VALUTAZIONE DEL CARICO ALLOSTATICO NELLE SCROFE PRE-PARTO

EVALUATION OF THE ALLOSTATIC LOAD IN SOWS PRE-DELIVERY

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RIASSUNTO

L’obiettivo di questo studio era quello di valutare il carico allostatico e l’effetto dell’ambiente su di esso nel preparto delle scrofe. Le informazioni ottenute potrebbero risultare utili per migliorare la gestione degli animali e, quindi, il loro benessere. Infatti, negli allevamenti intensivi gli *stressors* sono per la gran parte attribuibili alle condizioni ambientali in cui vivono gli animali e alle pratiche di gestione usate.

Lo studio è stato condotto su 296 scrofe gravide; le scrofe sono state allevate in un allevamento specializzato per la produzione del Prosciutto di Parma secondo il circuito DOP, in provincia di Brescia.

Per ottenere informazioni sullo stato ormonale della scrofa durante tutta la gravidanza è stato utilizzato un campione biologico retrospettivo di medio-lungo termine come lo è la matrice tricologica. Il prelievo del pelo è stato effettuato sul dorso; ogni campione è stato prelevato 2.6 ± 1.6 (media ± ES) giorni prima della data del parto prevista e complessivamente tutti i campioni sono stati prelevati tra febbraio e giugno. Pertanto, ogni animale poteva cadere nelle bande di febbraio, marzo, aprile, maggio o giugno. Le concentrazioni di cortisolo nel pelo sono state determinate utilizzando un radioimmunosaggio (RIA).

I risultati hanno mostrato una differenza statisticamente significativa nelle concentrazioni di cortisolo nel pelo (HC) tra quelle ottenute per i mesi di febbraio e marzo e quelle di aprile, maggio e giugno ($P < 0,001$); infatti, le concentrazioni di HC hanno continuato ad aumentare dall’inizio dello studio raggiungendo le loro concentrazioni più alte nel mese di giugno. Allo stesso tempo, il *Temperature Humidity Index* (THI) medio mensile è passato da un minimo di 43.01 a un valore di 73.48 raggiunto a giugno.

Considerando che l’intera gestione è stata mantenuta inalterata durante lo studio e per tutte le bande, l’unico cambiamento ambientale registrato è stato il THI. Pertanto, l’aumento delle concentrazioni di HC, biomarker del carico allostatico, sembra essere legato all’aumento del THI. Inoltre, i risultati sembrano suggerire che durante il processo di allevamento agisca un effetto aggregato di diversi fattori di stress che in questo studio sono stati esacerbati quando è stato registrato un THI più elevato.

Risulta, quindi, strategico assicurare condizioni di termoneutralità al fine di prevenire l’annullamento dei benefici apportati da tutte le strategie che vengono utilizzate per garantire e migliorare il benessere animale in allevamento.

ABSTRACT

The aim of the study was to evaluate the allostatic load and the effect of the environment on it in the pre-partum of sows. The information obtained could be useful to improve the management of the animals and therefore their welfare. In fact, in intensive farms the *stressors* are mostly attributable to the environmental conditions in which the animals live, and the management practices used.

The study was conducted on 296 pregnant sows; the sows were raised in a specialized farm for the production of Parma ham in according to PDO in the province of Brescia, Po Valley (Italy).

To obtain information on the hormonal status of the sow throughout the pregnancy it was used a medium-long term retrospective biological sample as hair is. The hair sampling was carried out on the back; each sample was taken 2.6 ± 1.6 (mean ± SE) days before the expected delivery date and overall all samples were taken between February and June. Thus, each animal could fall in the February, March, April, May, or June batch. The hair cortisol concentrations were determined using a solid-phase microtiter RIA assay.

The results showed a statistically significant difference in HC concentrations between those obtained for February and March and those of April, May and June ($P < 0.001$); in fact, the HC concentrations continue to rise from the beginning of the study reaching their highest concentrations in June. At the same time, the average monthly Temperature Humidity Index (THI) moved from a minimum of 43.01 to a value of 73.48 reached in June.

Considering that the entire management was kept unchanged during the trail and for all the batches, the only environmental change recorded was the THI. Therefore, the increase in HC concentrations, the biomarker of the allostatic load, seems to be linked to the increase in THI. Moreover, the results seem likely to suggest that during the rearing process act an aggregate effect of different *stressors* that in this study were exacerbated when a higher THI has been recorded.

Thus, it is strategic to assure conditions of thermoneutrality in order to prevent the nullification of the benefits brought by all the strategies that are used to guarantee and improve the on-farm animal welfare.

