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Influence of music on the welfare of dairy cattle:

towards the establishment of an experimental protocol

DOCTOR OF VETERINARY MEDICINE WORK:
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À ma Pépette,

ABSTRACT

Influence of music on the welfare of dairy cattle: towards the establishment of an experimental protocol.

A zene hatása a tejelő szarvasmarhák jóllétére: lépések egy kísérleti protokoll létrehozása felé.

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Contemporary concerns have been brought into the light of scientific research, emerging simultaneously with the intensification of animal production ushered by the industrialization of agriculture over the past five decades. This gave birth to the burgeoning science that is animal welfare. First and foremost, this thesis briefly reviews what the term welfare signifies and precisely entails, replacing it to its rightful place: a scientific concept, legitimately embedded in the heart of biological science, notably through its relationship to the notion of stress. By understanding its multidimensional nature, which renders its assessment so complex, we can better comprehend the disagreements it carries inside the scientific community.

Keeping all these concepts and views in mind, supplying us with a brand new precious toolbox, the review turns to the exploration of the literature on music, its various effects on animals and its possible incidences on stress and welfare. This thesis work is highlighting some substantial points, inter alia linked to the inherent nature of the stimuli that is music itself, that have been problematic when trying to draw definitive conclusions on the existence of beneficial effects of music on the welfare of animals. It is raising the curtain on important issues with a desire to clear the field for a future experimental program and to lay the first stones of a protocol that humbly wishes itself to satisfy both of the welfarists' and musicologists' concerns and demands.

A mezőgazdaság iparosodásának eredményeként egyre intenzívebbé váló állatitermék előállító módszerek új problémák és aggodalmak megjelenéséhez vezettek az elmúlt öt évtized során. Ez tette szükségessé az állatjóllét fogalmának megszületését. Jelen dolgozat elsődleges célja ennek a fogalomnak a körüljárása, hogy meghatározhassuk a tudományban elfoglalt helyét. Az állati jóllét egy tudományos koncepció, mely jogosan került be az élettudományok közé, elsősorban a stresszhez való szoros kapcsolata miatt. Ha megértjük összetett természetét – mely egyben olyan nehezen vizsgálhatóvá is teszi –, megértjük azt is, miért övezi annyi egyet nem értés a tudományos közösségen belül.

Ezen áttekintésbe foglalt elméletekkel és nézőpontokkal felvértezve a következő lépés a zenével kapcsolatos szakirodalom áttekintése és értékelése volt – annak vizsgálata, hogyan hat az állatokra, és milyen kapcsolata lehet a stresszel és a jóllét fogalmával. A dolgozat kiemel néhány lényeges elemet, többek között magának a zenének mint stimulusnak a természetét, mely sokszor komoly nehézségeket okoz, mikor annak az állat jóllétére gyakorolt pozitív hatását kívánjuk vizsgálni. Számos nehézségre rávilágít, melyek felmerülnek, mikor jövőbeli vizsgálati módszereket és kísérleti protokollokat szeretnénk megalapozni. Olyan módszereket, melyek mind a jólléttel foglalkozó szakemberek, mind a zenetudósok nézőpontjait és igényeit figyelembe veszik.

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ABBREVIATIONS

ACTH: Adrenocorticotropic Hormone

AMS: Automatic Milking System

BC: Before Christ

BDNF: Brain-Derived Neurotrophic Factor

BEHAVE-AD: Behavioral Pathology in Alzheimer's Disease Rating Scale

CNS: Central Nervous System

dB: Decibels

DFD: Dark Firm Dry

DVA: Danish Veterinary Association

EAEVE: European Association of Establishments for Veterinary Education

EFSA: European Food Safety Authority

FAWC: Farm Animal Welfare Council

FVE: Federation of Veterinarians of Europe

HPA: Hypothalamic Pituitary Adrenal Axis

HRV: Heart Rate Variability

NGF: Nerve Growth Factor

NK: Natural Killer

OIE: Office International des Epizooties

PSE: Pale Soft Exudative

SCC: Somatic Cell Count

SHR: Spontaneously Hypertensive Rat

UCCR: Urine Creatinine:Cortisol Ratio

WVA: World Veterinary Association

"What should we do about animal welfare?

The answer, ladies and gentlemen, is 'More'!"

Dr. Mike Appleby, Vice President for Farm animals and Sustainable Agriculture, Humane Society of the United States

At the inaugural annual lecture of the Sir James Dunn Animal Welfare Centre in October 2001

INTRODUCTION

To be fairly honest with you reader, I started this work with the motivation to figure out whether music could enhance the milk production of cows. This interest was instigated by hearing of Evans' report in the Hoard's Dairyman (Evans, 1990) by Pierre-Guy Marnet. At that time, in my mind, improving welfare was merely an interesting tool to enhance the animal production. Diving head first in the research about the burgeoning science that is animal welfare, I rapidly understood that I had been mistaken to confine welfare in such a tiny box. I was given the chance to understand how important this concept is, just like researchers in agricultural science have. In fact, an exponentially growing concern could be witnessed over the past few decades, placing animal welfare on top of the agricultural and political agendas in Europe and North America. This thesis work is well included in a veterinary student curriculum like mine, as the role of vet practitioners and the need for their education are both significant nowadays. As apprentice veterinarian, we are mainly taught to handle the animals' health and pain. Many of us might think that animal welfare is something insignificant compared to traditional medicine: to some it is a luxury we cannot always afford due to economic reasons, to some others welfarists are fanatics disconnected from the reality of the field. After two years of research and review, I now truly believes that animal welfare should be at heart of our generation's way to practice and the future generations' education. Quoting Estol: "*By teaching animal welfare we can kindle the light of hope for the profession's future: the young. These are young people who will ally passionate conviction with their skills and knowledge to join forces with serious protectionists who are equally passionate and bighearted, not fanatics*" (Estol, 2004). What's more, it is not a discipline that should be confined to research. The essence of this thesis work was knitted around this belief: from very formal concepts formulated by welfarists, but also lawyers, politicians, ethicists and philosophers, should emerge clear, intelligible and pragmatic applications intended for producers and consumers.

Hence, behind the thematic of music, the backbone of this thesis is anchored in the understanding of what is animal welfare and how it can be scientifically assessed. It is all the more crucial because many studies, aiming to elucidate music's role as environmental enrichment, and its impact on stress and its symptoms, disagree and fail to conclude on the basis of issues that are fundamentally linked to the conceptions of stress and welfare themselves and less often due to music-related issues.

I was also quite surprised because I would have expected a much more furnished literature on the influence of music on animals and especially on production animals. Besides welfare conceptual problematic, many technical problems arise from the reviewed studies and the need for more specific research and more elaborate experiments is blatant. This constitutes the second axis of focus of this thesis.

Because it works well on us humans, we have this intimate intuition that music could also work as a stress alleviator for animals and hence be beneficial to the modern farming systems. It almost seems obvious that it would. But can we extrapolate human concepts to animals? Research has many times proven us that it is a mistake. So, are we victim of anthropocentrism assuming that something created by men for men could affect the rest of the animal kingdom? It is the scope of this thesis: Are there specific effects of music on non-human animals? Specifically, could music improve welfare? Could music be part of environmental enrichment and improve the animals' quality of life, particularly in the case of dairy cows? How could we, humbly, elaborate better protocols to fulfill needs of both welfarists and musicologists in order to be able to draw definitive conclusions on the topic?

The thesis outline unfolds as follows. First and foremost, we shall proceed to a "classic" literature review about welfare to understand this concept and the ways to assess it. Then, the state of the art on the research of music's effect on animals is briefly reviewed, organized logically according to what has been learned in the previous chapter concerning the ways to evaluate stress and welfare levels. Both reviews are focused almost exclusively on production animals. Even though the literature is very (if not better) furnished about other captive animals such as laboratory animals (rats and mice, especially concerning biochemical and neuroendocrinological aspects, but also primates and birds) and wild fauna kept in zoos and parks, I voluntarily chose to exclude those studies, selecting the articles with the ultimate goal in mind to build a protocol that would take place in the theatre of the dairy farm. In a third and last part, I focus on some important points to consider when building a protocol. These are inspired by the articles previously reviewed: their methods, their failure, the different parameters used and the issues they encountered. This helps to form an experimental protocol embryo. All of this is nourished by the will to increase the validity of future researches.

1. General discussion about farm animal welfare

1.1. Understanding how the concept was built

1.1.1. A recently growing concern

The past five decades have seen two remarkable trends evolve simultaneously. Firstly, an outstanding change in animal agriculture has taken place transforming the old traditional, semi-outdoor production techniques into more industrialized, intensive, mainly indoor methods. The intensification of animal production industries over the last 50 years, accompanied by the development of animal production technology and the competitive pressures to reach lower production costs, has seen spectacular improvements in animal health and production in one hand, but has brought along some threatening challenges to farm animal welfare. Indeed, the deterioration of welfare standards have now been admitted, particularly in the sectors of intensive pig and poultry production. Nevertheless, whilst this quest of a highly efficient production was becoming the number one goal, a remarkably growing interest was given to animal welfare. According to Fraser, this trend could be explained by, inter alia, the collection of scientific knowledge about animals. Furthermore, there has been a rapid urbanization of the human population over the last half century. The growing urban population is way more confronted to pet animal populations and are removed from animals used to provide feed for them. Most of these people do not comprehend how animals are bred and kept and how food is derived from animals. Urban people live with pet animals that tend to be treated more and more like family members, and this has brought up the expectation that all animals should be well treated. We can also mention the role of the media in making the lives of wild animals accessible to people as never before. All of this results in a striking increase in the amount of attention and sympathy paid to farm animals (Fraser, 2003).

According to Broom, this phenomenon is part of a much bigger fashion. Indeed, public pressure is being born and spreading worldwide not only about animal welfare, but also about human health and the impact of human activities on the environment. In fact, a vast majority of people tend to agree in considering barbaric to let people become sick, to let animals be treated badly or to let the environment be spoilt. We now expect systems or procedures to be sustainable. It means they can only be acceptable now if their effects will

be acceptable in the future, in particular in relation to resource availability and morality of action. In this picture, animal welfare is far from being left behind as it is recognized to be one of the criteria used by the public when deciding whether a procedure or system is acceptable. Hence, it is an essential consideration for sustainability (Broom, 2010).

Moreover, all along the animal product industry chain, from the producers to the consumers, the concept of quality has changed. It has widened in the sense that good quality does not only imply good in taste anymore but also sustainable manufacturing: European consumers no longer want to pursue the tastiest possible food products but are seeking additional various characteristics such as safety, origin, environmental provenance and animal welfare standards. Schemes designed to answer these new concerns and emerging requirements are springing up like mushrooms across Europe. The French project named "Label Rouge" has led the way in this matter (Ouedrago, 1998). The proportion of French consumers who buy only according to the product's price is estimated to have dropped to 25%. We thus see appearing in our supermarket aisles some price premiums for "free-range" or organic livestock products. Another crucial evolution of the mindsets affecting the European consumption, is the certain backlash endured by industrialization and technology in general. It can be attested by the popular distrust of big business, global trade and genetic engineering and the recent scandals affecting the agri-food industry. Consumers seem to aspire to a return to more agrarian and ecological forms of agriculture. Lobbying and legislation programs are actively trying to remedy the welfare decline brought up by the industrialization of agriculture. We can cite some examples such as the attempts to improve the cages for laying hens, the debates about tethering and crating of pigs, and the measures taken to enhance transport conditions.

Meanwhile, in the research field, the number of animal welfare scientists is skyrocketing. It is of course undoubtful that research is a necessary preliminary step towards the improvement of the global welfare situation. However, science ought to build a solid bridge towards practical application of the concepts it lays. This bridge is made by education, particularly in the veterinary field, and has already started to be constructed worldwide. The first ever academical unit which has been created for teaching and researching exclusively into animal welfare was the Colleen Macleod Animal Welfare Chair in the Department of Clinical Veterinary Medicine of the University of Cambridge in the United Kingdom in 1986, held by Professor Donald Broom. Since then, in the European Union, the FVE

(Federation of Veterinarians of Europe) and the EAEVE (European Association of Establishments for Veterinary Education) have initiated to study how to teach key subjects such as the wellbeing of livestock during and the interaction between production animals and the environment, in order to meet the brand new societal requirements. The subject of welfare is now being taught in all European countries. In our university (University of Veterinary Medicine of Budapest), the animal welfare subject shifted from an optional course to a mandatory part of our curriculum. But this is not only contained in this continent. Across the Atlantic, in Brazil, who represents a major force in the global meat markets as it is a top giant meat producer country, the number of university courses on animal welfare has increased from one to over 60 in 15 years. In the United States, the decision by the American Veterinary Medical Association to encourage the teaching of the subject in all American veterinary schools already produces a substantial effect (Broom, 2011).

1.1.2. **New duties for vet practitioners**

It is essential to understand how decisive this promotion of welfare education to veterinarians and apprentice veterinarians is. In fact, it is now more than ever acknowledged that veterinarians should play a major key role in farm animal protection and welfare. Yet, veterinarians do not have clear guidelines for the extent of their role in animal welfare. Moreover, whatever veterinarians decide as a profession or as individuals, they must be knowledgeable. The failures often displayed by veterinarians to show leadership in animal welfare reflects the lack of veterinary education in animal welfare, the absence in the scientific field of a general consensus on the assessment of welfare and differing attitudes to animals within the profession.

The veterinary profession is, in its essence, dedicated to animal welfare. Actually, more than a dedication, it is a veterinary obligation marked by a solemn oath. Indeed, in a great number of countries, veterinary students make a very peculiar declaration when they graduate to spend their professional lives working under a code of ethics, especially regarding animal welfare. This commitment is overseen by the different organizations governing the vet profession. The World Veterinary Association (WVA) has developed a number of policies related to animal welfare. It is also actively operating with global organizations such as the OIE (Office International des Epizooties) to ensure that the veterinary profession is actively involved in the development of these policies. In Europe,

many national veterinary associations, such as for instance the Danish Veterinary Association (DVA), display the clear objective in their constitutions to improve the welfare of animals (Edwards, 2004).

The profession holds the great responsibility to take active steps to safeguard animal welfare partly because they are the ones that are the best placed to do so. Indeed, vets have the indispensable knowledge acquired through a long and intense training, to pursue a holistic and extensive animal welfare expertise. The range of veterinary skills includes anatomy and physiology, behavior, nutrition, health and illness; that is, what is normal and what is abnormal. This enables veterinarians to have an extremely good understanding of what an animal needs, what it is being exposed to, how it is reacting and what is required to return it to a good welfare state and be maintained at that level. They are also supposed to possess knowledge of the relevant animal welfare legislation. Besides education, veterinarians have an amazing opportunity to be welfare's Fifth Column: they can monitor it on an everyday basis and are often the first witnesses of cases of animal neglect or abuse. Being more than simple observers, they also have the opportunity to do something about it when providing high quality medical care for animals and alleviating suffering.

As Caroline J. Hewson reviews, when fulfilling their role towards animal welfare, veterinary practitioners should always remain independent. Although it is accepted that neither sentiment nor economic factors can be entirely divorced from welfare, they should never be paramount in its consideration. When veterinarians undertake their career at graduation, they accept the challenge to look after not only their clients' interests. David Morton, veterinarian and professor of Biomedical Science & Ethics, University of Birmingham, recently suggested (Morton, personal communication, 2002), *"The main way in which the veterinary profession fails to serve animal welfare is by not standing up and saying when they are unhappy about things, especially when it might upset their paymasters"*. In other words, even if vets work for a farmer who has an economic need to operate a successful, commercially sustainable business, they need to ensure that the animals are well looked after and that their welfare is not compromised beyond an acceptable level. A practitioner from Cardiff, Wales, expressed concern about veterinarians viewing farm animals as herds and not individuals: *"I have witnessed a vet walk past a sow with a prolapsed uterus without even a mention, let alone any action, during a 'herd visit'. Generally viewing the profession as a service industry, in which the paying clients' views*

trump all other interests, worries me a great deal. I believe the vet should at least put some effort into advocating for the animal. The other parties to the contract are able to advocate theirs without the vet's help, but the animal is voiceless. Being more aware of animal welfare science would help with this, but sometimes knowledge is adequate and there is a lack of willingness or empathy." Veterinarians need to harness an extremely hard task that consists in finding strategies that will attempt to satisfy all the stakeholders and maximize both animal welfare and productivity (Hewson, 2003a).

As we understood in this first development, besides the scientists, animal welfare directly involves a multitude of actors related from near and far to animal husbandry, because human beings have to care about the animals they rear. Consumers, producers, veterinarians, philosophers and ethicists, governments are all fully responsible stakeholders in this field. Moreover, the interaction among different disciplines is needed in order to carry on studies on animal welfare. In the list we can find social science, due to the role that animals play in the human society and the fact that welfare directly affect the products, hence the consumers. If anyone was not yet convinced by the stakes held in animal welfare research in regards to the respect owed to animal beings, it can be added that such research may be considered as a tool for humans who rear animals and rely on their performance and the products manufactured from them.

As a foreword, I would also like to stress a major outlining point in order to clarify what is underlying in the concept of animal welfare or rather what is not englobed in it. Much of the discussion about the use of animals, until relatively recently, crystallized on whether or not they should be killed. This is an important ethical issue. However, questions about whether or not man should kill animals or exploit them for food, clothing, and research or as unwanted pets need not be related to questions about welfare. Nevertheless, there is a fine line between those two considerations: if an animal is suddenly shot, with no previous warning, and it dies immediately, then there is a moral question about whether such killing should occur but there is absolutely no welfare problem. In contrast, if an animal dies slowly with much pain, or if the shot intended for killing results in pain and challenges in normal living, then its welfare is poor. The animal welfare issue is what happens before death, though including how they are treated during the very last part of their lives, often the pre-slaughter period and then the method by which they are killed. Haynes warns that this

position could represent a danger if it ends up in ignoring or inadequately considering the ethical question of whether or not it is acceptable to kill animals (Haynes, 2008).

1.1.3. **Brief history of animal welfare**

First and foremost, we must start with the moral origin building up the roots of the early welfare history. It is important to understand that animals have always had welfare. It is the knowledge humans possess about it that has changed over time, especially recently. According to Donald Broom, what is categorized by humans as a moral action or not has presumably changed little over thousands of years. Besides, not harming, or even helping others are effective strategies, especially for animals that live in long-lasting social groups. Ergo, the greatest evolution in this matter has been the broadening in categories of individuals who are considered to deserve to be treated in a moral way (Broom, 2003).

A major landmark in the timeline of animal welfare is Bentham statement, in the eighteen century, formulating that the key interrogation about animals was not can they reason but do they suffer (Bentham, 1789)? It resulted in upgrading the concept of "suffering" at the very top of the main issues involved in welfare. This view of suffering as a defining point was quite a commonplace up to the nineteenth century (Duncan I. , The changing concept of animal sentience, 2006) but came to be a source of reluctance due to the fact that is it tough to measure it.

