goats. An important aspect of its validating process is ensuring that the method is sensitive to discriminate between groups of animals managed in different ways. QBA found that goats’ demeanour on H and P farms differs along two dimensions. This supports the hypothesis that the possibility of access to pasture allows goats to express a more positive and natural behaviour and confirms that the method is able to discriminate between the two housing systems. These outcomes support previous studies in pigs (e.g., Temple *et al*., 2011), demonstrating that QBA can be a valid indicator to distinguish between different rearing systems. This is confirmed also by the statistically significant differences between housing systems highlighted by ANOVA in the QBA scores (higher scores in P farms *vs* lower scores in H farms). Although animals in extensive systems have to face a range of welfare challenges (e.g., variability in climate conditions, parasitic diseases) (Sevi *et al*., 2009;

Dwyer, 2009; Goddard, 2006, 2013), P farms showed a generally more positive situation as far as the emotionality of goats is concerned. Grazing goats seemed to be more content and calm than animals kept indoor. In fact it is recognized that access to pasture is beneficial to their behavioural needs promoting exploratory and active behaviour (Casamassima *et al*., 2001). On the contrary, in H farms goats lose some of their freedom to express their natural behaviour (Casamassima *et al*., 2001; Sevi *et al*., 2009; Dwyer, 2009; Braghieri *et al*., 2011). In these production system, negative moods such as 'aggressive', 'irritated' and 'suffering' obtained higher scores than in P farms, probably depending on the restrictions of space availability (Miranda-de la Lama & Mattiello, 2010). Moreover, in H farms another potential stress factor could be the relationship between the animal and the stockman (Muri *et al*., 2013): excessive or improper handling, such as sudden changes in rearing practices, may cause suffering to goats (Sevi *et al*., 2009).

Despite the observations reported above, in the present study some H farms were scattered on the left of PC1, suggesting the possibility to achieve good welfare standards also in intensive farms, although it is certainly more difficult to observe positive behaviour in animals kept indoor.

A second main result of the present study is that a good inter-observer reliability across three dimensions of goat demeanour was found, as reported by other studies on application of QBA fixed-list terms in other species (e.g., Wemelsfelder & Millard, 2009; Phytian *et al*., 2013). Pearson correlation ranks between the main PC for the two observers was above 0.7, indicating a satisfactory correlation coefficient for inter-observer reliability (Martin & Bateson, 2007). However, ANOVA highlighted a significant observer effect for the second PC and a significant effect of the interaction observer*housing system for the third PC. The observer effect on PC2 can be explained by the fact that this component is mainly represented by terms, such as ‘bored’ and ‘curious’, which were poorly correlated between observers. On the contrary, terms with higher loadings on PC1, such as ‘content ‘and ‘suffering’, were highly correlated between observers. In fact, Spearman’s correlations showed that observers agreed in their quantification of some descriptors, but they differed in their quantification of some others. These results on inter-observer-reliability are promising, but they suggest that there is a need for a refinement of the descriptors list and for further training.

Taking into consideration the correlations among descriptors for each observer, and considering the results of Spearman correlations between descriptors for Obs-A and Obs-B, some adjustments of the QBA list of terms can be can be proposed, in order to improve inter-observer reliability and to limit the presence of redundant information. For example, a negative correlation was found between ‘calm’ and ‘agitated’ (both for Obs-A and Obs-B; Tables 6-7), and this carries redundant information. As the correlation between observers was higher for ‘agitated’ than for ‘calm’ (Table 5), we suggest removing ‘calm’ from the list of terms. ‘Attentive’ also carries redundant information, being positively correlated with both ‘alert’ (both for Obs-A and Obs-B; Tables 6-7) and ‘curious’ (only Obs-A; Table 6). We suggest deleting ‘attentive’ from the list and including part of the definition of this term in ‘curious’, stressing the positive trait of reactivity, as opposite to ‘alert’, which describes attention in a negative emotional state. In spite of the fact that inter-observer agreement for ‘curious’ was not high, we suggest keeping it in the list, as it is very characteristic of goats. The high correlation observed between ‘curious’ and ‘attentive’ suggests that there is a bias factor in the definitions, that may lead to confusion. As ‘attentive’ can be eliminated and ‘curious’ can be refined, the interpretation should become easier.

‘Bored’ may also be removed, due to its negative correlation with ‘content’ (both for Obs-A and Obs-B; Tables 6-7) and to its low inter-observer agreement (Table 5). Moreover, both observers judged this descriptor difficult to be interpreted.

‘Apathetic’ was correlated with ‘suffering’ (both for Obs-A and Obs-B; Tables 6-7), therefore we suggest removing this term and improving the definition of ‘suffering’, including some features from ‘apathetic’: in fact, ‘suffering’ has a wider meaning (that may include ‘apathetic’) and seems to be easier to use in the field.

‘Playful’ was significantly correlated with ‘sociable’ (both for Obs-A and Obs-B; Tables 6-7) and ‘content’ (only Obs-A). As ‘content’ showed a higher inter-observer agreement (Table 5), we propose to redefine this term including ‘playful’ in this new definition.