1. INTRODUCTION

1.1. Animal welfare concepts

Animal welfare is a subject of increasing interest and public debate. This growing interest has led different countries to reconsider the current legislation on animal welfare to provide the best possible management (Korte et al. 2007; Marchant Forde, 2019). This largely derives from the fact that the market demand has led to the development of increasingly intensive farming systems, which due to their characteristics are in contrast with the ethics of many consumers (Špinka, 2017; Marchant Forde, 2019). It is therefore clear that we must first understand the meaning of the term "welfare". Animal welfare was defined as: "*the state of an animal as it attempts to cope with the environment*" (Webster, 2011), which in the case of farm animals becomes: "*the state of body and mind of a sentient animal as it attempts to cope with its environment*" (Broom & Johnson, 2019).

Welfare is a very broad concept and is connected to the concept of stress, in fact, they are both terms related to adaptive processes. When adaptation is possible without too much effort (in terms of energy cost) then well-being is safeguarded; on the other hand, if the adaptation is inadequate then the organism finds itself in the inability to cope with the stressful situation with a reduction in welfare (Veissier & Boissy, 2007; Colditz & Hine, 2016)

1.2. Sustainability concept

For animal production, as well as for other production systems, sustainability is important.

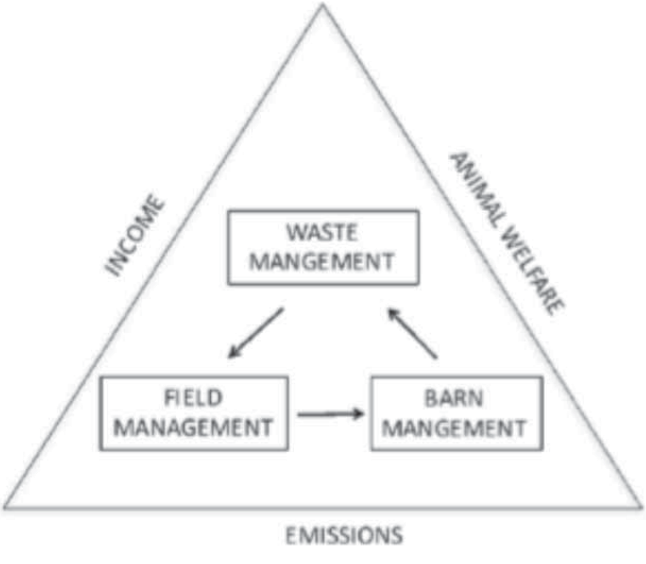
On the other hand it is not easy to define sustainability because it has different meanings and takes into consideration various factors, among which there is also the ethics of the production system (Galioto *et al.*, 2017; Broom & Johnson, 2019). A definition of sustainability is: *"a system or procedure is sustainable if it is acceptable now and if its expected future effects are acceptable, in particular in relation to resource availability, consequences of functioning and morality of action"* (Broom & Johnson, 2019)

Animal welfare is considered as one of the indispensable factors for the sustainability of animal production (Broom, 2010). In fact, in farms the concept of sustainability is divided into 4 different concepts:

- economic sustainability (profitability);
- internal social sustainability (working conditions);
- external social sustainability (animal welfare, landscape quality, etc.);
- ecological and environmental sustainability (GHG emissions, eutrophication, groundwater pollution, etc.) (Galioto *et al.*, 2017).

We can therefore consider the production process of livestock farms divided into 3 phases: field phase (involving field practices), barn phase (which concerns breeding practices) and waste management phase (management of livestock waste), which are not independent from each other but whose management influences the performance of the others in terms of: profitability, animal welfare, and emissions (Galioto *et al.*, 2017).

Figure 1- The nexus between the three production phases in livestock farms (Galioto et al. 2017).
Figura 1 - Il nesso tra le tre fasi produttive negli allevamenti (Galioto et al. 2017).



1.3. Effects of distress on the production process on farms

Considering that animal welfare refers to both the health and psychological state (Buller *et al.*, 2018), in intensive farms the *stressors* are mostly attributable to the environmental conditions in which the animals and the management practices used (Albernaz-Gonçalves *et al*. 2021). In fact some of the most frequent *stressors* in pig farms are: confinement, high temperatures, chronic hunger, painful mutilations, early weaning, high stocking density and subsequent social grouping (Velarde *et al.,* 2015; Albernaz-Gonçalves *et al.* 2021). All these *stressors* cause a reduction in animal welfare and this reduction in welfare is related to the following aspects:

- immunosuppression;
- reduced weight, growth, and body condition;
- reduced reproductive success;
- increased frequency of abnormal and stereotypic behaviours (Broom & Johnson, 2019).

In the following chapters we take into consideration the different aspects.