Later on, from the 1960s to the 1980s, we could witness an important pivotal period for welfare with, as a first footstep and trend launcher, the publication of Ruth Harrison's book "Animal Machines" in 1964 (Harrison, 1964). In the latter, Harrison emphasizes that those involved in the animal production industry were often treating animals like inanimate machines rather than living individuals. As a consequence of this book, in 1965 the British government set up the Brambell Committee, a committee chaired by Professor F. Rogers Brambell, to report on the matter (Brambell, 1965). This is considered to be the birth of animal welfare as a "formal discipline". The adoption of a conventional scientific approach, with experiments focusing on the effects of single factors under controlled circumstances (Sandøe *et al.*, 2003), allowed the new discipline to be established as a science, or as "a young science" (Millman *et al.*, 2004).

Amongst the Brambell committee members, Thorpe was the zoologist and ethologist who pointed out that a comprehension of the biology of the animals is important and stated that animals have needs with a biological basis, including some needs to express "natural" behaviors, and that animals would have problems if there were frustration of those needs (Thorpe, 1965). A long debate has been raised among researchers on animal welfare about the term "need". According to Fraser and Broom "*the general term 'need' is used to refer to a deficiency in an animal which can be remedied by obtaining a particular resource or responding to a particular environmental or bodily stimulus.*" (Fraser & Broom, 1997). Considering animal welfare in practice, the animal may be interacting with a variety of factors that may represent the fulfilling of the "needs" that is to say requirements for obtaining physical and mental health (Odendaal, 1998). Of course, the needs vary according to the species and their evolution, and may be divided into different categories, which may be stowed in two main shelves. First, the environmental needs, such as housing and management which comprise handling breeding, hygiene, transport, etc. Secondly, the physiological and behavioral needs, which include the opportunity to express the main specific biological functions as well as the behavioral repertoire.

The biological functional systems and the motivational state determine the variety of each animal's needs and their prioritization. (Baxter, 1988; Broom, 1988; Hugues & Duncan, 1988). According to those definitions, the impossibility of satisfying the needs raises welfare problems. Knowing about the animals' needs is logically directly related to the proposal of giving animals some "freedoms". This is how the latter notion has been thought and introduced by the British team as a major key to comprehend animal welfare. This came to be worded in the Brambell Report as the "five freedoms". It was revised by the Farm Animal Welfare Council in 1993 as follows (FAWC, 1993):

"- Freedom from thirst, hunger and malnutrition – by ready access to fresh water and diet to maintain full health and vigour;

- Freedom from discomfort – by providing a suitable environment including shelter and a comfortable resting area;

- Freedom from pain, injury and disease – by prevention or rapid diagnosis and treatment

- Freedom to express normal behavior – by providing sufficient space, proper facilities and company of the animal's own kind;

- Freedom from fear and distress – by ensuring conditions which avoid mental suffering."

According to Webster, *"absolute attainment of all five freedoms is unrealistic,"* but these freedoms are an *"attempt to make the best of a complex and difficult situation"*. (Webster, 1994). Another major thrust in the advent of animal welfare has been the research by ethologists and psychologists on motivation systems. The successive studies of Neal Miller, Robert Hinde, David McFarland and others helped ethologists to understand what has been named control systems and how animals came to take decisions (Miller, 1959; Hinde, 1970; McFarland DJ, 1975). In a review entitled "Biology of Behaviour" (Broom, 1981), Broom presented animals as sophisticated decision-makers in almost all aspects of what they did. It contrasted greatly with the then widespread but subsequently discredited opinion considering animals as automata driven by "instinct".

Besides being more complex than we previously thought, animals were then proven to possess "sentience". Even if the latter concept had been already given scientific validity by Darwin (Webster, 2006), it has only become an important issue after the publication of Griffin's book on it (Griffin D. , 1976). And, based on this view, animal welfare was slowly allowed to go once more beyond the realm of the scientific research and find itself in the legal field; another example illustrating that the evolution of science precedes and leads the evolution of legislation. In fact, thanks to the Amsterdam Treaty in 1997, animals are now officially acknowledged as "sentient beings", which confers special consideration for them under European Law (Millman *et al*., 2004).

1.2. Trying to define animal welfare

If I wanted to gather in a nutshell all the literature I could browse, and the state of the art on the research, I would retain the complexity to grasp and to define animal welfare as a scientific concept. The possibility to define the term has constituted a great debate topic since the 1980s.

Furthermore, the term welfare has been used extensively in a lot of contents like scientific articles, laws and more generally discussions about laboratory, farm and companion animals' husbandry and housing. Hence, the term "animal welfare" is more and more heard from various mouths and trades: corporations, consumers, veterinarians, politicians, etc. As all terms which are used and overused, welfare is very often badly used and ends up meaning different things to different people. Even in the scientific community

and in the available literature, the term is not uniformly used. This semantic mess could also be due to the different attitudes towards animals, but implies also the different methodologies used to evaluate welfare (Weber & Zarate, 2005). Among others, some confusion persist between the terms welfare and well-being, which in dictionaries are respectively: *"the state of being or doing well"* and *"a good or satisfactory condition of existence"*, which are linked to the concept of "quality of life" (Fraser, 1998).

According to the European Food Safety Authority, the term of welfare must reflect a clear concept, which can be scientifically assessed (EFSA, 2006). It should be possible for the word to be used by the scientific community and included in legislation (Broom, 1991). The definition should also make the meaning of animal welfare intelligible to the various categories of people previously cited (Hewson, 2003a).

1.2.1. **Defining welfare in order to assess it**

In this ocean of divergence amongst animal welfare scientists, there has been at least one fundamental agreement in the early 1990s and later claiming that animal welfare is measurable and hence is a scientific concept (Fraser, 2008). According to Broom "animal welfare" is used to describe a quality, thought to be measurable, of a living animal at a particular time. Those criteria allow to qualify welfare as a scientific concept (Broom, 2011).

The Brambell Committee did not offer a proper definition in their report. They stated it meant that the animal was in harmony with nature, or with its environment. Although this statement is biologically relevant, it is not a usable definition as it is a single state and does not allow direct scientific measurement (how can the animal be in harmony? How much it is?).

Broom then offered this definition: *"the welfare of an individual is its state as regards its attempts to cope with its environment"*, introducing the notion of "coping" in welfare (Broom, 1986). Coping means having control of mental and bodily stability (Broom & Johnson, 1993). But then again, how does the animal cope? There are numerous different ways to do so, using behavioral, physiological, immunological and other strategical mechanisms that are coordinated from the conductor that is the brain. The animal may use one or more coping strategies to face an environmental challenge. Hence, a wide range of

measures of welfare may be needed to assess it. This concept asks the very fundamental question of the precise biological bases of welfare.

As welfare appears to be a concept tricky to define, we therefore can logically foresee the difficulties of its assessment. A very eloquent example illustrating how thorny it is to comprehend and evaluate animal welfare, is the issue of the use of gestation stalls in pigs as David Fraser exposes in review (Fraser, 2008). A scientific committee, created by the European Union in 1997, had the mission to review the literature on the welfare of intensively kept pigs. Among other questions, they inquired whether there were welfare problems caused by the housing of sows in „gestation stalls" where they are unable to walk, socialize, or perform the majority of the species natural behaviors during most of the pregnancy length. The review concluded that, *"some serious welfare problems for sows persist even in the best stall-housing system"* (Borell von *et al*., 1997: section 5.2.11), and a directive was thus adopted by the European Union to ban the gestation stalls as of 2013.

Four years later, a group of Australian scientists reviewed much the same literature and asked much the same question. But surprisingly, they concluded with essentially the opposite culmination stating that *"Both individual [including stalls] and group housing can meet the welfare requirements of pigs"* (Barnett *et al*., 2001: page 13). And the team to add that *"public perceptions may result in difficulties with the concept of confinement housing"* but that *"the issue of public perception should not be confused with welfare"*. The United State swine industry has used this review to promote gestation stalls, stating that there is no scientific basis for eliminating them.

One can then wonder, legitimately, how those two teams of scientists could review the same scientific content and still end up with opposite outcomes. Is there a fundamental problem with the field of animal welfare science? Is the field less scientific than had been claimed? If we look deeper in both the analyses, we notice that they were based on different conceptions and value frameworks of animal welfare.

The Australian team started from the premise that basic health and biological functioning of the animals as substantial key to assess welfare. They used "*widely accepted criteria of poor welfare such as health, immunology, injuries, growth rate, and nitrogen balance*" (Barnett *et al*., 2001: page 3). They surely did not deny that affective states are involved in the concept of welfare as much as they are part of the animal's apparatus for

survival and reproduction. But, they stated that if there would be any significant risk to welfare, it would affect functioning variables, resulting in *"consequent effects on fitness variables such as growth, reproduction, injury, and health"* (page 3).

On the other side, the European team emphasized affective states such as fear and frustration and directly included them in addition to basic health in the welfare evaluation process. They did so claiming that, *"suffering is one of the most important aspects of poor welfare and we should investigate the existence of good or bad feelings wherever possible when trying to assess welfare"* (von Borell *et al*., 1997: section 1.2). The important view is they assumed that these problems would not necessarily affect functioning-based variables such as growth, reproduction, injury and health. They also included natural living and the possibility to carry out natural behavior in the assessment of welfare, stating that *"sow welfare will be worse in conditions where exploration of a complex environment, rooting in a soft substratum and manipulation of materials such as straw are not possible"* (section 5.2.1).

It looks very unsatisfactory to have scientists drawing opposite conclusions because they insist on using different criteria to assess the key concept in their field. As Fraser wittily notes, *"meteorologists would be in chaos if they used conflicting ways of measuring temperature, or cytologists if they disagreed on what a cell is"* (Fraser, 2003). The different conclusions reached by the European and Australian reviews were due, at least in part, to the different value frameworks adopted by the groups, which led to different criteria for assessing animal welfare. We can see clearly how different areas of emphasis in the concept of welfare, can lead scientists to use quite different criteria in its assessment. As noted by Mason and Mendl (Mason & Mendl, 1993), as long as there are conceptual disagreements about what animal welfare entails, it will of course be impossible to achieve consensus that any single measure adequately reflects all of the contributing variables and accords to each its correct weighting.

1.2.2. **Welfare and ethics**

As we have already learned at the beginning of this chapter (paragraph 1.1.2), the discipline of ethics is historically very strongly bound to the study of welfare and is it sometimes hard to delineate the extent of this philosophical branch inside the scientific reasoning tree. Furthermore, there is some kind of dissension between scientists studying animal welfare and philosophers writing about animal ethics, those two having essentially two distinct cultures (Fraser, 1999). In one hand, philosophers tend *"to focus only at the level of the individual, advocating single ethical principles and seeking solutions through ethical theory with little recourse to empirical knowledge."* From this viewpoint, some questions have been identified, such as *"what is the baseline standard for morally acceptable animal welfare? What is a good animal life? What farming purposes are legitimate? What kind of compromises are acceptable in a less-than-perfect world?"* (Sandøe *et al*., 2003). On the other hand, scientists were at first claiming that suffering and other subjective feelings experienced by animals are beyond scientific enquiry. It is easy to get that it might then be hard for those two groups of thinkers to stand on the same positions.

It even sometimes seems impossible for those two fields to coexist as studies are almost always leading to opposite conclusions. The fact is, these two schools of thoughts are inherently tangled and need each other, as shown in the two following examples, which are examples chosen by Fraser in his article of 2003 (Fraser, 2003):

The first example takes place in a fictional food hygiene laboratory. A team of nutrition scientists is trying to conduct a scientific evaluation of the quality of bread in order to steer consumers to buy good bread. They have equipment capable of measuring some basic nutrient levels such as protein and minerals. They do not have an assay for mould-derived toxins, and they are sceptical of the less objective yet usual methods used to assess freshness, texture, and flavour. Assuming that nutrients are important components of bread quality, they merge their various nutrient measurements into a "bread quality index" and end up concluding that stale, mouldy bread is equal in quality to freshly baked bread. Through this anecdote, we are able to grasp how the scientists failed to distinguish between scientific concepts, using the most objective measures available, and socially constructed concepts. Concepts such as metabolic rate are scientific concepts invented within, and taking exclusively their meaning from, a field of science. Such concepts might, of course, come to be in use in the popular culture. For instance, an overweight person might blame his

corpulence on a low metabolic rate. Whether this is a true fact is an empirical inquiry and can be verified by a physiologist through certain standardized measurements. Contrariwise, concepts such as the health of a person or the quality of bread are socially constructed: they arose in society and have meaning in everyday speech independent of their adoption into scientific discourse. Science can surely be applied to these subjects, but if scientists attempt to define socially constructed concepts in terms of scientific variables, they must be careful not to miss or misconstrue the social meaning of the term, or their research may prove irrelevant to its intended social purpose.

Undoubtly, animal welfare is a socially constructed concept. It belonged to social discourse before it fell within scientific research, and it is commonly applied to everyday speech to refer to the animals' quality of life. Hence, when scientists attempt to assess it, they need to ensure that their scientific measures reflect the socially constructed meaning of the term (Tannenbaum 1991; Stafleu *et al*., 1996). Ethicists, humanitarians, and consumers tend to bring to the fore affective states. By spotlighting these features in their assessment of welfare, the European reviewers came to align their work with this widely held social meaning of the term, thus dodging the moldy bread error.

Let's turn to another story with a whole other setting. We are now placed in an (also fictional) bedroom. A young boy has caught a frog and wish to make it his new pet friend. Of course, he wants to give the amphibian the best possible care. Scared that the frog would get chilly and exhausted after crawling in swamps and hunting flies, he tucked the frog into his own warm, dry bed with a handful of peppermints. Huge was his grief when he found his little companion dried off to death the next morning. This wistful anecdote can teach us something about animal welfare, reminding us that animals themselves have certain interests, and that, ultimately, these provide the ultimate criteria for welfare. Through scientific knowledge, we can make wiser judgements about what is good or bad for animal beings. Scientific reasoning fundamentally carries in the field a significant progress on uninformed opinion or simplistic extrapolation from humans to other species. It allows to understand the basic and applied meaning of animal welfare: animals show to us their welfare level through their physiological and behavioral reactions to treatment by humans, and these reactions can be measured and evaluated by science. The Australian reviewers, seem particularly mindful of the dry frog error. They emphasized the danger of relying on mere public perceptions of animal welfare, noting that "*public perceptions may result in*

difficulties with the concept of confinement housing... [but]... the issue of public perception should not be confused with welfare" (Barnett *et al.*, 2001). In relying on functioning-based variables, the Australian team adopted a meaning of the term that does not correspond well to the social meaning, deviating from the ethical views of philosophers, humanitarians and even consumers.

It is irrevocable that animal welfare has always to maintain a scientific position, if only in order to gain an increasingly precise role in animal science. It should follow the road of the scientific study and not the one of philosophy and ethics. That does not detract from the fact that those two matters are inherently interrelated, acknowledging that welfare was born in the cradle of ethics and essentially carries and needs values and judgements (Broom, 2011): no application of the science can occur without understanding arguments about ethical positions. Thus, animal welfare, as a scientific field, may be connected, although not necessarily, to the ethical viewpoints in an increasing convergence of science and philosophy. Indeed, lack of communication between ethicists and scientists should not happen, in order to avoid extreme views in either camp. This dynamic is illustrated by the fact that ethicists began to look at empirical research to solve ethics matters, while welfare scientists started to recognize the importance of subjective experiences (Lund *et al.*, 2006).

1.2.3. **Welfare and health**

As we already mentioned, in 1986 Broom introduced the notion of coping. It was instinctively first interpreted in terms of coping with pathology, putting health on top of the criteria to evaluate good animal welfare. It is the functional approach of welfare. Understandably, in the past, farmers and veterinarians have contained animal welfare chiefly in terms of the body and the physical environment such as shelter and feed: if an animal is healthy and producing well, it is faring well, and improving animal welfare then principally means curing or preventing disease. This is how clinical knowledge, and made I dare even say veterinary medicine, came to be developed in order to make sure that the health of animals was properly considered in evaluation of welfare (Blood & Studdert, 1988). McGlone defends that biological functioning is definitive of animal welfare, and snipes at attempts to relate animal welfare to subjective states such as suffering: *"an animal is in a poor state of welfare only when physiological systems are disturbed to the point that survival or reproduction are impaired."* (McGlone, 1993).

This corresponds to the Australian team's view we talked about in paragraph 1.2.1. According to this view, modern production methods, although they may appear unnatural and restrictive, are good for animal welfare as long as the animals are healthy, growing, and reproducing well. Thus, just like the Australian reviewers, one commentator espoused intensive production systems because: "*on balance... the animal is better cared for; it is certainly much freer from disease and attack by its mates; it receives much better attention from the attendants, is sure of shelter and bedding and a reasonable amount of good food and water*." (Taylor, 1972). This first perspective, emphasizing biological functioning, is commonly heard among those who are involved in animal production (te Velde *et al*., 2002).

However, there are limitations to seeing animal welfare only in terms of the body. One first argument against it is that the environment, or even genetics, can produce desirable physical and production outcomes, even though the animal's mental state is compromised. We can take the example of a dog breed champion which may have perfect conformation and be in perfect health, but which may be very anxious in its home environment. Terlouw *et al.* findings also illustrate this theory, this time with sows, proving that an animal can be healthy and physiologically normal, but sporting stereotypies suggestive of an unbalanced mental state hence poor welfare (Terlow *et al*., 1991). Another risky edge to this view is that some physical parameters - such as heart rate, plasma cortisol - are difficult to interpret, because they can be increased by both positive and negative experiences, such as the presence of a mate and the presence of a predator (Hewson, 2003b). Furthermore, the presence of the physiological state of stress does not necessarily induce reduced welfare. Likewise, the absence of a stress response does not always mean good welfare (Duncan, 2005). These arguments would suggest that animal welfare includes not only the state of the animal's body, but also its feelings. In the light of these arguments, already in 1993, Duncan became one of the trendsetter and first spokesperson claiming that affective states are definitive of animal welfare*: "... neither health nor lack of stress nor fitness is necessary and/or sufficient to conclude that an animal has good welfare. Welfare is dependent on what animals feel" (*Duncan, 1993*).*

1.2.4. **Welfare and feelings**

This brings us to the second scientific view that can be taken to define and assess animal welfare. This view historically bloomed thanks to the emergence of more and more knowledge about animal behavior. Yet, it has first been frantically rejected by the scientific community, arguing that feelings are impossible to be directly measured, making affective states neither available nor reliable for scientific investigation. It was stated by many that, although subjective experiences were inherently important, they could not be readily studied, leaving welfarists to use biological functioning measures as the most practical approach (Gonyou, 1993). This is how the functional approach got favored at first. It has also been subject to a fierce debate between behaviorists and ethologists: at the beginning the American school of Behaviourism did not welcome in the scientific terminology *"all subjective terms such as sensation, perception, image, desire and even thinking and emotion"* (Watson, 1928). Originally ethologists also confined their considerations to the behavior they could observe, although employing terms like "hunger", "pain", "fear" and "frustration" (Duncan, 2006). Nourished by the development of research in this field, psychology and ethology started to collaborate. Thus, picking up on the past belief that we could never know how animals feel, but only how they behave, some ethologists, such as Dawkins (Dawkins, 1980) and cognitive psychologists, such as Toates (Toates, 1986), have intensified all their efforts to try understand the animal minds by carrying out extensive research on animals' perception, decision making, self-awareness and capacity to learn from others. It has then become apparent that mammals and birds, and possibly other animals are capable of advanced thought processes (Dawkins, 1998; Varner, 1999; Griffin, 2001).

