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Stress and its impact on farm animals

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1. ABSTRACT

This article presents a brief overview of stress and its impact on growth, production, reproduction and disease susceptibility in farm animals. A single measure of stress might not be a reliable indicator and it is usually more informative to combine multiple indicators of stress to assess animal welfare. Popular measures of stress such as alterations in hormonal profiles can be complemented with behavioral and immunological changes. Traditional approaches are insufficient to unravel the role of large number of genes and interconnected genetic pathways leading to multiple responses to stresses. Modern genomic and proteomics methodologies used in identifying the biomarkers and molecular mechanisms of stress response have also been described briefly. With a better understanding of the basic biology, altered physiological processes and the genes/proteins involved in stress adaptability, it would be possible to develop methodologies for selection of animals with better performance and devise strategies for animal welfare.

2. INTRODUCTION

The term *stress* has been derived from the Latin word *stringere*, meaning to draw tight. In the 1920s and 1930s, the term was occasionally used for harmful environmental agent that could cause illness. Walter Cannon used it in 1934 to refer to external factors that disrupted homeostasis. The novel usage arose out of Selye's 1930s experiments wherein he referred stress as a condition and stressor to the stimulus causing it. Stress is a reflex reaction revealed by the inability of an animal to cope with its environment, which may lead to many unfavorable consequences, ranging from discomfort to death. It covers the behavioral and biological responses to a wide range of abiotic stressors such as social interactions or rough handling, common farm practices (castration, dehorning, teeth clipping, shoeing, weaning crowding etc) improper feeding, exposure to adverse climatic conditions, exercise, work and transport etc. Stressors can originate from within an individual (endogenous) or from the environment (exogenous). Stress-triggering stimuli are not

necessarily painful but psychological states, such as fear or anxiety also activate physiological responses. Once animal perceives a threat, it develops behavioral, autonomic, endocrine or immune response to maintain homeostasis. In case animal is unable to withstand stress, the consequences will be abnormal biological functions and development of pathologies. Stress responses are related not only to the nature and the intensity of the triggering stimulus, but also to individual response tendencies or temperament.

The body systems which are mainly involved in the process of adaptation to the environment are endocrine system for long-term responses and nervous system for sensory inputs and short term responses. When an environmental pressure exceeds to that which animal's adaptive mechanisms can accommodate, stress related disease occurs. Many theories have been postulated to explain the organism's physiological response to stress. Hans Seyle proposed the general adaptation syndrome which provided the first comprehensive biological theory of stress. He proposed three stages which constituted alarm, resistance and exhaustion. When the threat or stressor is identified or realized, the body's stress response is a state of alarm. In this stage that animals react by fighting or fleeing. If the stressor persists, the body tries to adapt to the strains or demands of the environment. Exhaustion is the third and final stage when all of the body's resources are eventually depleted and the body is unable to maintain normal function. If stage three is extended, the immune system is exhausted resulting to development of psychosomatic disease, immunosupression, reduced efficiency of production and reproduction. It affects ability to perform and make animals susceptible to physio-pathological disorders.

3. IMPACT OF STRESS

Impact of stress on growth, production, reproduction and disease outcome in farm animals has been studied by various workers. A concise review of the effects of stress on these parameters and underlying mechanisms involved are briefly presented.