1.3.1. Immunosuppression

When animals are found in chronic stress, their immune systems are compromised and therefore this impairment of the immune system resulting in greater susceptibility to diseases (Broom & Johnson, 2019; Albernaz-Gonçalves et al. 2021). Many studies have in fact shown both in humans and in animals, how a chronic activation of the HPA (Hypothalamic-Pituitary- Adrenal) axis induces immunosuppression (Broom & Johnson, 2019). This is because with the activation of the HPA axis there is the production of cortisol, which with prolonged *stressors* causes the reduction of lymphocytes, cytokines, and immunoglobulins (Martínez-Miró *et al.*, 2016) environmental, metabolic, immunological and due to human handling.

In farms, therefore, to maintain health and productivity and prevent the spread of diseases, the use of antimicrobials is used (Albernaz-Gonçalves et al. 2021). The use of antimicrobials on farms (including antibiotics, antivirals, antifungals and antiparasitics) is widespread and has been found to be the major contributor to the problem of antimicrobial resistance (Broom & Johnson, 2019).

1.3.2. Reduced weight, growth and body condition

One of the first signs of reduced animal welfare is a reduction in the growth of growing animals or a reduction in weight in adults. With the activation of the HPA axis and the production of glucocorticoids there is an increase in catabolism with degradation of glycogen, adipose tissue, and muscle tissue to allow the release of glucose necessary in the stressful situation since at the same time there was also a reduction of feed intake (Martínez-Miró *et al.*, 2016)environmental, metabolic, immunological and due to human handling. This metabolization of stored energy reserves leads to a change in body composition. A possible indicator of animal welfare is the use of Body Condition Score (Broom & Johnson, 2019). As also seen from the study by Pierozan et al. (2021)which were aged between 75 and 173 days, and were managed on an all-in all-out basis. The welfare indicators were evaluated once on each farm using the methodology of the Welfare Quality® assessment protocol for pigs. Multiple linear mixed models were used to assess the associations of welfare with FCR and DFI according to the production stage at which the pigs were evaluated on the farm. Key results: The welfare indicators with the highest average prevalence were frequency of coughing (35.7% animal performance (Feed Conversion Ratio) is reduced if there are conditions that cause a reduction in animal welfare.

1.3.3. Reduced reproductive success

The reproductive performance of farm animals can be predictive of a state of stress and therefore of a reduction in welfare (Broom & Johnson, 2019). This reduction of the HPG (Hypothalamic-Pituitary-Gonads) axis is due to the hyperactivity of the HPA axis, which with the production of glucocorticoids inhibited the release of LH (luteinizing hormone) from the pituitary and the secretion of estrogen and progesterone from the ovary (Martínez-Miró *et al.*, 2016)environmental, metabolic, immunological and due to human handling. This decline in fertility on farms results in a major economic loss.

1.3.4. Increased frequency of abnormal and stereotypic behaviours

In intensive farms, pigs are often housed in small environments (high stock density) which prevent them from exhibiting their natural behaviours and this leads to the development of anomalous and stereotyped behaviours (Broom & Johnson, 2019; Albernaz-Gonçalves et al. 2021).

These four aspects are the main factors related with pig production; in fact, with high levels of stress and poor welfare there are important effects on all of these. Another factor influenced by the stress level of the animals is the quality of the meat, with a higher incidence of PSE (pale, soft and exudative) and DFD (dark, firm, dry) (Martínez-Miró *et al.*, 2016)environmental, metabolic, immunological and due to human handling.

1.4. Promote health and welfare to improve animal resilience in farms

It is important to use strategies to improve the health and welfare of animals and consequently increase their resilience, that is their ability to deal with adverse events (European Commission, 2021). In fact, increasing the welfare reduces the risk of the presence of pathogens and therefore fewer pharmacological treatments will be made, limiting the onset of antimicrobial resistance ; furthermore, there is an improvement in the productive and reproductive performance of the animals (Broom & Johnson, 2019; European Commission, 2021). It improves the FCI (feed conversion index) and therefore there will be a better efficiency of use of food with a consequent greater growth rate of the growing animals (Martínez-Miró *et al.*, 2016; Broom & Johnson, 2019; Yuan et al. 2020)environmental, metabolic, immunological and due to human handling. It also improves ovarian function, with a consequent increase in the speed of reaching puberty in young animals and an improvement in fertility as well as in the reproductive success of adult ones (Moberg, 1985; Martínez-Miró *et al.*, 2016; Broom & Johnson, 2019)environmental, metabolic, immunological and due to human handling.

1.5. Pig farming in italy

The pig sector in Italy is an important component of the national agri-food system, both as regards the turnover of the agricultural phase alone (€ 3 billion in 2019), and as regards the value of processing (€ 8 billion in 2019). For about a decade the supply chain has been in crisis, in fact there has been a progressive decline in the number of companies present on national soil. Despite this, pig production has remained almost stable, showing a production concentration in the sector. This crisis is due to the increase in production costs and in particular to the increase in the prices of cereals and protein crops for the production of feed (Ministero delle Politiche Agricole Alimentari e Forestali, 2011).