Deepening our knowledge of animals' minds then made it possible to better grasp animals' subjective experiences, both positive and negative. These studies progressively painted a clear picture of the animals' perception of the world and how environment may affect welfare. Such research is to be seen in parallel to the emergence of the concept of fundamental behavioral needs, addressed in paragraph 1.1.3, which is deep-rooted in the construction of the concept of welfare. *(*Petherick & Rushen, 1997). If welfare consists in fulfilling these needs and if, as Duncan states, feelings have evolved to protect the animal's primary needs (Duncan, 2002), then it is logical to consider feelings as key criteria when assessing welfare. Duncan is a strong advocate of this view emphasizing the psychological aspects of welfare. The European reviewers clearly also regarded affective states as

inherently substantial for animal welfare. Indeed, they could discern signs that sows are "*frustrated*" (Borell von *et al.*, 1997: section 5.2.2) and find the installation "*aversive*" (5.2.1) to finally conclude that gestation stalls raise welfare problems.

Animals can indeed suffer from emotional disorders such as boredom, stress, and frustration if they cannot meet their behavioral needs, and that this suffering is detrimental to their welfare. According to Duncan again (Duncan, 2002), welfare corresponds to the absence of *"negative subjective emotional states, usually called suffering"*, probably accompanied by the presence of *"positive subjective emotional states, usually called pleasure"*. He defines suffering as *"a wide range of unpleasant emotional states" (*Duncan & Dawkins, 1983*).* According to Dawkins, suffering occurs *"when unpleasant subjective feelings are acute or continue for a long time because an animal is unable to carry out the actions that would normally reduce risks to life and reproduction in those circumstances"* (Dawkins, 1990).

Contrary to what had been alleged by its detractors, this feelings-based approach is not confined in theoretical lands and appears to be tangible and measurable. Typically what we measure are behavioral outcomes. Among those, we can name the willingness to "work" (for instance when an animal pushes open a weighted door) or the behavioral signs of fear or frustration. By knowing the biological needs of animals, we are then able to maintain a scientific approach in research, studying the links between these biological needs and the consequences on the organism when they are fulfilled or not. In this respect, it is however mandatory to preserve a critical attitude towards the scientific meaning of biological needs, avoiding by any means anthropomorphic interpretations (Morton *et al*., 1990).

As I am sure you reader already suspect, in the same way that defining welfare only in terms of body raised an issue, there is also controversy defining welfare only with the notion of feelings as they are just one part of an animal's catalogue of coping mechanisms. Pain, fear and the diverse forms of pleasure, may be part of the coping strategies. Feelings are thus a key part of welfare as we just exposed, but not all of it. To illustrate these misgivings, Broom gives the examples of an individual with a broken leg but asleep, a drug addict who has just taken heroin, an individual greatly affected by disease but unaware of it, an injured individual whose pain system does not function (Broom, 1998). Similarly, some pigs only show light avoidance of permanent exposure to high levels of ammonia, even though these levels are highly detrimental to the porcine respiratory system (Jones *et al*.

1998). These animals are compared to human smokers by Fraser *et al.* They appear not to experience any discomfort until the damage is serious. The damage may seriously affect the biological functioning of animals, yet the animals can show no evidence of harm, no sign of avoidance, nor any expression of suffering, at least until pathological changes are well established. In such cases, we witness the limit of an animal welfare defined exclusively by feelings as *"animals may undergo impaired biological functioning without necessarily showing evidence of effects on subjective feelings"* (Fraser *et al*., 1997).

1.2.5. **Welfare and natural-living**

We arrive now to the third view of welfare, the natural-living approach, which is quite coupled to the feelings-based approach. Indeed, we understood in the previous paragraph that the concept of welfare is linked to the deep creed that animals fare best if they can fulfill their "needs" that we can schematically summarize by living accordingly to their nature and species-specific behavior. The latter notion actually represent one of the earliest natural-living approaches, legitimate daughter the theory of evolution, stating that welfare would be reduced if animals could not express their whole "behavioral repertoire": *"If we believe in evolution... then in order to avoid suffering, it is necessary over a period of time for the animal to perform all the behaviors in its repertoire because it is all functional..."* (Kiley-Worthington, 1989).

This approach arises partly from a critic formulated against the functional approach. De Passile *et al.* use the example a suckling calf to illustrate the failure of the latter. A calf in an intensive farm is fed milk from a bucket. Although, the farmer may observe that the cub makes constant attempts to suck on neighboring the surrounding females (de Passille *et al.*, 1992). It is presumably due to the strong motivation to suck which used to be important for adequate milk intake in the environment of evolution. Preventing this behavior can leave the calf with an unfulfilled desire to suck. Yet, the behavior's original function is now met (by other means) and the calf is now replete. Thus, prevention of the behavior did not necessarily lead to clear impairment of biological functioning such as malnutrition but might lead to other detrimental effects for the offspring (de Passille *et al.*, 1993). This is how some "stereotypies" are explained by behaviorists: they express the persistent inclination of the animal to perform actions that are though no longer required for survival in the new environment (Rushen *et al.*, 1993). Animals then experience negative subjective feelings, or

fail to experience positive ones but do not necessarily show impairment of their "functioning". Sambraus (1981) called this phenomenon "immaterial suffering".

Animals should thus be allowed to live according to their natural attitudes and behavior, adopting and developing their natural adaptations. However, this third view goes even further in the reasoning stating good welfare is characterized by the degree of naturalness in which animals are kept and the ability of an animal to live according to its "nature". This third view is strongly and essentially bound to ethical concerns, stepping on the edge of welfare's realm, that philosophers formulate about the human impact on animals, their utilization and more generally the disruption of the natural global order. It is in that sense that it might be the view that bridges best the two cultures.

Respecting an animal's welfare entails the nurturing and fulfilment of the animal's genetically encoded "nature". What does this encoded "nature" consist of if not only a behavior set? More generally, it can be defined as the set of adaptations that an animal possesses as a result of its evolutionary history, and its individual experiences and the set of genetically encoded instructions that shepherd the animal's normal ontogenetic development (Rollin, 1993). Hence, to state that animal beings should be allowed to live according to their "natures" means that animals should be allowed to live in a manner that corresponds to their adaptations and to process to the type of ontogenetic development that is normal for the species they belong to. To put it in other words, it does not only revolve around the frequency of performance of certain behaviors, but consists in the set of conditional rules, blended in an internal machine, that govern the performance of the different subsequently expressed behaviors. For example, the adaptations of a pig to external temperature does not simply encompass the actions of panting and huddling, but the conditional rules deciding when he should pant (when it is hot) and when he should huddle (when it is cold). In the light of this, keeping a pig according to its natural adaptations does not only mean the occurrence of panting and huddling, but it carries the possibility for the swine to be able to use these adaptations when circumstances require them (Fraser *et al.*, 1997).

This view of animal welfare is the one largely favored by the general public. It was found to be the preferred one among consumers of animal products (te Velde *et al*., 2002). Lindgren expresses well in this quote what a great majority of them feel and think: *"(...) it might even be possible to guarantee that young animals... get a little summertime happiness, at least a temporary reprieve from the floors of barns and the crowded spaces where the*

poor animals are stored until they die. Let them see the sun just once, get away from the murderous roar of the fans. Let them get to breathe fresh air for once, instead of manure gas" (Lindgren: Anonymous, 1989). It is understandable how this perspective came to be adopted by many, considering the boom of highly restrictive and unnatural forms of animal housing we witnessed the past five decades.

However, when consumers and politicians value this view very much, the scientific community expresses reserve due to the fact that domestic animals differ in many ways from their conspecifics in the wild and from the past (Price, 1984). It may be very difficult to evaluate the impact of domestication processes and to discern the implications for welfare not to live like wild ancestors for animals that cannot be considered as identical to those. More generally, this view sometimes turns out not to be empirical enough and this causes the arising of conceptual problems when trying to define the "nature" of a given animal (Duncan & Fraser 1997). Besides, living in a natural way does no essentially guarantee the fulfillment of the full range of ethical concerns over the quality of life of animals. Indeed, even if an animal is kept according to its adaptations, it may still suffer and become ill if these adaptations are not sufficient to meet the challenges it experiences. Physical suffering, such as feeling cold, and mental suffering, such as the fear of a predator, are acceptable according to this precept but appears to be inacceptable to others. It is once more a value-laden issue. It is depicted by Fraser *et al.* with the two dog paradox: *"two dog-owners met one day to walk their dogs together. One owner had grown up in a small family that valued health, safety, and orderly, disciplined behavior. The dog of this owner received regular veterinary care, two meals a day of low-fat dog food, and was walked on a leash. The other owner had grown up in a large community that valued conviviality, sharing of resources and close contact with the natural world. This dog (the owner's third - the first two had been killed by cars) had burrs in its coat, was fed generously but sporadically, and had never worn a collar in its life. Each owner, judging quality of life from very different viewpoints, felt sorry for the other's dog."* (Fraser *et al.*, 1997). Last but not least, the concept provides little guidance on many important animal welfare issues, especially issues met "in the field", such as the use of analgesia, euthanasia and medication. Thus, although this natural-living view is a useful way to expand our notion of animal welfare, it unfortunately does not provide sufficient criterion for defining the concept in its entirety.

1.2.6. **Welfare as "Body and Soul"**

As we saw in the last paragraphs, we can count three overlapping major outlooks looming out of the stormy discussion about the best definition of welfare. To sum it up, in one view, animals' biological functioning constitute the most important assessment system: to state that animals fare well, scientists proposed to verify only that animals have a normally functioning physiology which is reflected by satisfactory health, growth and reproduction. The second outlook we mentioned is based on the fact that animals should feel well by experiencing pleasure or pleasurable feelings and contrariwise being free from prolonged and intense fear, pain, and other negative states. In a more recent view, the emphasis is placed on the necessity for animals to lead natural lives in accordance to the development and use of their natural adaptations. Up to now, most scientists have offered quite restricted conceptions of animal welfare that relate to only one these views. At the foot of these conceptions and the choice which is made alternatively in favor of one or the other, value-laden positions can be found about what is considered as substantially important for the quality of life of animals. *"It would be comforting to think that science could simply set things straight by replacing these different, value-dependent views of animal welfare with objective data about what is truly better for animals. In fact, however, scientists tend to bring to animal welfare assessment much the same three value frameworks outlined above"* (Fraser, 2003). Indeed, very often it is also blended with the attribute of human responsibility for animals under their care. Moreover, the choice is much done because of practical claims, in the interest of clarity and for the sake of the simplicity of measures. This is how the functional approach has been urged in the past as it was believed that subjective experiences fell outside the realm of scientific enquiry, and that evaluating the biological functioning was enough because subjective experiences and functioning are closely correlated and impact each other. Fraser argues that none of these positions, alone, provides a completely satisfactory guidance for animal welfare investigations (Fraser *et al.*, 1997). Furthermore not adopting broad enough definitions will always lead to disagreements amongst welfarists as we saw for porcine gestation stalls, which is inacceptable.

It shouldn't be a question of rejecting one or another of these views. They have to be placed all three at the same level of importance. Particularly, the contemporarily scorned functional view shouldn't be left aside. It is crucial in many ways: assessing welfare of animals having normal behavior according to their species and showing acceptable

emotional state and still presenting evidences of subclinical disease. Furthermore, the indicators offered by this view are often quite convenient for the science to measure them. Affective states are so closely tied to biological functioning that measures of biological functioning should be able to identify problems involving affective states. However, unlike what Baxter claimed (Baxter, 1983), it does not necessarily lead us to exclude the study of subjective emotions form welfare research. Rather, the functional approach should gently be shifting towards more subjective considerations, smoothly embracing the "feelings" approach in a will to see the organism's biological functioning in a "holistic" approach (Broom, 1998). Soaking up all the different information the three views are able to provide, the organisms can be studied in a more comprehensive way when all these information are assembled together. Indeed, the three views of animal welfare are by no means mutually exclusive, contrary to what their proponents sometimes seem to assume, urging that their own view of welfare encompasses the others. *"Thus, the most widely accepted definition of animal welfare is that it comprises the state of the animal's body and mind, and the extent to which its nature (genetic traits manifest in breed and temperament) is satisfied"* (Duncan & Fraser, 1997). All the physical, psychological and evolutional components, concurring in assessing the animals' welfare level, have to be studied and linked together. Not to mention that such an approach allows different parameters, picked from one or another view and uncertain when considered individually, to be validated. Physiological and behavioral measures for instance can thus be approved and legitimized by each other as they all match in the global picture of the animal's general state and welfare.

This comprehensive approach was recently proposed by Dockès and Kling-Eveillard (2006). They claimed that animal welfare should be examined according to four main issues. Firstly, the fundamental needs and freedoms animals should be provided with have to be highlighted and primarily judged as per biological and technical definitions. Secondly, they stress the importance of a regulation approach, in which animals are recognized as a sentient beings who need to be put in conditions compatible with the biological needs of the species. This point constitutes the junction point between science and law, guiding towards the translation of the concepts into laws. Thirdly, ethical values we discussed should be included through a philosophical approach, which acknowledges the "animal's status" and its role in the human society. Fourthly, they bring the communication between man and animal to the forefront, conferring much importance to the farmer-animal interaction and its effects on industrial breeding systems. Those four issues are thought by Dockès and Kling-Eveillard to

represent the whole meaning of animal welfare and its implications for animal husbandry as they include body, mind and the implications for humans in order to understand how to treat animals. It is based on this approach I choose to build the welfare assessment system used in the protocol as presented later (paragraph 3.2).

1.3. Stress and its relationship to welfare

When I dived into the literature on music and the studies on its effect on animals, I came upon the term "stress" a lot. Just like the term welfare, the term stress is very much used, in various fields such as physics, physiology, psychology and pathology with somewhat different meanings in each field. Alternately, stress is referred to as an environmental change which affects an organism, as the process of affecting the organism, as the physiological response mechanism or as the consequences of effects on the organism. Unfortunately this has led to confusion about the use of the word, especially in discussions about animal welfare. In the 70s and 80s, at the same time as the discussions about the definition of welfare, the scientific use of the term stress was being questioned. Despite this semantic confusion, the concept of stress is fundamental if we are to understand biological functioning in relation or not to welfare. Rather than definitely discarding it, we should refine and use it. It also appears essential to me to understand how this word has been used, is now used and what it precisely entails and implies for animal welfare. Quoting Moberg: *"understanding the biology of animal stress should be a primary duty of animal welfare scientists and students"*.

1.3.1. Another concept hard to define

The concept of stress was introduced by Selye at the beginning of the 20th century (Selye, A syndrome produced by diverse nocuous agents. , 1936). The Austro-Hungarian scientist, founding father of the concept, defined stress as the unspecific response of the body to external challenges such as pathogens or a harsh physical environment (heat, cold, electric shock, etc.). Besides some specific effects (such as sweat in response to heat for instance), any agent can trigger a nonspecific response needed to *"re-establish normalcy"*. Selye conducted experiments on rats, exposing them to different aversive agents. It appeared that all the rats showed the same symptoms, whatever the stressor. Selye called these symptoms the alarm response: enlargement of the adrenals, atrophy of the thymus and lymph nodes,

and gastric ulcers (Selye, 1973). He decided to name the whole range of modification the "general adaptation syndrome", qualifying stress physiology as the physiology of adaptation. Indeed, stress was thought to occur when the homeostasis of the animal is unbalanced or at risk i.e. when a gap digs itself between the actual environment and the ideal environment. The wider the gap, the higher the stress response (Selye, 1973). Gaining more and more knowledge of the functioning of the HPA axis, Selye and some of his contemporaries assumed that stress could be equated with the activity of this system. More precisely, Selye theorized that the stimulation of the anterior pituitary gland and the adrenal cortex, provoking an increase in the secretion of the hormone ACTH and glucocorticoids, had, as Mason summarized in 1968 *"a unique, pre-eminent, and non-specific*" relation to stress.

However, Mason pointed out the ambiguity of this conception where HPA mechanisms are presented as general situations when they are not (Mason, 1968; Dantzer & Morme`de, 1979). Indeed, he states there are many coping systems of an individual in front of adversity. Hence, it is incorrect to speak of „the stress response" if this implies that there is only one. There are in fact many different responses which are used by individuals in challenging and potentially adverse situations. Another meaning which has been ascribed by Selye to stress made it largely synonymous with stimulation. Nonetheless, if every impact of the environment on an organism is called stress, then the term has no value. Hence it is now pointless to speak of stress as the process of response to any environmental event. Rather, an effect of the environment on an individual which induces a response which is beneficial in the long-term should be qualified as stimulation rather than stress.

More recently, the concept of allostasis, defined as stability through changes, has been introduced. It states that any challenge an animal may be facing leads to modifications of the functioning of that animal; and these changes arm the animal to better cope with further challenges. In some circumstances, allostatic systems may not perform normally because of overstimulation or other causes. This phenomenon is called the allostatic load (McEwen, Stress, adaptation, and disease. Allostasis and allostatic load., 1998). A "new" definition of stress was thus introduced by Broom and Johnson: *„stress is an environmental effect on an individual which overtaxes its control systems and results in adverse consequences, eventually reduced fitness"* (Broom & Johnson, 1993). The environmental variable which has the previously described effect on the individual can be called a "stressor". Unlike a simple HPA activation and uniform consequences, it is now

acknowledged that the responses are showing an extreme variety: short-term and long-term effects including metabolic changes, cardiovascular malfunction, immunosuppression, increased incidence of parasitic bacterial and viral diseases as well as psychological disorders such as interludes of panic, anxiety or depression. Far from excluding the HPA axis from the concept, this definition states that it may or may not be involved but in both cases it ends up to be detrimental to the animal. There is no good stress. During the development of individuals, stimuli that result from somewhat difficult situations can be useful experience but these are best not referred to as being stressful. If the eventual effect is good, it is stimulation or challenge but not stress. This view was supported by Dantzer, von Holst, Moberg, Morme`de and Toates but was ignored by medical and most physiological researchers.