3.1. Growth and production

Growth and production performances of animals are adversely affected by different kinds of stressors. Increased levels of glucocorticoids in response to stress stimulate hypothalamic secretion of somatostatin, which inhibits growth hormone (GH) secretion from the anterior pituitary. It is obvious that growth of animal is affected by these stress hormones. Transportation stress in animals has been established to cause reduction in their body weight (1). During transportation the physiological alterations such as electrolyte imbalance, increased respiration rate and heart rate, dehydration, energy deficit and related catabolism have been reported (2). Besides transportation, heat stress has also been scientifically established to produce negative impact on the growth performance of animals. Different species, breeds and individuals depending upon their physiological states have comfort zones of temperature tolerance. Beyond these limits, the animals require extra energy for thermoregulation. Therefore, less energy is available for growth and production performances. Heat associated with high humidity or drought remains the most stressful condition for animals. In heat-stressed animals, there is reduced feed intake, which has negative impact on growth and milk production. Heat stress in lactating cow leads to decline in milk production and protein content (3). The fall in milk yield in a hot environment is higher for older and more productive animals especially at the peak of lactation. The animals experiencing cold stress also have reduced milk yield but the decline is less when compared to heat stressed animals. High ambient air temperature and solar radiation have been shown to have negative impact on dry matter intake, average daily gain, carcass weight and fat thickness in beef cattle (4). Decline in feed intake ranging from 40 to 60% in 15-month-old buffaloes by variation of temperature and humidity has been reported (5).

Milk production traits in small ruminants have also been reported to have negative correlation with temperature or relative humidity. Different breeds of sheep have been reported to have variable tolerance for temperature and humidity (6). Solar radiations have a lesser effect on milk yield, but a greater effect on yield of casein, fat and clot firmness in the milk of Comisana ewes (7). High air temperature also affects the milk yield and milk components in goats. Lactating goats deprived of water during heat stress activate an efficient mechanism for reducing water loss in urine, milk and by evaporation, to maintain milk production for a longer time (8).

In heat stressed pigs due to reduced feed intake the milk yield of the sow decreases, hence growth, viability and survival of piglets also decline (9). In high ambient air temperature the heavier pigs reduce more appetite and growth. Because protein deposits require more energy than fat deposits, the carcasses are leaner at slaughter. In a study, decreased growth, carcass lipid quality, and bacon quality in pigs housed at temperatures above the thermoneutral zone has been observed (10). Overcrowding further aggravates the condition whereas increasing the space allocation for housing may ameliorate the negative effects of heat stress. Multiple concurrent stressors like high temperature, high stocking density, and regrouping affect growth performance of pigs additively.

Environmental temperatures above 30°C cause reduction in feed intake, body weight, carcass weight, carcass protein and muscle calorie content and high mortality in broiler chickens (11). In hens there is reduction of body weight and feed consumption due to heat stress. Egg production, egg weight, shell weight and shell thickness are considerably compromised by heat exposure (12).

3.2. Animal Reproduction

Stress has been reported to influence the animal reproduction adversely. The impact of stress on reproduction depends on the type of stress, genetic predisposition of the animals, timing and duration of the stress. Stress conditions such as infection, strenuous exercise and malnutrition have been reported to predispose

to various reproductive pathologies like infertility or subfertility, defective oocytes and consequent reduction in conception rates. Reproductive processes like expression of sexual behavior, ovulation, and embryo implantation are controlled by neuroendocrine system. The alterations in neuroendocrine responses as a result of stress are likely to influence these processes.

Stress stimulates the hypothalamus, pituitary gland and gonads directly to affect gonadotropin-releasing hormone (GnRH) secretion into the hypophyseal portal blood. The hypothalamo-pituitary-adrenal axis is stimulated and produces corticotrophin-releasing factor (CRF) and arginine vasopressin. Corticotropin-releasing factor (CRF) interacts with GnRH-producing neurons, probably through an opioidergic pathway, suppressing gonadotropin secretion. Leptin and adiponectin also provide feedback to hypothalamus for GnRH release. The corticotrophs produce neuropeptides a adrenocorticotrophic hormone (ACTH), beta-endorphin and alpha-melanocyte-stimulating hormone due to stress impact. ACTH acts on the cortex of the adrenal glands to stimulate the synthesis and secretion of glucocorticoids. Glucocorticoid feedback action on the brain also suppresses overactivity of the hypothalamo-pituitary-adrenal (HPA) axis. In addition, glucocorticoid secretion is also believed to contribute to the stress-induced gonadal suppression by central actions on the pituitary or hypothalamus. The sympathoadrenal system consists of the sympathetic nervous system and the adrenal medulla. It is activated in response to stress and produces catecholamines (adrenalin, nonadrenaline and dopamine). Hormones that comprise components of the HPA axis, such as CRH, arginine vasopressin, ACTH, and glucocorticoids have all been shown to inhibit GnRH/gonadotropin secretion at the hypothalamic and/or pituitary levels. CRH inhibits GnRH release in hypophyseal portal blood or GnRH pulse generator activity. Arginine vasopressin and ACTH are also reported to inhibit LH secretion by decreasing responsiveness of the pituitary to GnRH as well as decreasing GnRH release.