In Italy in 2019 there were 32.137 farms, for a total of 8.608.000 pigs reared. The average size of national farms is about 268 animals/farm, but in larger farms it reaches 1500 animals/farm (ISMEA, 2021). The distribution of farms and animals by production orientation is as follows (Table 1):

Tabella 1 - Distribuzione degli allevamenti e degli animali per tipologia di allevamento (ISMEA, 2021).

***Table 1** - Distribution of herds and animals for type of farming (ISMEA, 2021).*

Type of farming	**Herds**	**Animals**
Breeding		
- closed cycle	52 %	8 %
- open cycle	11 %	26 %
Fattening	36 %	66 %

In 2019, 11.4 million of animals were slaughtered, showing a slight decrease compared to 2018 (-0.4%). In 2020, due to the COVID19 emergency, a sharp decline in slaughtering was recorded (- 13%) (Ministero delle Politiche Agricole Alimentari e Forestal, 2011).

National pig farming is focused on the production of heavy pig from industrial processing. In Italy the 90% of slaughtered pigs are consist of heavy pigs and the 80% of these will be destined for the protected circuit to produce hams and other PDO cured meats.In fact, Italy has a heritage of 21 PDO and 21 PGI products. Most of the pigs destined to produce PDO hams have been certified within the Prosciutto di Parma and San Daniele circuit. Indeed, in 2019 the turnover of the two hams was respectively € 720.9 million for Parma and € 313,0 million for San Daniele out of a total value of € 1,7 billion s for hams and cured meats of the protected circuit. (Ministero delle Politiche Agricole Alimentari e Forestal, 2011; ISMEA, 2021).

In 2019, imports from the pig sector reached a record value of € 2,4 billion. The 85 % of imports are represented by pigmeat (fresh, refrigerated, and frozen). Live animals are only the 3,5 % of the import value and are mainly intended for fattening. Exports, which in 2019 reached a value of 1.8 billion euros, mainly concern processed products (hams and cured meats) (ISMEA, 2021).

2. MATERIALS AND METHODS

2.1. Ethics

Although hair sampling is non-invasive and it is not a troublesome procedure, the study was carried out in accordance with Directive 2010/63/EU on the protection of animals used for scientific purposes.

2.2. Animals

The study was conducted in the province of Brescia, Po Valley (Italy), at a site of about 2000 Danish sows (Dan Bred International®, Denmark) specialized in production for Parma ham according to PDO (Protected Designation of Origin). The animals at the farm are sows and piglets till weaning and the weaning time is once a week with piglets of 24 days of life on average. The study included 296 pregnant sows with a mean parity order of 4.40 ± 0.15 (mean ± SE). The gilts were excluded from the study because their behaviour in the delivery room is more unpredictable than the multiparous ones (Roelofs et al., 2019).

2.3. Housing conditions

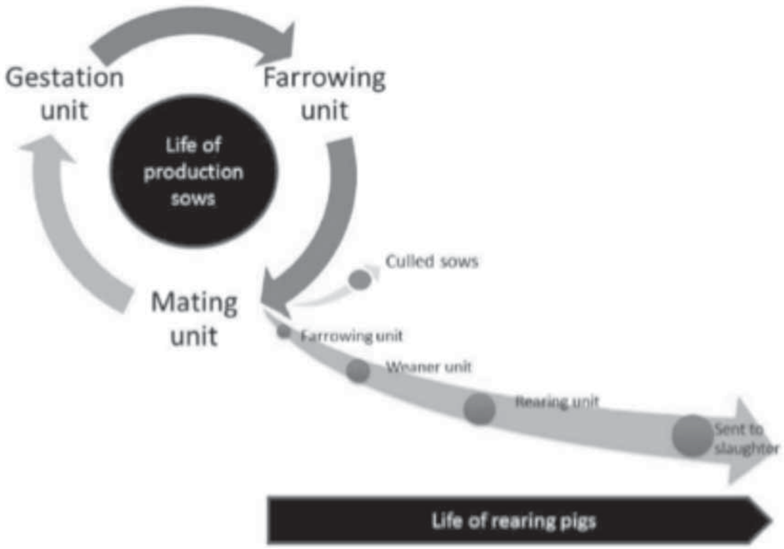
Animals were reared according to the current Italian legislation which implements Council Directive 2008/120 EC, laying down minimum standards for the protection of pigs.

After mating, sows remain in the gestation cages for 28 days. Here the animals received ultrasound pregnancy detection and those positive were transferred to multiple pens of 10-15 sows where they remained until 5 days before farrowing. In reaching the farrowing rooms the sows were randomly housed in the farrowing cages, where they were housed on paper strips bedding during farrowing.