1.3.2. **How stress is related to welfare**

As we have seen, the biological implications of animal welfare are not yet so well understood. The two concepts of welfare and stress are part of an intricate web. In fact, they share many common features and are often taken for one another in scientific research. Furthermore, stress is alternately assimilated to the consequence and the origin of bad welfare. What is it then?

Both concepts have been described in terms of physiological and behavioral responses. Cortisol release, tachycardia, startle, flight and fight are used to describe stress responses as well as they were traditionally used to assess state of welfare. As we have seen in paragraph 1.1.3, welfare has been defined in relation to the adaptation of an animal to its environment. In fact, individuals are thought to be capable of adaptation through physiological and/or behavioral responses, such as withdrawal, fight, or flight. While Broom (Broom, 1991) used to word coping to describe this adaptation, which would be possible at a low cost, Carpenter (Carpenter, 1980) used the term suffering, when this state of adaptation cannot be fulfilled. For both authors, poor welfare corresponds to a failure of the adaptation mechanisms.

And this failure to cope appears to match the notion of stress, how it has been described by Broom and Johnson. Indeed, the concept of allostasis, just mentioned above, is closely related to the concept of welfare. We can state that the welfare of an animal is safeguarded if the changes in its body functioning due to a given stimuli actually help to

anticipate further challenges, that is to say if these changes help the animal to cope with to its environment. On the contrary, if the body response is provoking adverse changes, we are in the presence of stress and this leads to bad welfare. Correct and proportional adaptation to the stimuli (which is then not considered as a stressor, as previously highlighted) with no exaggerated effort nor adverse consequences is accompanied by good welfare level, whereas a large discrepancy between the stressor and the response corresponds to bad levels of welfare. The bigger the discrepancy, the worst the welfare level. However, it is often difficult to relate the level of the responses to the intensity of stress or (poor) welfare.

1.3.3. **Measuring stress, a good way to assess welfare?**

It was long sensed by welfarists that stress forms a key notion to assess animal welfare. Already in 1985, Moberg urged: *"the only defensible measurement of well-being in animals is to determine if the animal is suffering from stress. Furthermore, I believe that the most appropriate indicator of stress is the appearance of a pre-pathological state"* (Moberg, Biological response to stress: key to assessment of animal well-being?, 1985). But is it really that simple?

Some problems were met in welfare research because it has been tried to assess welfare before correctly defining stress. Indeed, influenced by the HPA focused view, Barnett and Hemsworth (1990) have proposed that a sustained 40 per cent increase in plasma-free corticosteroids constituted a solid criterion for impaired welfare. However, research going on, it was then despised, as increased glucocorticoid output is not specific to unpleasant situations but can also occur after exposure to novel environments, exercise and such presumably pleasant activities as mating and nursing. In addition to the fact that increased glucocorticoid secretion does not necessarily indicate adversity, we can also claim that not all forms of adversity increase glucocorticoid secretion: there is no uniformity in the response of the body to stressors. Based on these considerations Moberg proposed that we *"abandon any attempt to identify a single biological endpoint that is characteristic for all stressors*" (Moberg, 1992).

Now that we have defined and comprehended better the concept of stress, we can proceed to the investigation of its diagnosis in the animals. How to diagnose of stress? What are the indices of stress? In order to determine the extent of stress inflicted to an animal, it is essential to understand the physiological basis and mechanisms of the stress response.

This will allow us to identify the variables that best reflect the disturbances in homeostatic control, i.e. the indices of allostatic load. For this, Moberg shapes up a model of animal stress (Moberg, 2000). This model divides the stress reponse into three general stages: the recognition of a stressor, the biological response to it and the consequences of this response. This last stage allows to figure out whether we are in presence of a what Moberg calls a "brief stress" (what we have outcasted as a stimulus without adverse consequences on the animal's integrity) or a "distress" (what we have simply called stress, carrying adverse effect on the animal's welfare).

The whole story starts in the CNS which is able to see and recognize a potential threat to homeostasis. The fact that the stressor is actually a threat is pointless and unrelevant. The perception of the stimulus is a fundamental point. This explains how physchological threat can be so devastating (McEwen & Stellar, 1993). Then, it is the turn of the biological response which corresponds to the association of four defence systems. In the case of many stressors, the first one chronologically and the most biologically economical is the behavior system. The later allows the animal to remove itself from the threat: for instance, avoiding a predator or seeking shade when dangeroulsy increased body temperature. However, it is not appropriate or not possible for all stimuli. Furthermore, how we have previously seen, the behavior expression is greatly limited by the animal's confinement, which represents a damage to welfare and also creates difficulty for its assessment. It should also be noted that even if the behavioral response is not capable of mitigating the stressor, some behavioral components may still be embedded in every stress response, making ot a useful indice to diagnose stress. The other three defence systems are the autonomic nervous system response, the neuroendocrine response and the immune response. They largely overlap and induce each other, making it impossible to distinguish them in the animal's biological response. The associated measures, considered as good indicators to evaluate the response to stress, include body temperature, heart rate, respiration rate, feed intake and eating pattern, digestibility of feedstuffs, body weight loss or gain, immune function. Secretion and peripheral concentrations of various hormones in plasma, milk yield, milk composition and quality, udder health, pregnancy status, and viability of offspring… The list is long.

As we have seen, the success of adaptive responses and the resultant effects upon the animal may be categorized in terms of the adequacy of the compensation in the face of a challenge unbalancing the animal's homeostasis. The response can be an adequate

compensation the animal successfully regains back homeostasis, there is no stress). Yet, if there is an inadequate compensation, this is when we can observe and measure perturbations in controlled variables, suggesting the presence of stress and indicating poor welfare. More dramatically, a decompensation can happen, meaning that there is a pathological failure of the compensatory mechanisms or direct deleterious effects of the compensation. Changes in the cited variables may be measured in all three cases of response but it not an easy task to match the measured physiological parameter to the exact corresponding level of stress. One should look for alterations in the slope of a plot of one of the variable and compare it to the magnitude of the stressor. A "catastrophic" step-change in one or more of the stress indices may also indicate transition from normal to abnormal compensation. Such observations may then be used to determine the severity of the stress experienced by the animal. But sometimes it is not easily distinguishable. What is sure is that the *"highly dynamic patterns of homeostatic response observed during stress make it difficult to deduce any simple relationship between stress and welfare*" (Wiepkema & Koolhaas, 1993). Hence, the parameters listed above, thought to be helpful in the diagnosis of stress and poor welfare, should not be expected to present a clear "cut-off" point at which welfare is deemed to be at risk. Furthermore, as we will see in the following chapter with studies specific to music, the use of single and isolated parameters should be outlawed. A more integrated approach to measuring stress reactions has been advocated, preaching the use of a spectrum of different stress indices or the development of stress profiles.

2. Can music have an influence on animal welfare?

2.1. Behavioral approach

2.1.1. Promises hinted by study results on humans

To explain the genesis of the scientific questioning about a hypothetical effect music could have on animal welfare, we must first present the findings collected by the scientific community on humans. It represents the logical instinctive approach, the premise leading us to think that music could be of any impact on the animals. Many people instinctively opt for music, with its popularly well-known soothing properties, to alleviate their stress. Historically the use of music as a method of stress relief could be traced back as far as 4000 BC. Indeed, it is estimated to stretch back to Paleolithic times (West, 2017). During the last decades, music has begun to draw scientists' attention, with their arms outstretched towards the hope for an alternative healthcare method.

Indeed, the value of music for psychological well-being is well documented in humans. First, it was noticed that our moods (*e.g.* Sousou, 1997; McCraty *et al.*, 1998) and our behavior (*e.g.* Ragneskog *et al.*, 1996; Yalch & Spangenberg, 2000) could be influenced by the type of auditory stimulation we were exposed to. In their studies, McCraty and his colleagues found out that 'Grunge'-type of music, for example, seems to produce an increased hostility, sadness, tension and fatigue. Contrariwise, 'designer music' (that is to say music created to have a specific effect on the listener) was found to enhance mental clarity, vigour and relaxation (McCraty *et al.*, 1998). Related to its proven mood modulating properties, music was even found to be able to increase the amount of time spent by customers in shops (Milliman, Using background music to affect the behavior of supermarket shoppers., 1982) and in restaurants (Milliman, 1986) and how much money people spend (Areni & Kim, 1993). More recently, it was studied how music can promote sleep (De Niet *et al.*, 2009) and increase prosocial behaviors (Guéguen *et al.*, 2010).

In a 2001 study, Knight and Rickard presented results suggesting that "relaxing" music could prevent the "symptoms of stress" such as increases in subjective anxiety, systolic blood pressure, and heart rate in healthy men and women. Berbel *et al.* (Berbel *et al.*, 2007) even found that listening to "relaxing" recorded music could reduce vital signs of

anxiety as well as a diazepam treatment. Those quite encouraging results on the effects of music on stress lead to study its effect on health in a wider frame (Haake, 2011). We can here mention some of the numerous studies that have successfully proved music's beneficial effect when used as a therapeutic intervention against pain (Siedliecki & Good, 2006), nausea (Ezzone *et al.*, 1998), anxiety and depression (Hanser & Thompson, 1994). In the same perspective of developing alternative care solutions, music was also studied that music could increase tolerance for uncomfortable procedures (Bampton & Draper, 1997), reduce pain perception during those (Nelson *et al*., 2008), and decreased need for sedative medications (Nelson *et al*., 2008; Schiemann *et al*., 2002). And even more astonishing, it was proposed as a treatment modality for a group of central nervous system pathologies such as senile dementia (Sung & Chang, 2005) or even schizophrenic-like disorders (Gold *et al.*, 2009) and Alzheimer's disease (Brotons & Marti, 2003). Recent studies have reported a possible beneficial role of music in some neurological disorders such as Parkinson's disease (Haneishi, 2001) and cerebral ischemia (Noda *et al.*, 2004). Some very promising evidences were even found about possible lasting properties of the impact of music. Indeed, Sakamoto *et al.* observed that improvements in the Behavioral Pathology in Alzheimer's Disease Rating Scale (BEHAVE-AD) persisted for 3 weeks after the end of a 10-week intervention during which patients listened to recorded music selected from memorable periods in their lives (Sakamoto *et al*., 2013).

All of those findings on the effect music can have on human kind gives a hint, or at least generates a hope making us wonder if results would be the same in the rest of the animal kingdom. Historically, this is how the interest in investigating the effect of music on other species emerged.

2.1.2. **Studies on animals over the past 20 years**

First and foremost, we can start by Muller's findings in 1987. He proved that animals do have behavioral response to music and that the sound threshold expected to cause this behavioral response is 85 to 90 dB (Muller, 1987). The investigations on animals' response to auditory stimulation is included in research about what is called environmental enrichment. The latter can be defined as a technique designed to enhance the functioning of an animal through modifications to its environment (Newberry, 1995). The utmost goal of research on enrichment methods and efficiency is to help animals to handle the challenges

of captive, “unnatural” environments, hence ultimately to increase their welfare. It is supposed that an enriched environment can indeed contribute to animals’ welfare. This environment comprises physical and social realms and the management regime associated with its care, including diet (Sheperdson *et al*., 1998). This field is not new. It can be traced back to works by Robert Yerkes in 1925 and Hediger in 1950, who were the first to discuss components of enrichment long ago. Historically, it was studied in zoo animals in the first place; and in this matter, Markowitz work in the late 1970s appears to be a crucial milestone. Hence, many articles can be found about exotic animals and especially primates.

With those concerns in mind, Wells and his colleagues (Wells *et al*., 2002) conducted a study aiming to explore the influence of five types of auditory stimulation (human conversation, classical music, heavy metal music, pop music, and a control) on the behavior of 50 shelter dogs. The canine subjects were exposed to each type of auditory stimulation for four hours, with a break period of one day between stimulations. The scientists monitored the dogs' position in their kennels, their activity (such as moving, standing, sitting, resting, sleeping), and their so-called vocalizations (barking, quiet, other). It was found that the dogs' activity and vocalization were substantially related to auditory stimulation. Interestingly, results similar to those found on humans (McCraty *et al*., 1998) were revealed. Indeed, classical music resulted in dogs to bark less and to spend more time resting than any of the other experimental conditions of auditory stimulation. Contrariwise, heavy metal music appeared to encourage dogs to spend significantly more of their time barking and standing than did other types of auditory stimulation, supposedly provoking agitation in them.

Rescue shelters are exceedingly stimulating environments. Animals housed in kennels are exposed to a wide range of psychological stressors such as noise, confinement, unpredictability and loss of control. It was suggested that the welfare of sheltered dogs could be improved through appropriate types of auditory stimulation and that the latter could be used as environmental enrichment for those animals. Classical music appeared to Wells to be particularly beneficial, ensuing in activities suggestive of relaxation.

In light of these encouraging results, Leeds and Wagner formulated in their book the idea that using auditory stimulation or enrichment could be a solid scientific way to affect positively the behavior and the health of dogs. Their publication was then accompanied by a CD which tracks were meticulously chosen by the authors to trigger relaxation in pet dogs

It consists in 45 minutes of classic musical said to be able to soothe and calm them (Leeds & Wagner, 2008).

This playlist was used in a second study about kenneled dogs conducted by an American team in 2012. The difference with the first study resides in the greater variety of behavioral observations: lowered body posture, panting, vocalizing, paw-lifting, body shaking, and repetitive or stereotypic behaviors. Results found were in accordance to those recorded by Wells and his team ten years before: classical music would promote more restful behaviors and might be associated with a reduction of stress levels whilst heavy metal music was found to have the opposite effect, provoking behaviors that suggest increased agitation. However, the psycho-acoustically arranged selection made by Leeds and Wagner, was found to have minimal effect on the dogs' behaviors. The reason for this is unknown. Surely more research into psycho-acoustically altered music selections designed to affect animals' behaviors is needed (Kogan *et al*., 2012).

Concerning poultry species, representing one of the most challenging animal production industry in terms of welfare, the possible beneficial effects of musical enrichment have been studied more intensely since the late 1990s. However, the study results related to music and poultry are quite erratic. In 1975, Christensen and Knight did not find any significant tranquilizing effect associated with neither high (85 dB) nor low (70 dB) music stimulation. Birds in the music treatments were frightened initially, although this reaction progressively declined through the first week and was not observed after that time (Christensen & Knight, 1975). Nevertheless, McAdie and his team reported that the sound of hens in a commercial poultry house at 100 dB was associated with the highest stress response. On the other side, a piece of music at 90 dB was associated with the lowest stress response. The fact remains that hens chose a noise-reduced environment when given the choice, suggesting that music, as other sounds, may have negative effects on behavior (McAdie *et al*., 1993).

Twelve years later, Campo *et al.* studied the influence of background noise alone and a mix of classical music and background noise on laying hens. There was no evidence of a change of stress response, as judged by heterophil to lymphocyte ratio, in hens exposed to a specific music treatment. Yet, they found a significant increase in fear response as judged by tonic immobility duration: hens exposed to specific music stimulus were more fearful than control hens. Birds in the music treatment group laid their eggs in the corner, far from

the cassette player. All those findings tend to suggest that music could negatively affect bird welfare, even though stress measures did not show any obvious positive or negative effects (Campo *et al*., 2005). We will engage later on (chapter 2.2) a discussion about the use of the heterophil to lymphocyte ratio as an indicator for stress and welfare and the successive results obtained from this ratio in such concerns.

In 2011, Dávila *et al.* tried in turn to assess the efficacy of different environmental enrichment including music on chicks of several layer breeds. Results did not show a consistent effect of auditory enrichment on tonic immobility duration (significant treatment by breed interaction). Indeed, the different avian breeds were affected differently by exposure to classical music. According to the expectations formed by the previously cited study, auditory enrichment reduced tonic immobility duration in 5 of the 8 breeds of layers studied, although only significantly in the Black Castellana breed. Control chicks in the Black Castellana breed had durations of tonic immobility that were more than 2 times longer than in chicks reared with classical music. In disagreement with this result, Campo *et al.* (2005) found a significant effect of music on tonic immobility duration in 36-wk-old hens from 2 Spanish breeds (Black-Breasted Red Andaluza and Birchen Leonesa). It was thought by the research team that this discrepancy might have been due to the age of the birds, the type of housing, and the breed. It was concluded that music auditory enrichment cannot be used as an effective method to alleviate fearfulness in layers (Davila *et al*., 2011).

Concerning pigs, no proof was demonstrated that music would be capable to decrease stress in pigs. In a study, Cloutier *et al*. focused on the influence of music during two simulations of stressful farm procedures (the five minutes the animals were held as if for castration and the first 20 hours after weaning). They recorded the piglets' vocal responses as indicator of well-being. It appeared that playing music had no significant effect on the number of vocalizations: the call rate was not below that heard during the control. It seems that playing music does not provide piglets with improvement to their welfare during handling and weaning (Cloutier *et al*., 2000).

Concerning cows, there are unfortunately very few studies on the effect of music focusing on behavioral observations. The study of music as a possible enrichment for cows gathered around the one that an auditory stimulation could have on the approach by the animals to the milking parlor. Researches were initiated by Wisniewski *et al.* in 1977 who studied behavioral responses of heifers trained to enter a parlor by conditioning methods

(Wisniewski *et al*., 1977). Twenty years later, the task was taken by Uetake *et al*. The methods of this experiment were close to Wisniewski's but it consisted more precisely in the study of the direct effect of music on the cow's voluntary approach to an automatic milking system. The results showed that music playing during milking had a stimulatory effect on voluntary approach of cows to the so-called holding area (pre-milking area). Furthermore, evidences were found about the existence of an influence of music on behavioral readiness of cows to enter the milking compartments. This leads us to think that music, as an environmental enrichment, would constitutes a non-negligible key to improve dairy cows' welfare. It should be noted that the results also suggests that some cows could be guided by other stimuli such as noises caused by the onset of milking (Uetake *et al*., 1997).

The different studies examining behavioral changes of animals exposed to music lead to quite contradictory results. A majority of the studies still seems to suggest that music would be able to alleviate stress and fear of captive animals, hence allowing hope for future developments in the field of auditory environmental enrichment and for new musical ways to improve welfare. The two main outcomes we can be sure of, when reviewing those experimentations, and that I want to stress here, are the following: firstly, it can be reasonably thought that the response to auditory stimulation is species specific and maybe even also breed specific. Secondly, the response seems to be extremely dependent on the type of music. We will focus on the consequences of this second point in the chapter 3 of this thesis work.