The effects of heat stress on the reproduction of dairy cattle have been studied extensively. Heat stress in the summer months lowers the conception rate of lactating dairy cows from 40-60% to 10-20% (13). It has negative impact on the anterior pituitary, preovulatory follicle, corpus luteum, embryo developments and endometrium resulting in low fertility and loss of fetus. Eestrus behavior in cattle and buffaloes is affected by heat stress in summers resulting in silent estrus or reduced estrus intensity and decreased duration. Heat stress damages ovarian follicles and causes a decrease in estradiol synthesis. This decrease in estradiol synthesis could influence expression of estrus, ovulation, and the corpus luteum (14). It has been demonstrated that dairy cows in the summer had approximately one-half the number of mounts per estrus compared to dairy cows in the winter (15). Stress results in disturbance of spermatogenesis, decreased sperm fertility parameters and disturbed folliculogenesis. Catecholamines interfere with transport of gametes and decreases blood flow (16). Early embryonic loss in livestock is common due to stress. Prenatal maternal stress results in increased incidence of spontaneous abortion, preterm delivery and low birth weight. Maternal prenatal stress may induce over-activity and/or dysregulation of the HPA-system in the offspring (17). Cattle, sheep in heat stress exhibit reduced uterine and umbilical blood flows, resulting reduced fetal oxygen. Heat stress has also shown to have deleterious effect on estrus incidences, estrus intensity and embryo production in ewes (18). The service period is increased because of stress arising due to milk fever or lameness in dairy cows (19). Stress due to transportation in dairy cattle reduces LH secretion in response to exogenous GnRH (20). In sheep, restraint and confinement enhances plasma cortisol concentrations and simultaneously decrease the pituitary's response to GnRH administration (21). Conditions, in which nutritional status is suboptimal, such as eating disorders, exercise-induced amenorrhea, and functional hypothalamic amenorrhea, are associated with low serum leptin levels (22). GnRH pulse is highly affected by weight loss, decreased energy availability, altered body fat ratio (23). Although short-term fasting of adult cows in healthy body condition does not affect LH pulse frequency. Short-term fasting of peripubertal heifers leads to significant reductions in leptin gene expression and circulating leptin, along with decrease in LH pulse frequency (24).

3..3 Disease susceptibility

It has been reported by many workers that various stressors increase the susceptibility to infection. Under stress conditions, pathogens like viruses or mycoplasma predispose the animals to secondarily bacterial infections, allowing opportunistic bacteria to become pathogenic. Increased risk of fatal bacterial respiratory infections following a primary viral infection has been observed in a wide variety of species (25). Viral–bacterial synergy has been established following human influenza epidemics and secondary bacterial respiratory infections leading to increased mortality. Stressors such as transportation have been associated with susceptibility to bovine respiratory diseases (BRD). The most intensively explored relationship of this kind has been that of exposure of calves to weaning and transportation and their subsequent susceptibility to shipping fever. BRD can be caused by a primary infection with a virus, commonly bovine herpesvirus-1 (BHV-1), followed by a secondary bacterial infection with *Mannheimia haemolytica* (26). The relationship between stress and mastitis in cattle is well documented. Infection by Mycobacteria, causative agent of tuberculosis is known to be increased by stress. Glucocorticoids have been shown to increase the susceptibility of experimental mice to infectious *agents L. monocytogenes, S. aureus, P. aeruginosa, Candida albicans* etc. Nutritional imbalances, deficiencies can create various types of metabolic diseases like acetonemia, preganancy toxemia of cattle and sheep on deficient diets, hypocalcaemia of sheep, hypomagnesemia of cattle The sensitivity of animals to the environmental stress is greatest at times when they are already affected by metabolic stresses, late pregnancy, lactation etc. Three closely related stress syndromes occur in pigs. The porcine stress syndrome is common in commercial breeds of pigs used to raise low fat pork. It is characterized by acute death