For all the sows included in the study only a spontaneous farrowing took place. During gestation the animals were fed with a liquid feeding through an automatic system. Nipple drinkers were available for make water available *ad libitum* to animals.

The management was organized in one week batch farrowing system and followed the standard operating procedures (SOPs) regarding vaccinations, placement, cleaning, waste management and biosecurity measures. The health status of the animals included in the study was considered "conventional": *Mycoplasma hyopneumoniae* positive, Circovirus positive, PRRS (Porcine reproductive and respiratory syndrome) positive but stable-inactive at time of trial.

Figure 2 - Schematic general illustration of life of production sows and rearing pigs (from Marek Špinka, 2017).
Figura 2 - Illustrazione schematica generale della vita delle scrofe in riproduzione e dei suini da ingrasso (da Marek Špinka, 2017).



2.4. Environmental data

The environmental temperature and the relative humidity were recorded in collaboration with ARPA, Lombardia (Agenzia Regionale per la Protezione dell'Ambiente della Lombardia, Italy). A meteorological station that is recognized by the World Meteorological Organization regulations and located 15 km away from the farm provided the environmental data throughout the study; the station recorded the daily average for each factor. With these data the Temperature Humidity Index (THI) was calculated according to Mader's formula (2006):

$$THI = (0{,}8 \times T) + \left(\frac{RH}{100}\right) \times (T - 14{,}4) + 46{,}4$$

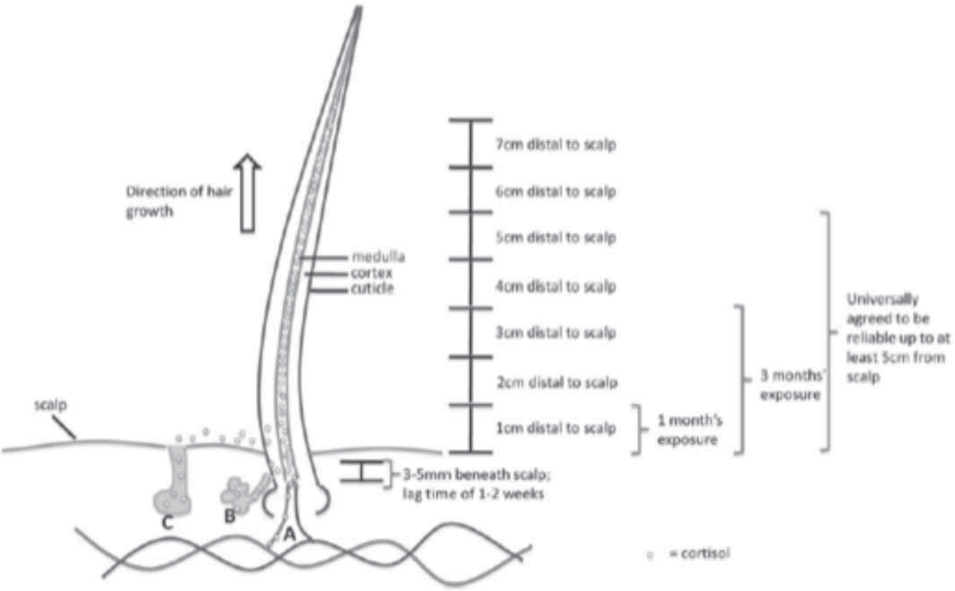
where T is the environmental temperature in °C and RH the relative humidity in %. Then, for all the data obtained the month average ± SE was calculated.

2.5. Hair sampling

The collection of the hair was carried out on the back at the level of the last rib and for about 10 cm at the side of the vertebral column; this area was chosen because of its cleanliness. The animal has been shaved as close as possible to the skin with a *Bosh* electric razor for large animals. Samples were then stored in paper envelops, in the dark at room temperature until analysis.

A sample was taken for each sow included in the experimental test, in relation to the expected date of farrowing. The sampling (mean ± SE: 2.6 ± 1.6 days before the expected delivery date) was done between the months of February and June, therefore each animal could fall in the February, March, April, May, or June batch.

Figure 3 - Mechanisms for incorporation of cortisol into hair via blood (A), sebum (B) and sweat (C) (Russell et al. 2012).
Figura 3 *- Meccanismi di incorporazione del cortisolo nel pelo tramite sangue (A), sebo (B) e sudore (C) (Russell et al. 2012).*



2.6. Washing procedure and extraction

In accordance with what described by Bergamin et al. (2019) the hair samples were previously washed before proceeding with the extraction. The hair samples were washed twice with 3 min/wash in 3 ml of isopropanol to minimize the risk of extracting steroids from the surface of the hair, which were deposited by sweat and sebum. Hair was than extracted according to the method described by Bergamin et al. (2019). In brief, approximately 60 mg of hair was put in a glass vial with 3 ml of methanol for the extraction and the vials were incubated at 37° C for 16 h. Next, the vial with the extract is placed under an airstream suction hood, where the liquid is evaporated to dryness at 37° C. The sample residues were dissolved in 0.8 ml of 0,05 M phosphate-buffered saline (PBS, pH 7.5).