2.1.3. **Discussion on the scientific validity of behavioral tools**

The first point I just stressed carries practical consequences for the experimentations. Indeed, if we want to use behavioral observations to study the changes happening (or not) when music is played, and if the changes are genuinely species-specific, then we can affirm that a thorough knowledge of the specific behavioral repertoire of the animals concerned is a sine qua non condition for such welfare studies. In this paragraph, we then logically come to raise the following questions: is behavior analysis a good indicator of the animals' stress and welfare? Are the implications possible to be determined?

Behavioral indices may seem quite attractive on the first look. They are quicker and technically easy. Studying behavior bears the significant advantage that it can be carried out non-invasively (it does not involve breaking the skin), and non-intrusively (it does not even disturb the animal), especially if the observations are performed "on-site" that is to say at

the farm. It allows to subtract the stress of the monitoring itself and withdraw any other additional environmental variation in the final equation. Moreover, behavior can give a direct insight into the view of the situation from the perspective of the animal. There are arguments and study results that are in favor of such indices and have led us to consider them as satisfying tools to reflect more directly the animals' feelings and emotions which are, as mentioned previously, crucial for the assessment of welfare. Dawkins even imagines welfare monitoring systems helped by cheap video technology and computer processing of the obtained images. It is inspired by commercial company technologies which are able to differentiate, for instance, people walking to their car to drive away from people lurking around with the intention to steal a vehicle, or people waiting on the pavement to cross from people standing in the same area but intending to stay on that side of the street. This leads us to envision a comparable technology to be used for subjective assessment of animal behavior (Dawkins, 2004).

Nevertheless, there are some objections to the use of behavioral tools. The first and main one lies in our too little knowledge of the behavior causation system and the implication in the understanding of stress responses. The very root of the problem posed by the use of behavioral parameters is that their interpretation is often based on a too simplistic scheme, especially in open-field tests. According to J. Rushen (Rushen, Some issues in the interpretation of behavioural responses to stress., 2000), the interpretation of behavioral responses to stress lacks a crucial understanding of the causal mechanisms underlying the behavior. There are three main battle fields aiming for the understanding of the causation of behavior. First and foremost, the neuroendocrine basis of it, namely the role played by corticotropin-releasing hormone in controlling behavior and here, more specifically the responses to stress. This latter is far from a straight line and a simple matter. But, if that was not enough, it is added to another fundamental facet of behavior causation: the motivational control of the behavior corresponding to the postulate made my ethologists that a given behavior is the results of a competition between a mix of different motivations (McFraland, 1989). *"Behavior is (…) the result of all of the animal's own decision-making processes, the final common path"* as Sherrington states (Sherrington, 1906). Last but not least, we shall grasp what is called the functional role of behavior (communication, anti-predator behavior especially in response to acute stressors) and also how those three all interact to be able to examine how a certain behavior, let it be in response to stress, is provoked. *"The control of the behavior of animals in response to stress is complex and we cannot interpret behavioral*

responses, nor use them as indicators of stress until we understand the underlying causes of the behavior."

Beyond these considerations, there are also some issues concerning the subjectivity and thus the scientific respectability of the behavioral methods of welfare assessment. Variables such as disease incidence, growth rate, litter can be recorded quite objectively: hence, should the observer be changed, the results would still be the same. On the other hand, when it comes to affective states such as pain, frustration, and suffering, there is much less consensus on how to assess them, and we can imagine that different results could be obtained by different experimenters. Yet, as scientists must always strive for objectivity, in the sense of making measurements that represent the object under study, not the subject making the measurement, it has been often recommended to use as objective measures as possible. This explains why many scientists urged that animals' affective states fell outside the realm of the scientific study (Burkhardt, 1997). Today, despite the growing scientific interest in such subjective matters - as we have seen with the feeling-based approach (paragraph 0) - the subject remains relatively new and keeps on raising skepticism.

Objectivity is also at stake in a technical matter encountered when music is involved together with comportment. Indeed, another objection to the use of comportment studies as tools to assess stress, or the relief from it provided by music, is the influence of music on the experimenter. Indeed, it is a non-negligible fact that environmental features such as music can have a significant effect upon people's perceptions of the animals' behavior. In a 2006 study, Wells and his co-researchers showed that visitors deemed the gorillas to look less aggressive and more 'natural' during exposure to recorded sounds from the rainforest. Earlier work has also proven that the visual environment of rescue shelters can have an impact upon visitors' sensations, and the subsequent adoption rates, of kenneled dogs (Wells & Hepper, 1992). Although it has not been investigated, it is possible that auditory stimuli like music could play an equally important role in shaping perceptions. This highlighted subjectivity represents a real problem when studying and drawing conclusions about the influence of music on animal behavior, allowing us to doubt their scientific validity. This ultimately brings in a supplemental issue when building an experimental protocol.

Some solutions to the problems posed by the use of behavioral tools have already been provided. Indeed, to bypass the concerns raised by the lack of knowledge about the behavior causation system and to subvert any critics related to the assessment subjectivity,

it was proposed to use conventional choice tests. Those can indicate what animals want and how much they want it. We put the animal in the presence of situations and we determine its explicit choice regarding the situations (for instance going away from it or, on the contrary, enjoying it), we can learn what the animal likes or dislikes. In doing so, we can identify behavior or vocalizations that are characteristic of those two situations. Once we have identified these correlates of positive and negative choice, we can leave the controlled environment of the laboratory and defy the farm setting. There, we can attempt to detect and recognize evidence of those same behaviors in situ and interpret their meaning in terms of animal needs and behavioral coping strategies. *"Because of the work we have previously done, we are now in a position to interpret the behavior we see as indicating that animal is in the presence of something it likes or conversely, something it does not like. The behavior we observe on a farm is a surrogate for the choice that the animal would make if it could*" (Dawkins, 2004). For instance, this is how the meaning of piglets' vocalizations was established: according to Weary and Fraser, it means they are hungry (Weary & Fraser, 1995). Such signals can be interpreted because they have previously been validated against the physical health of piglets and also related to what they would like to eat if given the opportunity. They are known to be good surrogates for what the animal wants.

More generally, instead of purely rejecting the subjective measures that are behavioral observations, we can support an experimental system in which behavior is to be confronted with other parameters such as physiological measures to make sure these behavioral information are correctly used and interpreted. Vice versa, as Dawkins exposes, all of the measures that we might want to use, let they be physiological, autonomic, immune or neuroendocrinologic, must be validated in terms of how well they tell us about animal needs and the fulfilling of those needs. Advocating for behavioral methods, he declares in his article: *"Theodosius Dobzhansky famously said 'Nothing is biology makes sense except in the might of evolution'. With apologies to Dobzhansky for misquoting him, it is equally true that 'Nothing in animal welfare makes sense except in the light of health and what the animals want'. We are entering a new era in which I believe behavior will become even more important as a tool in welfare assessment than it has been up to now, and will be even more widely used, not only by academics looking at small numbers of intensively studied animals, but also farmers, veterinarians, zoo keepers and people wanting to do on-farm audits."* (Dawkins, 2004).

2.2. Effect of music on physiological parameters

2.2.1. How to choose the parameters to study?

We now fully understand how behavioral tools are fundamental to any experimentation aiming to assess the effect of an environmental stimuli, such as music, on animal welfare. We also realize, facing the complexity of the behavior causation system, that such observations should be confronted to other parameters in order to be validated as good indicators of any change in the animal's quality of life. These other parameters are the physiological parameters, related to the functioning of the body. The next logical question that comes to our mind is how to select those physiological parameters? In other words, amongst the multitude of measurable data, which ones should we pick?

Traditionally, the measures chosen to assess welfare were parameters linked to the biological functioning of the animals as we saw in the paragraph 1.2.3. Moreover, they usually were parameters related to the biology of stress, as we saw in the paragraph 0. It is also this family of parameters that is most often picked when studying the impact of music on living beings. A very interesting systematic study gives a state of the art on the study of the psychoneuroimmunological effects of music on humans and a good overview of the various parameters used up to now (Fancourt *et al.*, 2014). Three major subgroups of parameters can be sketched out. Indeed, out of the sixty-three studies included by the British team in this review, twenty studies focused on recording basic vital signs such as blood pressure, heart rate and respiratory rate, allowing to monitor the sympathetic nervous system activation. This forms the first subgroup which consists in evaluating the autonomic response to the stimuli that is music. Then, fifteen studies examined the neurological response to music. For instance, have been studied the effect of music on the opioid peptide neurotransmitter beta-endorphin (McKinney *et al.*, 1997), on mu-opiate receptor (Stefano *et al.*, 2004) or, in twelve other studies, on the neurotransmitters epinephrine and norepinephrine. What's more, thirty-two studies focused on the endocrine effect of music. The main hormone monitored (in twenty-nine out of thirty-two) is cortisol; the other one being oxytocin (Nilsson, 2009). We can fuse those in a second subgroup of parameters: the so-called neuroendocrine subgroup. Last but not least, the last subgroup of analyzed data is the immunology subgroup in which were mostly measured leukocytes (white blood cell counts, T cell killer activity), cytokines and immunoglobulins.

In the following paragraphs, we turn to the review of the different studies that evaluated the impact of music to animals' lives through various physiological parameters. This review is for us doubly interesting: it allows us to understand in one hand how welfare can be assessed by these parameters, as the ultimate goal of many of these studies is to determine the influence of music on the animals' quality of life, and on the other hand how music seems to influence the functioning of animals.

2.2.2. **Autonomic output parameters**

We can find a furnished literature concerning the variations of so-called autonomic output parameters (i.e. cardiovascular and respiratory parameters) induced by music exposition in humans. Animal studies are quite limited to the lab and mostly focusing on rodents. Amongst those studies, we can mention Sutoo & Akiyama's experimentations in 2004. They found out that, similarly as in humans, Mozart compositions (here precisely Divertimento K.205) are able to significantly reduces the systolic blood pressure of spontaneously hypertensive rats (SHRs). The latter are animals used as a research model of primary hypertension, used to study the cardiovascular system and its defects. Interestingly, the effect was also found to last in time: the decrease in pressure was measured in as little as 30 min and for up to 2 h after the music had ceased (Sutoo & Akiyama, 2004).

Besides blood pressure, scientists also examined the influence of music on the heart rate. For instance, in another study, were compared the effects of music by two composers, Mozart and Ligeti, on heart rate and blood pressure in both normotensive rats and SHRs. The results obtained after exposure to Ligeti's String Quartet number 2 were quite variable. Indeed, they recorded that SHRs' blood pressure increased when music was played, and this for more than 12 hours after exposure. Appearing as contradictory, the heart rate of normotensive rats decreased during music exposure during the light phase. Mozart's Symphony number 40 induced a decrease in the heart rate of SHRs but had no effect on normotensive rats (Lemmer, 2008). We can note that exposure to Mozart failed to reduce blood pressure in either rat strain, as we would have expected given Sutoo & Akiyama's results (Sutoo & Akiyama, 2004). This conflict in results can be thought to be due to the fact that different Mozart pieces were used in the two studies. What's more, different methods of measuring blood pressure were used in the two studies. Thus, they cannot be reliably compared.

There are few studies studying other animals than laboratory rodents and daring to wander outside the laboratory controlled environment. For instance, another study examined some physiological changes in African green monkeys exposed to harp music. The following parameters were monitored before, during and after the harp stimuli: heart rate, blood pressure, respiratory rate and body. Contrariwise to the previous studies cited, no significant differences were reported in any of the evaluated parameters. It has been suggested that African green monkeys are relatively calm animals in captivity and it was hypothesized that we might obtain different results with more excitable non-human primate species (Hinds *et al.*, 2007). When baboons were exposed to intermittent radio music, their heart rates were measured significantly lower when the music was played compared to when it was turned off (Brent & Weaver, 1996). Given the disparity in the results between the two monkey species, it appears that the species and the type of music played can influence the effects of music exposure on autonomic parameters in nonhuman primates.

In a recent study, a group of Scottish scientists put dogs under their magnifying glass. The canine subjects were exposed to a 6.5 hour playlist of 259 tracks from a CD named '300 Classical Favorites'. They monitored the dogs' cardiac activity with the help of a human portable heart rate monitor placed on their thorax. This equipment allowed them to measure the Heart Rate Variability or HRV. This interesting measure has been largely used to assess autonomic nervous system function in humans and a variety of animal species. Many of these studies have used HRV data to examine responses to psycho-physiological and mental stress (Von Borell *et al.*, 2007). The method consists in parsing the variations in the R–R interval. The initial response to music was characterized by an increased R–R interval (hence an increase in the HRV) and a consequent reduction in the average heart rate. It can be thought that music is able to increase the animals' vagal tone, inducing such changes in the cardiac rhythm. However, it was also stated that upon initial exposure to music the subjects spent more time lying (rather than standing) and in silence (rather than barking). It is reasonable to think that the decrease in heart rate and increase in R–R interval may not only reflect the direct influence of the auditory stimulation on the vagal tone, but also the reduced physical activity. Nevertheless, the recorded effects of classical music on both HRV and behavior were identical regardless of whether the music exposure period followed or preceded the silent period. This seems to demonstrate that the observed effects of music on both the physiological and behavioral responses were in fact due to the auditory stimulation and not as a result of habituation to the new kennel environment or the technical

experimental procedures. This reasoning is backed up by the fact that few if any changes were seen in either HRV parameters or behavior within the silent week (Bowman *et al.*, 2015).

Overall, music seems to possess the potential to decrease autonomic parameters such as blood pressure or heart rate. Those parameters are also indicators of stress, leading us to think that music could reduce the later or at least its symptoms. However, in some cases, exposure to music appears to increase those parameters, suggesting an increase of stress. Hence, of course, it should not be immediately assumed that playing any kind of music for any animal species will always have a calming effect. As we will see in paragraph 3.1.5, it is clear that further research is needed to determine which types of music have the strongest stress-reducing and thus welfare-increasing effects for each species. Moreover, such results should always be interpreted in the light of behavior, as we already discussed. Unlike in humans, where some divergence were found between autonomic (and endocrine) data and psychological states, it seems that results are in better alignment in animal studies. Although, changes affecting such parameters remain hard to interpret because they are part of the adaptive way in which animals response to their environment and because activities such as sex and prey hunting, apparently pleasurable, were found to lead to similar changes to apparently unpleasant events like escaping a predator (Rushen, 2000). It is to be highlighted that a deeper knowledge of the physiological bases of these phenomena and pathways through which music exerts its effects on the autonomic nervous system is an absolute pre-requisite before drawing any conclusions on the possible virtue of music to animal stress and welfare.

2.2.3. **The insights brought by neuroendocrinology**

In order to investigate those pathways, welfarists and musicologists turn to neuroendocrinology. Sutoo & Akiyama, for example, going beyond a simple blood pressure monitoring, endeavored to determine the pathway through which music could modify brain function resulting in a change in blood pressure. They noticed that the serum calcium levels in SHRs were greater by 5–6% within 15 min after music exposure and remained at those increased levels for as long as the music was played. Additionally, dopamine values in music-exposed SHRs were also raised, but only in the lateral neostriatum region of the brain. They concluded by proposing a mechanism in which the activation of dopamine would

inhibit sympathetic nerve activity (via D2 receptors), which results in decreased blood pressure (Sutoo & Akiyama, 2004).

Regarding endocrine parameters, just like in humans as we discussed previously (Fancourt *et al*., 2013), I mostly came across studies focusing on the effect of music on the animals' corticosteroid levels. As we saw in 0 they are fundamentally linked to the concept of stress and hence, the concept of welfare as well.

In a 2007 study, repeated restraint, considered here as a stressor, was induced in mice. The rodents were then exposed to different types of auditory enrichment or no enrichment. Mice exposed to "serenade" type music had lower levels of adrenocorticotropic hormone (ACTH) and noradrenaline, suggesting a decrease in the animals' stress level. Contrariwise, mice in non-enrichment control groups where no music was played and mice exposed to "march" type music did not show such trends (Hu *et al*., 2007).

Besides monitoring the cardiac activity, Bowman *et al.*, recorded the amount of cortisol in the dogs' saliva. The changes noticed in the later were not robust enough to be interpreted as they presented a too significant inter-subject variability. What is interesting and still worth mentioning concerning cortisol in this study, is that it was then concluded that the correlation between cortisol and the behavior observations was lower than the correlation between the HRV and behavior (Bowman et *al*., 2015). In the study they conducted two years later, they chose to measure this time the urinary cortisol:creatinine ratio (UCCR). This choice of measuring methods is motivated by the fact that, in the urine, excretion products such as cortisol, accumulate progressively over several hours. Hence, it could help correct some of the natural fluctuation in cortisol disturbing the interpretation of this parameter. Compared to the silent period prior to the music exposure, the UCCR values were significantly higher during Soft Rock and the second silent control period following music playing. Indeed, the UCCR measured during all other genres was lower than Soft Rock but higher than the silent period prior exposure, suggesting that auditory enrichment did not reduce HPA activity in this study. Thus, although the UCCR was significantly higher following the cessation of auditory enrichment, the overall results of UCCR analysis seem to contradict HRV/behavioral parameters, which suggested the dogs were less stressed when played music. The team grounded there are several possible explanations for the discrepancy between parameters detected in this study. Firstly, as the subjects urinated exclusively during allocated sample collection times, urine samples were only obtained from half the subjects.

Therefore, the gathered data may not illustrate the true mean UCCR for the sample population. Secondly, prolonged HPA activity induced by chronic stress can substantially modify HPA activity itself, meaning that some UCCR values might not reflect the actual state of the animal. Finally, it can be wondered whether the UCCR, representing the accumulation of cortisol in the urine over several hours, may be compared to instantaneous data that are HRV and behavior (Bowman *et al*., 2017).

Other than corticosteroid measures, sexual hormones were also studied. In particular, research came to focus on the role of ovarian hormones in mediating the observed anxiolytic effects of music on mice. In fact, music was found to be anxiolytic only for females with intact ovaries and ovariectomized mice being administered progesterone as a supplement. It was thus concluded that ovarian steroids, and especially progesterone, may be involved in the pathway through which music exerts its effect in female mice (Chikahisa *et al*., 2007).

Going even deeper in the neurobiology of music, Angelucci *et al*. studied the influence of music on two neurotrophins: the brain-derived neurotrophic factor (BDNF) and the nerve growth factor (NGF), which are proteins produced in the peripheral and central nervous systems and involved in the growth, survival and function of CNS neurons (Weisenhorn *et al*., 1999). The effect of music on neurotrophin production was investigated in young adult mice. The rodents were exposed to 6 hours daily of music with a slow rhythm for 21 consecutive days and the scientific team measured the levels of BDNF and NGF in the hippocampus and other brain regions. Simultaneously, behavioral performance in a passive avoidance task was assessed in the mice exposed to music. It seemed that mice exposed to music had a significantly higher BDNF production in the hippocampus as compared with control mice. This effect was specific, as no changes in BDNF were observed in the striatum and frontal cortex. Moreover, in the same brain regions, the structurally related neurotrophin NGF was not affected by music exposure. Furthermore, it appeared that music significantly enhanced the responses in the passive avoidance test. All of these findings tend to show that, under selected circumstances, music can influence neural substrates implicated in learning and memory processes (Angelucci *et al*., 2007). These data are coherent with other human studies, carried out in children, showing a better response in spatial–temporal or verbal tests after music training (Rauscher *et al*., 1997).