The definition of ‘lively’ also needs to be revised, in order to be well distinguishable from ‘content’. In fact, these two terms are significantly correlated for both Observers (Tables 6-7), but active animals are not necessarily content. A refinement of the definitions will be useful in order to avoid possible confusion, that may affect inter-observer reliability. For further studies, before defining a fixed list of descriptors, another option could be to apply a procedure, called Free Choice Profiling (FCP) and used to generate QBA descriptors in several QBA studies (e.g., Rutherford *et al*., 2012). FCP allows observers to generate their own terms, making them completely familiar with the meaning of the descriptors to be used on farm (Phytian *et al*., 2013).

Certainly, a clear definition of descriptors is essential and can help to improve inter-observer reliability (Meagher, 2009), but further training is required too. Training aims to ensure that observers record measures with a consistent rate of accuracy (Kazdin, 1977). Training sessions based on video clips, as those adopted for this study, are easily achieved, but the vidoes do not represent the real situation faced by the observers when they visit a farm. In field conditions, the observer should be able to observe the animals without disturbing them and to see their expressions from an optimal position, avoiding to be confused by other surrounding factors. Training directly on farm requires more efforts in terms of time and resources, but it would probably be the better choice.

It will be interesting repeating this study after refinement of the descriptors' list and further training, possibly on-farm, in order to verify if an improvement of inter-observer reliability can be achieved.

CONCLUSIONS

Based on the expression of natural behaviour, QBA was able to discriminate between extensive and intensive goat farms, and therefore appears to be a valid method for evaluating positive emotional state in dairy goats.

QBA could be a practical tool to easily detect early signs of declining or improving welfare, particularly in extensive production systems, where more invasive welfare assessment are difficult to perform.

This study reported the first attempt to evaluate the inter-observer reliability of QBA in dairy goats. The two observers achieved a good level of inter-observer reliability applying QBA fixed list descriptors, even though further refinement is necessary.

Although further studies are required, QBA, together with other health and welfare indicators, could make a valuable contribution to the assessment of dairy goats' welfare.

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GENERAL CONCLUSIONS

"The game of science is, in principle, without end. He who decides one day that scientific statements do not call for any further test, and they can be regarded as finally verified, retires from the game"

Popper (1959)

In recent years, public concern towards animal welfare has been steadily increasing becoming of primary relevance especially in organic farming.

While high animal health and welfare are implicit goals of organic principles and standard, very little is known about how animal welfare can be adequately assessed and promoted in practice.

The studies compiled in this thesis investigated two fundamental health and welfare issues related to the use of pasture in organic dairy goat farming: gastrointestinal nematodes and positive emotional states.

Chapter 1 includes an in-depth review of the literature on sustainable control strategies of gastrointestinal nematodes. This review aims at providing a general assessment and a state of the art of the knowledge around what has been identified as the main health problem in grazing goats. Particular attention has been devoted to phytotherapy as a feasible alternative to allopathic anthelmintics, particularly when preventive strategies are limited. The complementary medicine has been encouraged by EU standards and many organic farmers are interested to adopt this medicinal approach.

Chapter 2 presents two studies that evaluate the antiparasitic effect of herbal remedies. Both the trials show that phytotherapic remedies lack in controlling GIN in terms of faecal egg counts in organic dairy goats. Although the negative results, it is worth noting that the goats included in the studies did not shown any clinical sign related to infestation, suggesting a high tolerance with respect to parasites.

These trials were field studies carried out in a commercial farm studying naturally infected dairy goats. Further experiments are required to assess the efficacy of herbal remedies under controlled studies, which allow monitoring the animal health status by assessing physiological parameters under experimental infestations. Also the quality of pasture - in terms of grass species and nutritional values - should be evaluated as it is recognized that feeding represents a valuable tool to enhance the animal tolerance to GIN. Furthermore, the fecal egg count and the fecal egg count reduction test might be inappropriate indicators to test the anthelmintic properties of medicinal plants from a methodological perspective, as already reported by some authors.

The overall effects of medicinal plants should be evaluated through the welfare assessment of parasitized animals. For instance, monitoring goat behaviours might be a relevant tool to evaluate whether medicinal plants improve the resistance or resilience of the parasitized goats.

Preventive strategies, including grazing management, are the most important tools for GIN control, suggesting that the great tolerance to infections might be attributed to the access to pasture. In this sense, pasture represents a risk factor as well as a “remedy” for GIN infections. Grazing goats at pasture can perform their natural behaviour and experience positive emotions, contributing to enhance a good health and welfare status.

In Chapter 3, the QBA approach found the goats’ demeanour on extensive and intensive farms, showing that access to pasture may have a positive effect on goats' emotional state. This study points out that QBA might be a valid indicator to assess the goat's "quality of life" and be worth incorporating in a welfare assessment protocol focused on organic farming.

Step forward might be the use of QBA in goats with GIN and no clinical sign related to infestation in order to confirm the positive effect of pasture on goats with gastrointestinal nematodes.

Based on the studies presented in this thesis and according to the organic principle of health and welfare, pasture represents a valuable element for the welfare of organic goats. The harmony between all living organisms on the farm, including parasites, is one of the goals of organic farming. As a broader conclusion, these studies raise further questions on the extent to which GIN actually represent a serious problem in organic goat farms. This would have also practical implications to orientate the most adequate treating strategy both with phytotherapy as well as with traditional methods.

In light of these results, further controlled studies are encouraged to develop this field of research and assess health and welfare in organic grazing goats from a multidimensional and holistic perspective.

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