2.7. Hormonal analysis

The hair cortisol concentrations were determined using a solid-phase microtiter RIA assay for pigs developed in house as described by Bergamin et al. (2019).

In brief, a 96-well microtiter plate (OptiPlate; PerkinElmer Life Sciences Inc.) was coated with goat anti-rabbit- γ globulin serum diluted 1:1000 in 0,15 mM sodium acetate buffer (pH 9) and incubated overnight at 4 °C. The plate was then washed twice with RIA buffer (pH 7.5) and incubated overnight at 4 °C with 200 μL of the antibody serum diluted at ratios of 1:20.000 (Analytical Antibodies, Bologna, Italy).

The cross-reactivities of the anti-cortisol antibody with other steroids were as follows:

- cortisol, 100%;
- corticosterone, 1.8%;
- aldosterone, < 0.02%.

After washing the plate with RIA buffer, the standards (5–200 pg/well), the quality-control extract, the test extracts and the RIA buffer were added, and the plate was incubated overnight at 4°C.

The decanting period allows the formation of the antigen-antibody bound which occurs with the same affinity for both the labeled and the unlabeled antigen. According to the law of mass action, the most concentrated antigen (both the labeled and the unlabeled one) will be the one that will occupy the most antibody sites (Kubasik, 1984).

The unbound antigen (labeled and unlabeled) constitutes the free fraction, while the bound antigen constitutes the bound fraction. The bound fraction is separated from the free fraction by washing the wells with RIA buffer. After the addition of 200 μL of scintillation cocktail, the plate was counted on a β counter.

The intra and inter-assay coefficients of variation for cortisol assay were 3.7% and 10.1%, respectively. The sensitivity of the assay for cortisol was 24.6 pg/ml.

2.8. Statistical analysis

The statistical analysis was performed with the R vers. 3.4.0. The normality of the data distribution was tested with the Shapiro-Wilk test. In case of non-normal distribution, the data has been transformed. The model adopted was a mixed model for repeated measures (Wang & Goonewardene, 2004) where the batch were considered between subject factors whereas the day of the sampling was considered as a repeated measure. The post hoc test was conducted according to Holm-Sidak.

2.9. Aim of the study

The objectives of this research were as following:

- to evaluate the allostatic load in the pre-partum of sows by the hair cortisol assessment;
- to assess the effect of environment on the allostatic load of pregnant sows.

3. RESULTS

Table 2 shows the monthly mean values of temperature and relative humidity that were detected during the study. The month average THI ranged between a minimum of 43.01 in the February batch and a maximum of 73.48 in the June batch.

Table 2 - Temperature, Relative humidity, and THI mean values from February to June.
Tabella 2 - Valori medi di temperatura, umidità relativa e THI da febbraio a giugno.

Batch	Temperature (°C)	Relative humidity (%)	THI
February	5.32	91.77	43.09
March	4.68	87.22	41.86
April	14.00	82.35	57.15
May	20.75	76.74	67.81
June	25.03	66.17	73.48

As reported in Table 3 the April, May and June batches showed the highest hair cortisol (HC) concentration in comparison with those assessed for the February and March batches ($P < 0.01$).

Table 3 - Estimated marginal means (±SEM) of hair cortisol concentrations (pg/mg) recorded in sows (n=296). [a,b] Means within a column and within mean effect not sharing the same superscript differ at $P<0.05$.

Tabella 3 - Medie marginali stimate (±SEM) delle concentrazioni di cortisolo nel pelo (pg/mg) registrate nelle scrofe (n=296). [a,b] Le medie all'interno di una colonna e all'interno dell'effetto medio che non condividono lo stesso esponente differiscono per $P<0,05$.

Main effects	Cortisol
February	12.67^{a}
March	9.31^{a}
April	16.25^{b}
May	27.83^{b}
June	28.55^{b}
SEM	*1.349*
P - value	< 0.001

As also shown in the next graph (Figure 4) there is a statistically significant difference in HC concentrations between the months of February and March and those of April, May, and June; in fact, the HC concentrations continue to rise reaching its highest concentrations in June.

As shown in Figure 5 the month average THI ranged between a minimum of 43.01 in the February batch and a maximum of 73.48 in the June batch.

Figure 4 – Estimated marginal means (±SEM) of hair cortisol concentrations (pg/mg) recorded in sows (n=296) in the different batches.