2.2.4. **The acumen of immunology studies**

Before diving in the research concerning the immunological effect of music, must be brought up some crucial findings concerning the connection between this subgroup of parameters and the previous ones. Indeed, research in this field has shown that direct relationships exist between the neuroendocrine and immunological systems in relation to stress and welfare. Notably, it is stated that frequent and/or prolonged adrenal activity is able to suppress the functioning of certain aspects of the immune system. Siegel introduced this theory when studying such phenomena in poultry populations. He claimed that when glucocorticoid hormones bind to protein in lymphoid cells, they induce an alteration of enzyme activity and nucleic acid production. This alteration results in reduced glucose uptake and protein synthesis causing a reduction in the production of interleukin II. Siegel observed that this immunological effect of corticosterone seems to primarily affect T-cell populations in chickens (Siegel, 1987).

Generally, such immunological effects are studied in the context of adverse conditions inflicted to the immune system of animals i.e. in the context of bad welfare. In such cases, farm animals are found to present decreased immunological capabilities. For instance, a study reported a significant reduction in the antibody production of recently tethered sows (tethering representing a "stressor") in reaction to sheep red blood cells inoculation (Metz & Oosterlee, 1981). In another study, calves displayed reduced delayed-type hypersensitivity to foreign protein and reduced contact sensitivity to dinitrofluoro-benzene when exposed to thermic stress (Kelley *et al*., 1982). It was hence proposed to use various immune-system measures as indicators of welfare. It composes one of the pillars of the assessment system we called the functional view of welfare (paragraph 1.2.3).

Getting now into the swing of things, the possible effect of music on such parameters, we can mention three major studies. One study provided strong evidence that exposure to music could decrease the immunosuppressive effects of stress and decrease the metastatic activity of tumor cells in rodents. In fact, the experiments consisted in injecting rats with Walker 256 tumor cells. Those mice and naïve (not injected) mice were subjected to one of four conditions: noise stress (a fire alarm bell) at night, 5 hours of music in the morning, both noise stress at night and music in the following morning, or neither (control). Compared with the control group, the animals exposed to the noise stress showed a decrease in the immune parameters recorded: thymus weight, T-cell proliferation and natural killer (NK)

cell activity. These results are concurrent with the endocrine ones: the plasma ACTH levels were also significantly higher in the noise-stressed mice compared with controls. Compared with the ones exposed to noise stress alone, rodents exposed to music after the so-called stress had significantly lower plasma ACTH levels. What's more, rats exposed to music after noise stress or music alone showed significantly lower number and percentage area of metastatic lung nodules in comparison to rats exposed to noise stress alone. All the results summarized here strongly suggests that music may be able to cut down the immuno-suppressive effects of stress and the metastatic activity of tumor cells in rodents (Nuñez *et al*., 2002).

Campo *et al.* studied the influence of background noise and a mix of classical music and background noise on laying hens. As we already saw (paragraph 2.1.2), they found a significant increase in fear response as judged by tonic immobility duration. Besides this behavioral parameter, they also measured the heterophil to lymphocyte ratio. Like cortisol level in mammals, heterophil to lymphocyte ratio is considered a very satisfactory indicator of stress in chickens (Gross & Siegel, 1983). The results obtained by the Spanish team showed no evidence of a change in the stress response when the poultry was exposed to music, as judged by the heterophil to lymphocyte ratio (Campo *et al*., 2005).

In 2011, Dávila *et al.* tried in turn to assess the efficacy of different environmental enrichment including music on chicks of several layer breeds. These experimentations consisted in rearing layer strain chicks with or without music enrichment until they were 8 weeks old. Contrariwise to Campo *et al.* results, evidences were found that classical music could reduce stress in chicks. This was suggested by the significant difference in heterophil to lymphocyte ratio found between layer chicks reared with auditory enrichment and control layer chicks reared without enrichment. More precisely, the heterophil to lymphocyte ratio, equal to 0.32 for the chicks with auditory enrichment, indicates a moderate effect from music, keeping in mind that ratios of 0.2, 0.5, and 0.8 are characteristic of low, optimal, and high degrees of stress, respectively (Gross & Siegel, 1993). In comparison, chicks from the control group had a heterophil to lymphocyte ratio that was 15% higher than chicks reared with classical music, showing significant heterophilia, but no significant lymphopenia. It is important to note that these results were consistent in all breeds. It is possible that the conflicting results with the 2005 study, obtained on adult laying hens, arose from the differences in the birds' age at the time of the exposure or in the duration of music exposure. Davila's

team concluded that auditory enrichment could be considered a reliable method for reducing the stress level, heterophil to lymphocyte ratio but it cannot be considered an effective method for reducing fearfulness (Davila *et al*., 2011).

2.3. Effect of music on the animal production

2.3.1. Production and welfare of animals

What we call here production parameters are parameters linked to the production yield. This link between the animal's quality of life and the production it returns can be direct, i.e. characterizing the final product with parameters like body weight or product content, or indirect, gathering parameters such as daily food intake of the producing animal or its readiness to enter the milking parlor for dairy cattle for instance. Concerning meat production, we can cite measures such as the animal's growth, its daily weight gain, but also the carcasses' weight and quality. Concerning dairy production, can be recorded the quantity of milk obtained from a cow, the content of the milk, the presence of somatic cells, etc. to cite the least.

An animal's welfare and its production are two interdependent entities: they mutually implicate each other in the sense that satisfying production often means good welfare and that good welfare levels allow good production. Indeed, as we have seen in paragraph 1.2.3, production parameters can be used as tools to assess welfare. It is included in a welfare assessment system where animal health and production are the main judging elements, in the so-called functional view. The later states, inter alia, that the risks to the welfare of the animal by environmental challenges can be assessed at two levels: firstly, the magnitude of the physiological responses (this englobes the parameters described in paragraph 2.2); and secondly, the biological cost of these responses. The production data is included in this second point. Historically, the functional view has been the view instinctively adopted first and long-time favored by the agricultural world and veterinarians. This is what we learn on the vet school benches: if an animal fares well, it produces well.

The converse of this is also true: besides the ethical arguments, improving welfare could constitute an amazing non-invasive way to enhance husbandry and improve production. The motivation to improve welfare can be economically driven, pointed towards

an improvement of the product quality and a reduction in animal losses. These benefits of improving welfare are assertions we can also find in the literature: cutting down losses through reduced mortality (Dawkins, 2017), lessening damage to carcasses via the reduction of bruising, injuries and the incidence of pale soft meat (PSE) in pigs (Hambrecht *et al.*, 2005) and dark cutting (DFD) in beef cattle (Gruber *et al.*, 2006), both being signs of an important stress caused to the animals before their death. We can also find some studies showing the improved productivity of animals (Aguayo-Ulloa *et al.*, 2014), or the improved reproduction and thrift in livestock (Green *et al*., 2012).

In fact, the ethical and value-laden arguments sometimes take a back seat in the discussion between the different stakeholders, especially in emerging countries. In a study, livestock industry leaders of China, Vietnam, Thailand, Malaysia, India and Bangladesh were asked about animal welfare issues, potential solutions and attitudes. Particularly, they were asked to list the benefits to improving animal welfare and to rank them according to their importance. At the top of the most frequently cited and most highly rated potentials, were found the increase in the productive output and the improvement in meat and product quality, so primarily financial motivations. Some differences were found, shifting the focus in some countries towards food safety (China and Vietnam), and people-focused benefits such as human health and improved community livelihood (India and Bangladesh). Animal-centered reasons were not compelling benefits in any of the investigated countries, other than India. Hence, it was stated that, for the asked population, improving animal welfare for the sake of the animals is unlikely to be a compelling argument. It is quite interesting and leads to think about the creation of a meaningful mutual ground between those that advocate improvement of animal welfare and the stakeholders that have the ability to implement it (Sinclair *et al*., 2019).

In Europe, as we have seen in paragraph 1.1.1, the motivation to develop animal welfare seems to be more linked to ethical concerns. However, in European countries, welfare provides some non-negligible strategic business benefits to the food industry. In fact, it is stated that improving welfare offers significant commercial opportunities to market products as being from higher welfare systems. Research shows that consumers are willing to pay more to purchase meat that makes them feel better about the life the animal had (Bennett *et al*., 2012).

2.3.2. **The state-of-the-art on the matter**

Surprisingly, the most publications I could find concern the influence of music on fish production. Amongst this furnished literature, I choose to mention here three studies about carps. Firstly, Vasantha and his team studied the influence of music on the growth of koi carps. Carps exposed to music were found to have a significantly greater growth in comparison to fishy mates not exposed to the melody (Vasantha *et al*., 2003). In 2007, the carps (common carp) are made to listen Mozart's music (sol major, K525). The research team could subsequently monitore an increase in growth and an improvement in carcasses and their fatty acid composition (Papoutsoglou *et al*., 2007). In 2010, carps exposed to one Mozart composition showed a greater growth (weight and length) than did those exposed to no music. It was noticed that one of the two compositions played induced better feed efficiency than did the other (Papoutsoglou *et al*., 2010).

There is limited evidence of improved growth of poultry as a result of music exposure. In 1975, a flock of meat type chicks was exposed to four different music treatments: high level dinner music, high level rock and roll music (both reaching a maximum of 85dB), low level dinner music and low level rock and roll music (both reaching a maximum of 70dB). The chicks feed consumption and individual weights were evaluated. No significant difference was found about the poultry feed consumption. Final live weights and carcass weights of chickens in the high level rock and roll group were lesser than those of the others, with the difference between low level dinner and high level rock and roll approaching statistical significance *(*Christensen *et al*., 1975*).* The results of Gvaryahu *et al*. fourteen years later happened to be more conclusive. Indeed, they found that when broiler chickens were exposed to classical music intermittently, they ate significantly more often and had significantly improved feed:weight-gain ratios compared with chickens not exposed to music (Gvaryahu *et al*., 1989). However, these results were later questioned as concerns have been expressed over the potential interlacing effects of other environmental variables present in this experimentation (Newberry, 1995).

Results obtained on pigs were not especially more compelling. In a study, it was even found that exposure to some types of music could be detrimental to swine production. In this study, fifteen growing burrows (Landrace X Large White X Duroc), were randomly allocated into the following treatments: no music, slow rhythm music (light music between 80 and 85 dB) and fast rhythm music (rock and roll between 80 and 85 dB). The pigs exposed

to fast music treatment presented a decreased daily weight gain and higher feed conversion ratio compared to the other two groups. It was proposed that the animals with the rock and roll music treatment may have been stressed to a higher level than the other two groups, thus requiring a higher maintenance energy. However, exposure to slow music did not diminish nor improve these parameters. These results suggest on one hand that music may not always have the hoped beneficial effect to animal production and, on the other hand, that such effects are highly dependent on the genre of music employed. Indeed, it seems that not only the level of noise but also the rhythm or sound frequency can influence the pigs' growth performance (Ekachat & Vajrabukka, 1994).

Concerning cows, attention has been drawn onto a report in the Hoard's Dairyman. The later stated that music exposure would be able to increase milk yield and quality in dairy cows. There has been no official articles published including Evan's methods or results analysis (Evans, 1990). This constituted the starting point of my thesis, giving birth to the fierce motivation to investigate the difficulties of setting up a scientifically accepted protocol to study the actual influence of music on the dairy production. In 2006, another study found that playing Indian classical instrumental music immediately before and during the evening milking increased the milk yield by 12.64%. The authors also reported that the cows were more *"calm…quiet…and were more docile during milking when the music was played*". Yet, no precise data were provided to support these observations (Moregaonkar *et al.*, 2006).

As we can notice, boosting animal production with music is far from being the overwhelming success one may have hoped for, the results being quite variable and the peer-reviewed literature being sparse in the matter. The studies described in paragraph 2.2 tend to support the fact that autonomic, neuroendocrine and immunological changes can result from music exposure. As we have learned from welfarists, such data can only be studied in the light of behavior which is the animal's ultimate phenotype. These physiological and biochemical evidence, linked to behavioral observations described in paragraph 2.1, seem to suggest that music could benefit animals by reducing stress and its symptoms, hence ameliorate their welfare status. Other studies, however, found that music had no observable effect or even, could wield a detrimental effect on animals. It appeared to me that this inconsistency in results must be investigated in the light of additional notions, and especially notions linked to the very nature of the stimuli applied: music, its perception by animals but also its properties and varying parameters. The absence of sufficient evidence is what

animated the writing of this thesis work and motivated me to search for a protocol that could clearly study the possible potential for music to enhance dairy cattle's welfare. The following third part is all wrapped up around this ultimate goal.

3. Towards the establishment of an optimized protocol

As a first step, to study the possible influence of music on welfare, it was imperative to understand the concept of welfare and the ways to assess it. We could notice the bag of bones it has been in welfare history, and still is nowadays, to agree on the methods of assessment of welfare. By choosing to adopt Duncan and Fraser's "body and mind" view, one can hope to satisfy a majority of the stakeholders: gain scientific approval and also be in accordance with the value-laden demands of the ethicists and consumers. Then, the second part reviewed the different studies on the impact of music on various behavioral and physiological parameters. It strengthened the belief formed in the first part, comforting the idea that welfare is best assessed when an array of diverse parameters is used, allowing us to compare and confront them always keeping in mind the animal's needs and feelings. However, this second part raised substantial interrogations such as, for instance, the influence of the type of music on its effect or the challenge to perform such a study in a long-term frame. These can be grouped into three major points.

In this third and last part, we intend to tackle these three points that are fundamental to the research on this topic. They are fundamental because they are deep rooted in the nature of music itself: an auditory stimuli which we are trying to understand the perception of by animals and the scope of consequences on their functioning. They are all the more fundamental because they are posing problems in the interpretation of many study results and have been sources of disagreement when concluding about the effect of this stimuli. These points are accompanied by some axis of thinking on how we could remedy that practically in a protocol and a draft of experimental protocol composed with the elements discussed.

3.1. Important notions to consider when building the protocol

3.1.1. Music versus Noise

"Music is meaningless noise unless it touches a receiving mind."
Paul Hindemith, composer (1895-1963)

In this paragraph, we come back to the very beginning: why do we call music music? What allows us to grant a sound the qualification of music? What makes it different from a noise? Do all species agree on this sorting out of sounds?

In fact, music is an artificial stimuli produced by humans for humans. Thus, it should not be taken for granted that a different species, having different auditory and perception abilities, perceives music the same way humans do.

This ability to distinguish specific sounds and consider them as a melody (or even different melodies) is called discrimination. The first experimentations to investigate animal's discriminative capabilities were conducted in 1984 by Porter and Neuringer on pigeons. They trained pigeons to discriminate the music of Bach and Stravinsky, using an operant paradigm, where pigeons received a food reward after pecking one of two discs during presentation of excerpts. With time, the pigeons learned this discrimination and if they were making few errors, they were presented with novel excerpts from the same composers, and similar excerpts from other composers. Amazingly, the pigeons reacted to all of these novel stimuli by their disk choice in a way that mirrored that of human participants (Porter & Neuringer, 1984). Even more fascinating, a recent study was executed using a similar operant paradigm but this time using carps. The fishes, exposed to blues and classical stimuli, showed comparable results, being able to correctly classify stimuli into their own music genres even if they had never heard it before (Chase, 2001). In fact, after decades of research, it appears that a great variety of species, from fish to songbirds and primates, has been proven to present musical discrimination. Although negative results might be not published, this ability to discriminate complex auditory stimuli seem to be widely spread in the vertebrates regardless of their different audiograms (Watanabe, How animals perceive music? Comparative Study of Discriminative and Reinforcing Properties for Infrahuman animals, 2009).

Another fundamental property of music worth mentioning in this development is its ability to have a pleasurable or "reinforcing" effect on the listener. In fact, music has reinforcing properties for humans, meaning that hearing music is capable of creating a pleasurable experience in humans. Contrariwise to discrimination, reinforcing properties of music have not been thoroughly investigated in non-human animals; and in the little bunch of studies that were published, most of them failed to obtain a clear reinforcing effect of music. It does not mean that music was proven detrimental each time. Indeed, even though strict experimental works did not show such effect, we have seen (paragraph 2.1.2) it can be used as a tool of environmental enrichment for captive animals (for example, Howell *et al*., 2003 or Wells *et al*., 2002). Although these observations are not controlled experiment, they still suggest some positive effect of music for the animals. One exceptional result concerning reinforcement was obtained from songbirds. Indeed, it was demonstrated that Java sparrows can show a preference for particular music style (Watanabe & Nemoto, 1998). Interestingly, one common characteristic of humans and songbirds is that both have well developed vocal communication. Besides, similarities between music and animal songs have been pointed out. Aside from this particular case, it seems the property of reinforcement is not widely spread throughout the animal kingdom, but rather to limited species like humans and songbirds. It was proposed thus that phylogenetic contingency may be a crucial factor for the reinforcing properties (McAdie *et al*., 1993).

It can constitute an issue for research on welfare in relation to music. Indeed, as we only have limited understanding of bioacoustics in different species, results arising from such studies are hence far from conclusive in terms of their implications for the animals' welfare.

3.1.2. **The cost of noise**

Here we must first define what we call noises: they are environmental sounds that exist without controls for volume, duration, or cause and effect relations. These sounds are very numerous and various inside the animal facility. Noise experienced during housing of farm animals can be short-term or chronic. The sources of noise are extremely various: technical devices, routine works (such as opening and closing doors, changing pens, feed dispensing), basal sound levels caused by mechanical ventilation, animals activities (such as climbing and chewing on fences), and by their vocalizations (Mihina *et al*., 2012). It is said

that what causes the loudest sounds are husbandry procedures, especially if metallic material is involved or if the work is performed in a hurried manner (Burn, 2008). Sounds produced by humans might also be stressful for farm animals. Admittedly, loud cry does cause stress responses in them (Hemsworth, 2003).