induced by stressors such as transport, high ambient temperature, exercise and fighting which lead to hyperthermia, dyspnoea, disseminate vasoconstriction and rapid onset of rigor mortis. These pigs are particularly susceptible to stress and if frightened may collapse or even spontaneously die. Malignant hyperthermia is drug induced stress syndrome characterized by muscle rigidity and hyperthermia occurring in the susceptible pigs following the use of halothane. Black muscle necrosis of pigs is another manifestation of porcine stress syndrome. Stress from metabolic problems may decrease the animal's resistance and compromise immune system function.

4. INDICATORS OF STRESS

Biological responses to a stressor have been used most frequently as indicators of stress. It is usually more informative to combine multiple indicators of stress to assess animal welfare. Behavioral and the immunological responses also serve as indicators of stress and welfare of animals.

4.1.Behavioral changes

Changes in vocalization, motor activity or the expression of stereotypic behavior may be an early indication of a stressful situation. The effect of acute stress on the behavior of dogs was evaluated (27). Among the 58 examined dogs, 50 displayed at least one type of activity or posture indicative of stress. Among the activities or postures that indicate stress, vocalizations were the most common, followed by flattened ears, low tail, lowered body posture, paw lifting, digging, oral manipulation of the cage, hiding, yawning, unresponsiveness and licking and grooming. Most dogs displayed multiple activities or postures. Males spent significantly more time vocalizing than females, while females adopted stress-related postures more often than males. Females displayed behavioral and physiological indicators of stress more often and for longer periods of time than males.

Ewes and lambs temporarily separated from each other express their distress by an increase in bleating and locomotor activity. Foals have been reported to increase general motor activity and vocalizations (28, 29). Young piglets separated from their mother give distinctive and frequent squeals and sometimes try to jump out of their pen. These behavioral modifications help the animals to cope with the stress-inducing situation and communicate with each other. Dairy cows showed an increased percentage of the whites of their eyes, when their 4-day old calves were temporarily removed from them. Weaning creates a state of frustration, among young ones. It is a complex stressor as it includes dietary change, environmental change, social change, and it prevents the animal from performing suckling.

Pigs, calves and cows try to avoid humans after they have experience of stockmen who hit, kick, prod, shock or threaten them. Pigs can be severely stressed by anxiety and fear caused by human handling. They can collapse and even die as a result. Hungry cows that were prevented from eating grass that they could see and smell showed their frustration by rolling their tongues, shaking their heads and opening their eyes abnormally wide. Vocalization, frequency of urination and defecation are increased in cattle due to acute stress. Incidence of stepping increases in isolation chamber or while being milked in unfamiliar surroundings. Increased movement is considered as sign of agitation in cattle (30). Hens have a particular 'frustration' call (the gakel call) when they are thwarted in getting to food, water, a dustbath or a nestbox. The birds show boredom and frustration by hyperactivity, aggression, stereotyped pacing and pecking. When stress becomes long-term, animals kept in confinement have been reported to carry out repetitive, apparently purposeless actions. These include the 'weaving' of horses kept in stalls, the bar-biting, tongue-rolling and head-waving of sows confined in narrow 'sow stalls' (gestation crates), the self-licking and tongue-rolling of calves confined in narrow 'veal crates', the repetitive pacing of zoo animals from one end of the cage to another. The cats show defensive vocalizations, inappropriate urination/defecation, piloerection, pupil dilation and hiding. Signs of stress in birds include depression, irritability, feather picking, increased pecking, abnormal vocalization and ruffled feathers

4.2. Hormonal indicators

During stress, various endocrinal responses are involved to overcome stressful situations. The immediate endocrinal responses are symphatho-adrenomedullary system releasing catecholamines. The main biological stress responses are related to the HPA axis releasing corticosteroid *i.e.* cortisol, corticosterone, aldosterone in to blood. Corticotropic-releasing hormone (CRH), which acts on the anterior pituitary to synthesize and release ACTH, which in turn is released into the peripheral circulation to cause the release of glucocorticoids from the adrenal cortex. Plasma ACTH provides the direct determination of stress whereas cortisol provides the indirect criterion of stress.