Figura 4 – Medie marginali stimate (±SEM) delle concentrazioni di cortisolo nel pelo (pg/mg) registrate nelle scrofe (n=296) nelle diverse bande

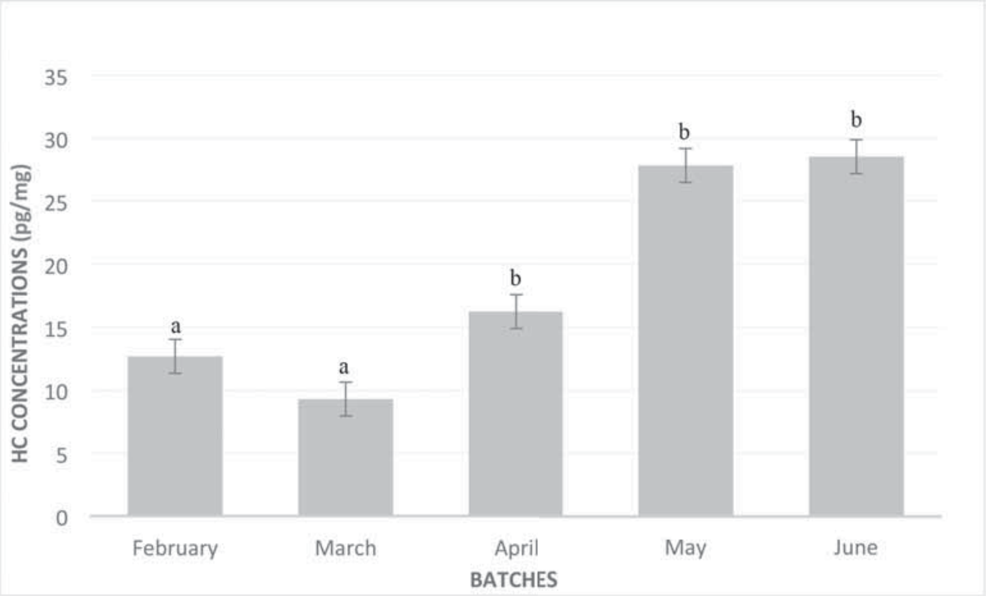
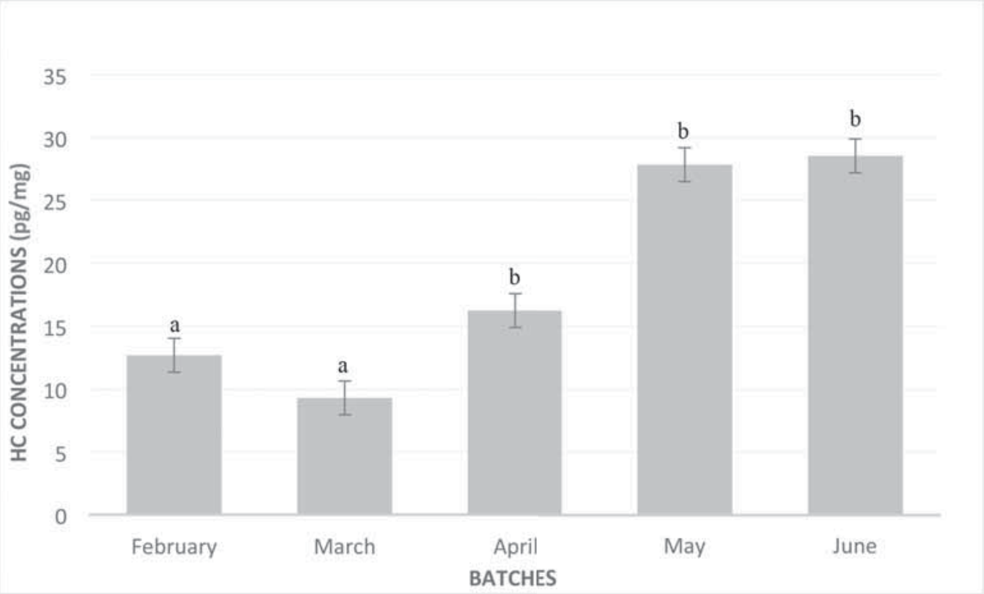


Figure 5 - Temperature, humidity, THI and estimated marginal means (±SEM) of hair cortisol concentrations (pg/mg) recorded in sows (n=296) in the different batches.
Figura 5 - Temperatura, umidità, THI e medie marginali stimate (±SEM) delle concentrazioni di cortisolo nel pelo (pg/mg) registrate nelle scrofe (n=296) nelle diverse bande.