The list of physiological functions and parameters noise could affect is as long as this thesis. The most obvious effect is to provoke a general stress reaction accompanied, as discussed already, by higher secretion of ACTH giving an increase of adrenocortical hormones in the blood (Burrow *et al*., 2005). Autonomic reactions then unfold in the circulatory gastrointestinal systems via the sympathetic nervous system. Noise was proven to directly affect energy consumption and reproductive function (Escribano *et al*., 2014). What's more, sound emission at the frequency of 2 kHz in noise of 75 dB, 85 dB, and 95 dB was found to contribute to a reduction in the animals' appetite (Cwynar & Kolacz, 2011). When responding to noise stressors, these animals may increasing vigilance, hiding and/or retreating, hence decreasing the amount of time they spend foraging. However, noise exposition was found to lead to weight gain, as observed in rats submitted to noise stress for 30 days (Alario *et al*., 1987). Furthermore, noise is also thought to have indirect effects on population dynamics via changes in habitat use, courtship and mating, reproduction and parental care (Rabin *et al.*, 2003). Other effects we can cite are sleep disturbances, changes in the glucose metabolism of the liver, changes in the enzymatic activity of the kidneys, and immune-wise an increase of eosinophils percentage in blood and immunosuppression (Algers *et al*., 1978).

Concerning cattle specifically, the noise threshold expected to cause in them a behavioral response is 85 to 90 dB (Manci *et al*., 1988). Stimuli greater than this threshold have been found to induce retreat, freezing, or strong startle response (Morgan and Tromborg, 2007). Arnold *et al.* examined the effect of noise on the choice behavior of dairy heifers in a maze. They found out that the percentage of heifers choosing the quiet side of the maze was constantly increasing as the experiment progressed. Furthermore, the animals exposed to milking parlor noises showed escape-type behaviors, suggestive of a fear response. They incrementally learned to dodge the noise (Arnold *et al*., 2008). Similar conclusions had been already drawn by Pajor *et al.* in 2000. Indeed, when studying responses of dairy cows to various handling treatments, they found that exposure to noise increased avoidance behavior such as increases in stopping and amount of required handler

intervention. What's more, shouting on dairy cows appears to be very aversive (Pajor *et al.*, 2000). Noise made by humans shouting and slamming of metal gates increases heart rate and activity in cattle (Waynert *et al.*, 1999). Lanier *et al.* (2000) also noted that cattle appeared more stressed by intermittent loud human vocalization, particularly when high-pitched like a child's.

According to multiple studies, such responses observed in cattle are very dependent on the nature and especially on the suddenness of the sound. Admittedly, Kovalčík and Šottník (1971) stated that noise as high as 80 dB had no negative effect on dairy cows: feed intake was increased, milk yield was unchanged, and indices of the rate of milk-releasing were enhanced. However, immediate exposure to a high-intensity noise (105 dB) resulted in the decrease of these three measured parameters. What's more, a gradual increase of noise to 105 dB resulted in a less-negative response. Many other studies indicate that sudden, novel sounds seem to affect cattle behavior more than continuous high noise: (Grandin, 1998; Arnold *et al.*, 2007; Head *et al*., 1993). The later aimed to evaluate effects of jet aircraft noise before milking on milk yield and composition, amount of residual milk, and behavior of thirty-six Holstein cows. Cows were exposed to jet noise up to four times daily on 10 to 12 days per period. It resulted that cows showed no behavioral or productivity responses during the 21 day treatment periods. The cattle's activity or movement in the holding area before and after noise was similar to that of control cows. Such foundings were unexpected as the noise was greater than the sound level threshold for response. It was proposed by the team that, as cows on dairies are constantly exposed to a wide array of noises from farm equipment, farm machinery, and work activities, they may be used to such noise exposure, explaining the lack of aversive behaviors or decreased productivity (Head *et al*., 1993).

The physiological responses of dairy cows to noise were reported by Broucek *et al*. (1983). For instance, the sound of a tractor engine (97 dB) significantly increased glucose concentration and leucocyte counts and markedly reduced the level of hemoglobin in the blood. The same authors also recorded significant increases of glycaemia, non-esterified fatty acids content and creatinine in dairy heifers under the effect of acoustic exposition. Hemoglobin level was also found to drop significantly. In another trial, the same team also recorded a slight decrease in thyroxin in plasma in response to noise stimuli (Broucek et al, 1988b). Unexpected high intensity noise was also found to provoke an increase in peripheral or mammary release of catecholamines (Albright & Arave, 1997).

Concerning the effect of noise on dairy production, Gygax and Nosal (2006) conducted a study on fifty Swiss dairy farms about the effect of vibration and noise on somatic cell counts (SCC) in milk, one likely indicator of udder health (Harmon, 1994). They measured SCC on farms using bulk tank milk samples. What was recorded is that somatic cell counts increased with the increasing intensity of vibration but not with acoustic noise. Furthermore, there was no statistical correlation between the measures of vibration and noise hence, allowing the scientists to estimate the influences of vibration and noise independently in the data set. The team proposed that the negative effect of vibration on SCC could be mediated by reduced oxytocin levels under stressful conditions, leading to a reduced milk yield. This would in turn increase residual milk, which could result in subclinical mastitis and increased SCC (Bruckmaier & Blum, 1998; Stelwagen *et al*., 2000; Rushen *et al*., 2001).

In conclusion, noises and especially sudden high-level ones, such as noises generated in the course of routine dairy activities, seem to act like stressors that may have adverse effects on cattle welfare. Regarding our research, two main hypothesis stem from these conclusions. Firstly, we can postulate that music could serve as a "mask" buffering animals from these noises, hence improving farming methods. In fact, very often, what motivates farm workers to listen to music is to cover up unpleasant working noises for themselves. We can thus think of a similar use of music for the animals. However, if this were the case, one might expect an equally effective impact of all types of auditory stimulation. As reviewed in this thesis work, this is not always the case. Hence, *"the possibility that there is something specific and enriching about certain types of auditory stimulation must also be acknowledged*" (Wells, 2009).

3.1.3. **Preference and motivation**

Secondly, we can hypothesize that music could be equated to noise by animals, thus being an additional stressor and hence a disadvantage to welfare and production. Indeed, although specific types of auditory stimuli might be considered enriching, there is the potential for this extra noise to do more harm than good. For instance, McDermott and Hauser found that whilst laboratory-housed cotton-top tamarins and common marmosets exhibited a preference for music of a slow, over a fast, tempo, when presented with a choice between slow tempo music and silence, the animals preferred silence. It was then concluded by Cambridge team that, if additional auditory stimuli are added as an enrichment, then it

seems essential to introduce a possibility for the animals to exert control over the sound i.e. turn it on and off (McDermott & Hauser, 2007).

This leads us to the principles of preference and motivation which have been considered as fundamental insights into animal welfare. They essentially consist in attempts to understand the animal's own priorities and needs. This kind of experiments have developed rapidly over the past 20 years after the pioneering work of Hughes who tested the animals' preferences for different environments. In a 1973 study, Hughes and Black housed hens in cages where they could move freely between compartments with different types of flooring. Based on the time the birds spent on the different grounds, the two scientists concluded that the birds showed a significant preference for a particular flooring product (Hughes & Black, 1973). More recently, rats have been observed to press a bar more for the reward of being granted access to a cage containing other rats than they will for an empty cage. This suggests that being with social companions is something they want (Patterson-Kane *et al*., 2002). In another study, caged mink were allowed to push doors to gain access to a water bath. They were found to do so even when the doors were loaded with weights. However, they did not exert this behavior for an empty cage or for a cage full of novel objects, suggesting that access to water to swim in is something that is important to them (Mason *et al*., 2001). From such simple experiments, were quite rapidly developed more and more sophisticated tests of environmental preferences. We now have several different ways of 'asking' animals what they want and whether they find the situations they are in pleasurable or distressing.

Dawkins *et al.* showed the advantages and accuracy of such methods in welfare research. Indeed, they studied three different measures of welfare in laying hens: levels of corticosteroids (measured in the birds' feces), changes in the quality of egg shells and what the birds themselves wanted. This latter measure consisted in an experiment in which pairs of birds were placed in one of two similar choice boxes for a period of 5 days. Each box was made of two attached compartments: one being filled with food, water and a nest box and the other one being covered either with a bare wire floor ("barren" treatment 1) or with wood shavings plus a box of sprouting wheat ("enriched" treatment). They evaluated the birds' preference by measuring the relative amount of time the animals spent in the second compartment. The results showed that, right from the beginning of the experiment, the birds with access to the enriched compartment spent significantly more time in their second

compartment than did the birds with access to the barren compartment. This seemed to indicate that the hens had a preference for the enriched environment. However, the birds with the access to the enriched environment also had higher levels of fecal corticosterone and a greater loss of shell thickness (Dawkins *et al.*, 2004). In the light of a functional approach to welfare, as exposed in paragraph 1.2.3, one could conclude from these results that the barren environment is better for hen welfare than is the enriched one. A more holistic approach is proposed by Dawkins. He suggests that any results should always be interpreted according to the two major following questions: 1) Are the animals healthy? 2) Do they have what they want? Such a perspective gives more credit to preference tests (and also to behavioral studies) and would lead to conclude that the enriched cage is the one being the best one for the animals' welfare, admitting that the two other measures were simply indicating that the birds were more aroused by the environment that they liked. *"What the birds wanted is thus not just another measure of welfare, but a necessary piece of evidence that gives valence and meaning to the more physiological measures of corticosterone level and shell quality*" (Dawkins, 2004).

However, as noted by Duncan (1978) and Dawkins (1980), preference testing by itself does not indicate the degree of importance that the animal attaches to the preferred option. Hence, it was urged that measuring the strength of preference by examining the strength of motivation for a certain option makes preference tests even more valuable. This is how were born new kinds of experimentations designed to measure the strength of animals' motivation to obtain preferred options or to avoid unpreferred ones. Hence, going further than the simple litter choosing tests described above, Dawkins and Beardsley (1986) tried to determine the strength of the preference by requiring hens to perform a task in order to gain access to the preferred litter. It should be mentioned that significant concerns exist about the influence of experimental procedures on the results of such studies. Indeed, it is natural for poultry to find food by pecking and to enter a new area by walking. If we require a hen to peck a key in order to activate a barrier and thus to enlarge its cage, can we trust the observed behavior as a true reflection of the hen's motivation to gain additional space, or is the link between pecking and access to space just too foreign to the hen? Arguments in favor of these concerns are illustrated by Hutson and Arey's successive experiments. In 1988, Hutson imagined a protocol in which sows could perform an operant response to open a box that contained l kg of straw. The females did little of this behavior, even during the time before farrowing when they normally build nests, leading Hutson to conclude that the sows had

little motivation to use straw (Hutson, 1988). However, 4 years later, Arey used a similar protocol where sows could perform an operant response for access to a pen, but this time furnished with 18kg of straw. The animals demonstrated strong motivation for this reward as the time of farrowing approached. Arey suggested that the discrepancy in the conclusions of the two studies was due to procedural differences i.e. that 1kg of straw was too little to be functionally significant to the sows (Arey, 1992).

Besides preference and motivation tests, Baumans affirms that, more generally, assessment of welfare should ideally be performed in a positive way, such as by the preference tests just described, or by measuring pleasure and by behavioral observations in animals' environment. Indeed, welfare is very often evaluated in the negative way: we observe more readily and easily a lack of welfare. According to Baumans, this constitutes a problem in welfare research. Furthermore, he stresses the importance of anticipatory behavior i.e. behavior expressed by an increase in activity prior to an announced reward. *"Anticipatory behavior has been described as a typical arousal with goal-directed activity that occurs in the appetitive phase when the actual reward is not yet present (e.g. food, water, sexual contact, access to enriched housing)*" (Baumans, 2005).

It must finally also be mentioned that such tests, however, could present some limitations. For instance, it was claimed that preferences assessed in those tests might not always reflect the animals' long-term priorities. What else, the animal may be forced to choose between non-valued commodities or the choice may be too complex. Nevertheless, Fraser advocates that when the right "question" is asked in terms of the animal's sensory capacity, cognitive ability and natural history, it is reasonable to assume that natural selection has equipped the animal to make such choices (Fraser, 1996).

In the case of our experimentations, I came to wonder how the cows' preference and motivation could be evaluated in the protocol. I first thought it would be judicious to design a device giving the cows the possibility to choose whether they want to hear music or not, in the way that McDermott and Hauser (2007) did with their monkeys. I came to think of another way to assess cows' preference towards music when reading Wells *et al.* article on shelter dogs (Wells *et al.*, 2002). They highlighted in their results that the dogs' position in the kennels was not influenced by the type of auditory stimulation to which they were exposed. They concluded that dogs did not actively seek out the source of the auditory stimulation. Indeed, if this were the case, the dogs would have spent more of their time at

the rear of their kennels, closer to the source of the music. In order to investigate whether this would be true for dairy cattle, it should be decided to put the music source in a place of the stable where the animals can choose to go or not and where we are sure that the movement is not motivated by something else: that is to say neither next to the feeding trough nor next to the milking machine.

3.1.4. **Habituation**

Habituation can be described as the diminishing of an innate response to a frequently repeated stimulus. In a great number of studies investigating the influence of music on animals, this phenomenon of habituation has widely been observed. It is expressed in the experimental results as a decrease in the observed response (let it be stress response or beneficial effect) over time. The interpretations and conclusions outcoming from this are multiple.

On one hand, we can hypothesize that this phenomenon of habituation could be beneficial regarding the influence of music and its interpretation. It is indeed natural that such an auditory stimulation might be perceived by the animals as a stressor or even an assault, due to its unknown origin and the animal's natural survival instinct to protect itself from any external event they are unaware of. On this reasoning, habituation could be able to incrementally lower the stress response; allowing us to observe, once the primary panic and flight reflex passed, the actual response to music, let it be an absence of response or a positive effect. This reasoning motivates the conduct of the experimentations on a long-term run.

On the other hand, we can hypothesize that habituation of the animals to the music stimuli is a bad thing in our research. Indeed, we can imagine that, once the animals are habituated to it, music becomes part of the background sounds or auditory environment of the animals such as any other noise, hence, not inducing any particular effect. Bowman *et al.* faced such observations in their results in 2015, exposing repeatedly the dogs to the same classical music playlist over 7 days (Bowman *et al.*, 2015). In order to determine whether they could prevent this habituation, the team conducted a study two years later investigating the physiological and behavioral response of 38 kenneled dogs to five different genres of music including Soft Rock, Motown, Pop, Reggae and Classical. They found out that the physiological and behavioral changes observed in this study were maintained over the 5 days

of auditory stimulation, suggesting that increasing the variety of auditory stimulation was able to reduce the level of habituation to auditory enrichment.

In our protocol, we could consider increasing the range of music types we expose our dairy cattle to. What's more, it appears crucial to analyze the outcoming results while taking care to differentiate in the data set the response of cows on one side and of heifers on the other side. It would be interesting to see if there is a significant difference between those two, suggesting a possible habituation of the pluriparous animals.

Furthermore, in 2002, Wells *et al.* highlighted how determining this notion is to the interpretation of the results. It was noted that neither pop music nor human conversation had any apparent effect on the dogs' behavior. It was thought that dogs may be more accustomed to these types of auditory stimulation than to the others. Indeed, shelter staff regularly listen to radio programs involving a mixture of human conversation and pop music whilst undertaking their husbandry duties whereas very few, however, listen to classical or heavy metal music. It remains unknown whether the effects of these types of music would have remained significant over the other, perhaps more familiar, forms of auditory stimuli had the dogs been adapted to them in the same manner. Thus, music to which farm handlers usually listen will also be commonly heard by their animals and a "preference" may therefore develop due to habituation rather than any innate musical preference (Rickard *et al*., 2005). In our protocol, it might then be interesting to perform a quick preliminary survey on what the farm workers usually listen to, or have already listened to.

Another consequence of habituation concerns the validity of a long-term monitoring of corticosteroid levels. The adrenal medullary responses are very brief and adrenal cortex responses, although considerably more prolonged, decline after a few hours. We understand how problematic could be the use of measures of adrenal function as indicators of long-term welfare problems. Furthermore, it now appears that the non-specific nature of the glucocorticoid response to environmental challenges has perhaps been misconstrued. Mason has shown that increased glucocorticoid secretion in response to various stressors occurs mainly because the experimental situation evokes an emotional reaction in the animal (Mason, 1968). If the emotional reaction is eliminated, then the increased glucocorticoid secretion in response to adverse environments may not occur. This may explain why glucocorticoid secretion appears to lose its link to adverse conditions in some longer-term studies. For example, Ladewig and Smidt found that the altered secretory patterns of cortisol

in bulls had returned to normal levels after four weeks of tethering in uncomfortable stalls despite other evidence of continued discomfort (Ladewig & Smidt, 1989).

3.1.5. **Mozart or Bon Jovi?**

Quite diverse, not to say contradictory results have been obtained about the influence of music on autonomic output parameters such as the heart rate and the blood pressure or on production data, as we have seen in chapter 2. Furthermore, when exposing animals to different genres, scientists could observe common trends in the reactions, irrespective of the different species studied. For instance, Van Loo *et al.* found out that "new age" music had a "calming" effect on mice while classical or pop music did not (Van Loo *et al.*, 2004). There is a good bunch of other studies with similar results. Concerning dairy cows in particular, it has been found that country music could make them enter a milking parlor more readily than "rock 'n roll" music (Wisniewski, 1997). As already mentioned in paragraph 3.1.4 about habituation, this more marked calming effect, suggestive of an apparent preference, could be simply explained by the animal handler's own music preference: if the farm worker listens to country music every morning during the milking, the cows are more used to this genre and thus show less sign of stress when exposed to it. Furthermore, it can also be thought that handlers enjoying one genre better than another could be more relaxed during their work, alleviating their relationship to the animals. This subsequent change in handler behavior could affects the animals. A consequence for a future protocol would be for the farm worker to write down if he enjoys the cow's mixtape and how much he does, on a scale from 1 to 5. Thus, we could study the correlation.

More precisely, it was reported that the difference in animals' reaction did not only depend on the genre of music but also on its intrinsic parameters: some differences have been seen between tracks of the same genre. For example, when weanling mice were exposed to either Mozart or Beethoven for 12 hours each night for 10 weeks, learning abilities as tested using a T-maze were significantly better in mice exposed to Mozart than in mice exposed to Beethoven (Aoun *et al.*, 2005). Hence, it seems to be more subtle than just a music genre discrepancy. What is it then?

In 2007, Videan *et al*. reported instrumental music to be more effective than vocal in increasing social interactions in laboratory-housed chimpanzees. In fact, vocal music was more likely to decrease aggressive patterns of behavior. The speed of the music was also

found to exert a role upon the animals' behavior, with slower tempo vocal music being more efficacious in reducing aggression in male animals than that with a faster tempo (Videan *et al.*, 2007). In 2009, when reviewing the use of auditory stimulation as enrichment for captive animals, Wells *et al.* observed that the style and speed of the music could influence the value of music as environmental enrichment.