4. DISCUSSION

Considering that hair is a retrospective matrix that allows us to obtain information on the medium-long term period, the hormonal concentrations of the hair samples obtained in this study (a few days before the expected date of farrowing) represented the hormonal status of the sows pre-partum, with the exclusion of the last 15 days.

Sampling operations started at the beginning of February and lasted until the end of June; during this period the average monthly value of THI increased progressively, reaching the highest levels in June.

From the hormonal point of view in our study an interesting progressive increase in HC concentrations throughout the batches was found ($P < 0.01$).

As described previously, after mating, sows remain in the gestation cages for 28 days. Here the animals received ultrasound pregnancy detection and those positive were transferred to multiple pens of 10-15 sows where they remained until 5 days before farrowing.

Thus, considering that the entire management was kept unchanged during the trail and for all the batches, the only environmental change recorded was the THI. Therefore, the increase in HC concentrations, the biomarker of the allostatic load, seems to be linked to the increase in THI.

Being cortisol a metabolic hormone, it is released in response to metabolic demands that can occur facing any stressor. The stress response is not a reflex type but depends on the assessment of the stressful situation and the potential threat that the event represents, in addition depends on the way in which the individual can deal with it, based on personal resources and previous experience at the same type of stressor (Lucassen et al., 2014; Dantzer, 2016).

Moreover, the increased THI observed in this study appeared to have increased the allostatic load in our sows even if some authors would classify it just as mild stress (Wegner et al. 2016; He et al. 2019).

It has already been reported that the resilience of animals is influenced by various environmental factors and in particular an exposure to increased thermal load reduces their ability to cope with stressors (Colditz & Hine 2016). In fact, so far, the literature reports a higher susceptibility of animals to high environmental temperatures than to lower one (Borges et al, 2020; Bate & Hacker, 1985; He et al, 2019). Sows are particularly sensitive to a hot environment not only because of their lack of effective sweat glands, but also their thick layer of subcutaneous adipose tissue that impedes radiant heat loss (Ross et al., 2015).

Heat stress in pig farms is very important because as described by several authors (Lucy & Safranski, 2017; Mayorga et al., 2020) involves the onset of production problems (reproductive problems associated with inadequate ovarian function such as: anestrus, weak expression or irregular estrus, delayed puberty, irregular oestrus cycles, low farrowing rates, increased abortion rates and reduced litter size, reduction of feed intake leading to a negative energy balance with loss of body condition and reduction of milk production which negatively affects piglet growth and weight at weaning).

Moreover, Sear et al. (1978) observed that the duration of the potential action is decreased by higher temperatures and thus the neuronal transmission and the consequent physiological functioning of the entire organism could be compromised.

Considering, therefore, that the THI, as we have seen, has progressively increased and that this was the only environmental/management change recorded during the study, it seems likely to suggest that during the rearing process act an aggregate effect of different stressors that were exacerbated with a higher THI. There are many stressors in a farming process but the most important stressor to consider is the “social factor”; in fact, as seen by many authors (Martínez-Miró 2016; Li et al. 2017; Verdon et al. 2015; Anil et al. 2006), the movement of sows from the individual pen (during insemination and the beginning of gestation) to the multiple box (during the rest of the gestation period), represents a high-impact stressor: animals are regrouped with unfamiliar conspecifics and, therefore, in this situation the animals fight to determine a new dominant hierarchy. A possible solution to this problem could be the regrouping of familiar individuals or keeping animals in multiple boxes for the entire production process.

Another aspect that can be taken into consideration is the genetic selection of animals. The selection focuses on increasing the growth rate of lean tissue and reproductive capacity but are both accompanied by increased endogenous heat production (Seibert et al., 2018); therefore, the genetic selection should focus considerably more on the robustness of the animals, in order to increase their resilience (Hermesch et al. 2015; Mormede & Terenina 2012).

Anyway, particular attention should be paid also to the environmental temperatures and humidity in which animals are reared. A comfort THI would allow them to be more resilient and to better cope with the other stressors that could affect the rearing process in the different phases; in fact, it would not result as an additional stress to the inevitable ones.

5. CONCLUSIONS

In the present study, the biomarker used to assess the allostatic load in sows allowed us to observe that with an increased THI the animals were subjected to a higher allostatic load; this could lead to a loss of resilience.

Thus, at farms would be necessary to assure a condition of thermoneutrality to avoid that those benefits brought by strategies adopted to improve the animal welfare (as e.g., multiple boxes) could be nullified. Once again, hair allowed us to study the HPA axis without interference of acute changes in hormone concentrations and with an easy sampling procedure.

Finally, based on the data collected by this study, new research could be focused on assessing both allostatic load and resilience (including new markers as DHEA/DHEA-S are and the cortisol/DHEA(S) ratio) in sows pre-and post-partum. The post-partum is another important phase of the reproduction cycle from which depends the farm performances

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