More precisely, it was found that frequency could play an important part in the effect of music. For instance, it was reported that the physiological effects of Mozart's compositions could be attributed to the higher frequencies and the greater number of modulations within the frequency domain in these compositions (Akiyama & Sutoo, 2011; Shaw *et al*., 2000). Indeed, Akiyama & Sutoo conducted a study on the effects of music on blood pressure regulation and tested over one hundred pieces of classical music. They determined that Mozart's Adagio from Divertimento No. 7, K.205, produced the strongest preliminary results. More generally, it was observed that higher frequencies (4–16 kHz) were able to create the most significant effects (Akiyama & Sutoo, 2011).

More studies are needed to determine precisely the respective influence of music parameters such as timbre (the quality of musical sound that distinguishes different sound sources such as voices and specific musical instruments), rhythm (a non-random repetitive temporal auditory pattern), pitch (a perceptual attribute related to the fundamental frequency that enables comparisons of sounds as higher or lower) or beat (the underlying pulse, or unit of time). Hence, one might think that, in the literature on the topic, there is a lack of rigorous selection and analysis of the chosen music stimuli itself before processing to the study of its effect. Eventually, such an approach could allow to determine which parameter(s) of the music has or have the most significant effect, allowing us to determine subsequently how we should modulate these parameters and ultimately choose compositions in accordance to the findings, in order to obtain a collection of tracks exerting the strongest welfare enhancing effects for each species.

3.2. The protocol's blueprint

3.2.1. The setting

The difficulty resides in the fact that such a study is conducted in a much uncontrolled environment, hence many factors are involved inducing difficulties in the interpretation of the results. Indeed, the farm environment is different from the lab, in which we already encounter variability issues. As Fraser states, in experimental science, we are able to study single variables or treatments at one time, with other variables being either controlled or included in a planned or balanced manner. Furthermore, are usually examined the average effects of the variables on a reasonably homogeneous sample of animals, and the individual differences tend to be minimized as outlier in order to clear out the central tendency. Here, however, we are asked to take on a much more complex mission: the assessment of animal welfare at the farm and group level. Instead of single variables and controlled treatments, we are required to consider a range of diverse variables and to combine these somehow in a scoring system or what we will call thereafter the assessment grid. Instead of researching average trends, we will have to deal with quite different responses by different animals and somehow incorporate them in an overall evaluation of the animals making up the group or farm (Fraser, 2003).

It is nowadays more and more acknowledged that proceeding to research on commercial farms in cooperation with farmers is a powerful weapon in the conquest of an applicable and practical welfare science. It is well understandable how facilitated the results applicability is compared to small-scale studies carried out in a laboratory. Such studies can take the form of epidemiological studies of what is currently happening on farms (Nicol *et al*., 2003) or can consist in farm-level experiments. For instance, 11 major broiler chicken producers in the United Kingdom and Denmark recently agreed to manipulate the crowding i.e. stocking density of whole chicken houses in a coordinated experiment that involved more than 2.7 million birds (Dawkins *et al*., 2004). This study granted policy makers a better preview of the effect that stocking density legislation would induce. Small-scale laboratory could never provide such information. It is also stated by Fraser that working directly with farmers *"has the further advantage that other factors, such as food safety, can be studied simultaneously so that policy decisions can be based not only on what is best for animal*

welfare, but also in the wider context of human health, effects on the environment and what the public wants to see happening" (Fraser, 2003).

How to choose the farm(s) we will conduct the experiments in? First of all, accordingly to the concerns just cited above, the farm size should be chosen in accordance with the national average farm size in order not to fall into a laboratory unrealistic frame. The majority (44,1%) of the French livestock is allocated in farms comprising 51 to 100 cows. This is the farm size we should aim for when looking for our experimental setting. The herd should be uniform in breed.

We should also aim for farms equipped with milking robots or automatic milking systems (AMS). Thanks to a computerized system and a herd management software, we could then collect a complete data set including the cow's identification, allowing to determine its reproduction status, parity, and day into lactation, but also a great range of milking parameters (milk yield i.e. liters of milk per milking, speed of milk ejection, strength of the vacuum) and of milk parameters (protein and fat content of the milk, conductivity, somatic cell count, indicative of the quality of the milk but also of the presence of subclinical disease in the animal). As we have seen previously (paragraph 3.1.4), it would be interesting to differentiate the data of the heifers from the data of the cows when analyzing the results we can identify. Although being a source of additional noise stimuli, the AMS represents a great opportunity for us in these experimentations to withdraw a great part of the sample collecting stress in the experimental proceedings. If we were getting to work with several farms, it appears essential that they would use the same AMS.

Ideally, the feed ration should be fixed and identical for the entire duration of the experiment, in order not to blurry out the interpretation of the milk quantity and quality parameters.

3.2.2. **The cow's mixtape**

The level of the auditory stimuli played should always be comprised between 85 and 90dB. As we have seen in paragraph 3.1.5, it would be fairly interesting to select the playlist to which the cows will be exposed with the help of musicologists. For instance, these professionals could help to choose different tracks having the same tempo but different frequencies or vice versa, in a perspective to determine which musical parameter(s) exert the most effect on the animals. In addition, we should introduce to the animals a sufficient variety in the auditory stimuli to avoid habituation. In relation to habituation also, as we have seen in paragraph 3.1.4, a survey of what the farmers usually listen to, or have ever listened to in the past in their working place, should be conducted prior to the experimentations to acknowledge the cows' possible already formed preference. Also, there should be a notation system available for the farm workers to note down if they enjoy the music played and how much they are enjoying it, on a scale from 1 to 5. Hence, we could evaluate the possible correlation between the workers' preference, which might impact his/her way of working, and the animals' behavior and welfare level. The music should be played gradually until it reaches the wanted sound level at the beginning of each session. Indeed, as seen previously (paragraph 3.1.2), it could limit the startle reaction and the stress response linked to the suddenness of the stimuli that do not interest us in this study.

As discussed in paragraph 3.1.3, the music source should not be placed next to the milking spot, the feeder or the water trough. It should be placed in a strategic place to allow the interpretation of the animals' preference towards it (it should be placed in an area where animals can "choose to go"). We can wonder if we should design a way to grant the cows the choice to turn music on and off, forming a more sophisticated preference test. However, the simple preference-test experiment has been criticized in two ways in terms of welfare relevancy. Firstly, as Duncan (1978) pointed out, the animal may not select what is best for it. In many situations the successive expressed choices made by animals are those which increase their biological fitness. Yet, in some cases, some animals do choose to do things which harm them, for instance over-eating. Hence, choice tests alone are sometimes inadequate. A much more general criticism is that the action required to make the choice in an experiment is often very easy. Thus, there is little indication of the importance of the choice to the animal. It is the case with our protocol: the cow just has to take a few step to hear the music better. This leads Broom to urge that *"in order to be able to apply data from*

preference tests to practical situations where an improvement in welfare is sought, the strength of a preference must be assessed by discovering what costs the individual is willing to incur in order to be able to express the preference" (Broom, 1988). The technique should then not only consist in observing their preferences, but also in measuring how hard they will work for the preferred event or object. In our protocol, we could imagine that the cows would have to work to turn the music on, or that they would have to push a weighted fence to get closer to the music broadcasting device.

3.2.3. **The welfare assessment grid**

The essence of the experiment we are trying to set up is to build a model in which the welfare level prior to music exposure corresponds to the level 0. The animals are then exposed to the music stimuli and the potential effects are observed, measured and recorded in order to determine whether music brings the welfare level of the dairy cows up or down from the previously observed basal level.

In this part, I make a stand on the welfare assessment system to be chosen in this study and have to select relevant parameters in the light of all the literature on welfare and on music previously reviewed. As a veterinary student, I truly believes that animal health is a fundamental pillar of animal welfare. Also, after 8 years of scientific studies, which allowed me to form a solid scientific knowledge and methodology scientific, I tend to turn to objective and measurable parameters. Before processing to that literature review on animal welfare, I would have instinctively sided with the functional view developed in paragraph 1.2.3. However, in the light of Duncan's and Dawkins' eloquent work, I discovered how substantial behavior observation and analysis are for welfare assessment, setting the fear of subjectivity in the measure to the background. What's more, to assess welfare, it is best without being too intrusive in the animals' routine and body integrity. I would now tend to turn to Dockès and Kling-Eveillard's more holistic approach, praising the importance of a "body and mind" approach to the issue (Dockès & Kling-Eveillard, 2006). It also allows different data (such as physiological and behavioral) to be read together, confronted to each other, in order to be validated by each other or at least to be understood better in relation to each other. Hence, for a protocol with dairy cows, I would choose an array of measures. Such an approach is even more interesting when the experimentation is done at the farm level. Indeed, each individual animal has several alternative methods of

trying to cope with adversity and individuals differ in the methods which they favor. Hence, the use of one physiological measure of response to apparently difficult conditions might give the impression that most animals are unaffected by the conditions. If other physiological, behavioral and fitness measures had been taken, however, it might have been apparent that all animals had been affected but that they had used different coping procedures. The strategy chosen here is thus to take the sum of as many measures as possible (behavioral, health, physiological) and to "triangulate" on what is good welfare.

In our plethora of measures, first come the behavioral observations. As stated in paragraph 2.1.3, the use of such tools requires a good knowledge of the species comportment in general, and of the individuals in particular. Behavior assessment should be done by farmers on one side, as he/she remains the one person knowing his/her animals the best, hence more prone to detecting the delicate changes in the cows' behavior; and by an experimental operator on the other side, as it could bring in a newer sight. The observations should be compared. The use of video technology could be very interesting, allowing a wider observation period.

A question is brought in the lens when behavior is in question: should we use stereotypies as indices when assessing welfare? Their use are indeed still controversial. They are defined as fixed, often repeated sequences of behavior with no obvious function. Broom and Johnson (1993) argue that an animal's welfare is poor if stereotypies take up more than 40% of its active time. However, Mason and Latham (2004) show that stereotypies can under different circumstances indicate neutral or even good states of welfare. Indeed, some stereotypies actually seem to benefit the health of the animal. For instance, repeated biting of wooden doors or food troughs observed in some stabled horses is associated with reduced gastric ulcers, hypothetically because the "abnormal" behavior may stimulate the production and the swallowing of saliva which could improve the protection the stomach from excess acid. In other cases, such as bar-biting in restricted sows, where the animal rubs its mouth until it bleeds, indicate poor welfare on the most basic health grounds. As emphasized earlier, one measure of welfare taken alone does not mean anything: it is the same for stereotypies. Whether a given stereotypy indicates good or bad welfare is therefore not an inherent property of it being a stereotypy; and as any other welfare indices, it needs to be judged against its effects on animal health.

It is important to underline that not only behavioral indicators of bad welfare should be payed attention to. It is crucial to be able to discern indicators of good welfare levels in the behavior. For instance, it was suggested to use play behavior by a 2013 study (Mintline *et al*., 2013). Other quite easy to monitor non-invasive measures should be recorded to assess the welfare level: food intake, body condition score, disease occurrence, injuries, deformities, walking, changes in the sex ratio of offspring, which is resource dependent. In addition, the production data should be recorded with the help of the AMS, as explained in paragraph 3.2.1: milk yield i.e. liters of milk per milking, speed of milk ejection, strength of the vacuum, protein and fat content of the milk, conductivity, somatic cell count, indicative of the quality of the milk but also of the presence of subclinical disease in the animal.

We could also monitor the autonomic output by measuring the HRV (Heart Rate Variability), defined as the difference in beat-to-beat intervals (R–R interval) which is derived from the non-additive input of the two branches of the autonomic system. Indeed, the principle of the measure is to investigate the functioning of the ANS by analyzing the balance between sympathetic and vagal activity. HRV analysis is based on the fundamental principle that healthy cardiac function comprises irregular time intervals between consecutive heart beats. Studies in humans has shown that higher resting HRV corresponds to an improved control of emotions, thoughts and behavior. HRV has emerged as a very useful tool in humans for both research and clinical studies on cardiovascular diseases, diabetic autonomic dysfunction, hypertension and psychiatric and psychological disorders. Over the past decade, HRV has turn to the animal kingdom and is used increasingly in animal research to analyze sympathovagal imbalances related to diseases, psychological and environmental stressors or individual characteristics such as temperament and coping strategies. In a 2007 paper, von Borell *et al*. reviewed the current and past HRV research in farm animals and highlighted that HRV is a promising approach for evaluating stress and emotional states in animals: "*It has the potential to contribute much to our understanding and assessment of the underlying neurophysiological processes of stress responses and different welfare states in farm animals*" (von Borell *et al.*, 2007). Furthermore, it has the great advantage of being a non-invasive technique.

Concerning endocrine parameters, the measurements of corticosteroids could be an interesting insight. Admittedly, taken in isolation they are often difficult to interpret in welfare terms because levels rise not only when the animal is in a situation we assume to be

stressful but also when engaged in activities such as eating and copulation (Toates, 1995). Here too, we should ask ourselves whether or not the animal's health is at risk, and whether the animal shows evidence of wanting to escape from the given situation: we need to link these data to disease prevalence index and behavioral observations. In welfare studies, there are three accepted ways to measure these hormones' levels: in plasma, urine or saliva.

Plasma cortisol concentrations have been shown to correlate well with stress. However, due to the fact that it experiences pulsatile release and that it is sensitive to experimental procedures such as restraint and venipuncture, concentrations can be variable and unreliable. Ladewig (1984) reported that after bulls were tethered, a peak of cortisol in the blood could be measured every few hours. Free-moving bulls also showed peaks of cortisol at intervals, but these were less pronounced and less frequent. It was concluded that frequent sampling is needed to discover that more cortisol peaks are occurring in a given condition. Single or occasional samples are of little use because of the "natural" diurnal variation of cortisol. We can see how impractical ad too intrusive such frequent blood sampling would be. Another method has been proposed: the ACTH-challenge technique. If an animal is often challenged and stimulated, then it will have a frequently activated adrenal cortex and well developed cortical enzymes. The later are likely to be more active than are those of an animal which uses its adrenal cortex less often. Hence, an injection of a large dose of ACTH would reveal the maximal amount of glucocorticoids which can be produced by the organism. This method has been used on farm animals (Friend *et al*., 1977; Dantzer *et al*., 1983). However, it should be stressed that negative results do not necessarily mean the absence of welfare problems. Although quite invasive, as the samples are taken less often than a regular corticosteroid measure, this technique is to be considered.

However, it seems to me we need to pursue the least invasive methods possible to be able to exclude as much as possible any experimental stress. In this perspective, we can consider the use of urine samples and UCCR (Urine Creatinine to Cortisol Ratio) measurements like in Bowman *et al*.'s study (Bowman *et al*., 2017; paragraph 2.2.3 in this thesis). Besides the fact that this would be quite impractical in a 100 cow herd, the introduction of urinary catheters could be too stressful for the cows, not to mention the risk of microbial contamination that would distort the disease prevalence index and other immunity parameters of the herd. The least invasive way to measure stress hormones I could find in the literature review consists in the use of saliva samples. This method was used by

Bowman *et al*. (2015) who collected the samples using cotton swabs (method described by Kobelt *et al., 2003)*. Using such a technique has two major advantages: firstly, cows would be lightly restrained and secondly it represents cortisol secretion over a longer time period of minutes to hours rather than the single time point measurement obtained from a plasma sample. However, the collection technique and associated handling required is still very much able to elicit a stress response. The best way would be to measure cortisol from milk samples as the samples would be taken by the milking machine, hence it would not change the dairy cows' routine. The AMS should be equipped with a special function allowing the derivation of some mL of the milk obtained from each cow in order to collect it in proper tube and freeze it for later ELISA testing and corticosteroids titrating.

Concerning immunology, in a concern to proceed to the least intrusive methods possible, it should be decided to use various immune-system output measures as indicators of welfare: disease prevalence index in each group, kind of diseases reported, occurrence of diarrhea, body condition score. Indeed, the welfare level of most diseased animals is quite low, and a system which leads to a higher occurrence of disease is less satisfying in terms of welfare. However, all the animals of the assessed herd should be follow the same vaccination program.

A question still lingers: how to record correctly all these data? We could imagine a software or application synchronized with the AMS software. The later would fill automatically the data readily available during the milking (listed in paragraph 3.2.1). Then, a farm worker, which should be the same all along the experimentation, fills the rest of the wanted date in his version of the software. Additionally, an experimental operator independent from the farm should fill up the same info in his version of the software. They can be later confronted.

CONCLUSION

To answer our primary question, we can state that the intuition we had concerning music and animals turns out not be completely anthropomorphic. Indeed, discrimination, defined as the ability to distinguish specific sounds and consider them as a melody, has been proven in many animal species including cattle. Furthermore, there are signs that music can specifically exert some effects. Indeed, other sounds and noise do not provoke similar reactions and also, the response depends greatly on the music genre the animals are exposed to, suggesting a complex mechanism of action depending on music intrinsic parameters. We are lacking research on this matter. Another aspect of music perception is decisive for us: reinforcing properties, meaning that hearing music is capable of creating a pleasurable experience. It is the case in humans. The whole issue resides in determining whether or not animals could appreciate similarly such an auditory stimulation. Once more, there is an important lack of research in this field. However, the approach brought by this thesis could help clear the field, by combining musicology and welfare research. The key notion that bridges the two is preference. It is highlighted as a major component of animal welfare and one of the cornerstone of the protocol's blueprint.

Admittedly, when scouring the literature, we notice indices which seem to show that music could have the ability to enhance welfare levels. But, if no agreement is found on the view of welfare to adopt and on the parameters to select, it is complex to draw valid conclusions on the impact of music on it. Our sketch of a protocol, made strong by the acquired knowledge on welfare and inspired by the failure of its peers, is matching the emerging consensus regarding welfare assessment: it embraces a more holistic or "body and mind" approach. Inter alia, in endeavors to give behavioral tools back their nobleness and rightful place. The later should be placed on equal terms with physiological parameters such as autonomic, neuroendocrine and production data in accordance to three major concerns. Firstly, the experimentation should be the least invasive possible, using besides behavior observations some other measures like disease occurrence or reproduction function evaluation for instance, that do not need any sampling, hence not exerting additional stress. Secondly, such an approach allows to include feelings and subjective emotions, such as fear and frustration, which are fundamental in the assessment of welfare. Thirdly, the experiments designed in this way would tend to be more realistic and feasible compared to

laboratory fully-controlled studies. This adopts the spirit of animal welfare science, which is best conducted on site and at the farm level and that should be an empirical by nature, useful and readily applicable by producers and intelligible and readily readable by consumers.

Lacking of time, I unfortunately could not conduct the experimentations this year, but I sincerely hope this does not mark the end of my work on the subject. And that the experiments will one day see the sweet light of the French countryside.

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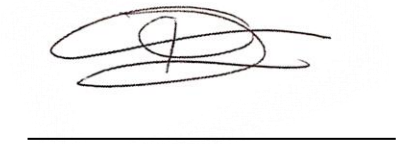

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