Besides plasma, salivary and urinary cortisol levels are good indicators of stress (31). The disadvantage is that plasma cortisol levels naturally increase in the morning and decreases around midnight (32). Another disadvantage is that all types of stressors do not induce an increase in cortisol levels (33). Changes in cortisol levels do not occur in response to weaning stress. The complexity of diurnal activity, natural increase in cortisol, individual variability suggests the use of other markers as well to evaluate the stress response.

Fecal glucocorticoid analyses have been used in a wide range of studies as this is non-invasive measure of these stress hormones. Salivary alpha amylase has also been used as marker of stress.

4.3. Oxidative stress biomarkers

Oxidative stress is a condition associated with an increased rate of cellular damage induced by oxygen and oxygen derived oxidants commonly known as reactive oxygen species (ROS). Uncontrolled production of ROS that exceeds the antioxidant capacity of cells leads to

oxidative stress. These ROS result in damage to nucleic acids, proteins and lipids leading to tissue damage cell death. Stressors, such as heat, toxins, ultraviolet rays, inflammation, infections, etc. can lead to the oxidative stress, with important consequences over the function, life and death of the affected cells.

Oxidative stress can be assessed by three methods
Quantification of the reactive oxygen species ROS
Estimation of the antioxidant enzymes and redox molecules.
Measurement of oxidative damage

4.3.1. Quantification of oxidants and antioxidants

Oxidative stress describes an imbalance between oxidants and antioxidants. It occurs in the presence of ROS in excess of available antioxidant buffering capacity. A free radical has unpaired electrons in the outer orbital and thus makes the specie unstable with affinity to react with other molecules for stability. Oxidant and antioxidant action can be observed directly by electron spin resonance and indirectly by quantification of dichlorofluorescein, a fluorescent product formed by reaction of non-fluorescent diacetate dye with oxidants. It can be measured by using a fluorescence plate reader, by microscopy or by flow cytometry. Electron spin resonance is not routinely used.

4.3.2. Estimation of the antioxidant enzymes and redox molecules

Changes in oxidative stress biomarkers, including superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, glutathione levels, vitamins, lipid peroxidation, nitrite concentration, nonenzymatic glycosylated proteins can be estimated. The two most important antioxidant biomarkers are GSH and ascorbate which are capable of regenerating other antioxidants to their active state. Decreased redox ratios of GSH–GSSH or ascorbate–dehydroascorbic acid indicates increased oxidative stress and decreased antioxidant status. The changes in lipophilic antioxidants vitamin E and coenzyme Q may also be measured along with GSH and ascorbate which are also biomarkers of oxidative stress. These compounds can be measured directly by high-performance liquid chromatography (HPLC) and spectrophotometry. Assays are also available to determine the activity of some antioxidant enzymes, such as catalase and superoxide dismutase. The overall antioxidant capacity can be determined by performing various tests like total antioxidant status, total radical-trapping antioxidant parameter, Trolox equivalent antioxidant capacity, ferric reducing-antioxidant power colorimetric assays and cyclic voltammetry.

4.3.3. Measurement of oxidative damage

Measures of oxidative damage have typically focussed on the three major macromolecules: DNA, proteins and lipids. Oxidative modifications to DNA include base misincorporations, mutations, DNA strand breaks and cell death. Quantification of oxidised DNA damage is done by COMET assay. It assesses the number of DNA strand breaks in single cells semiquantitatively using gel electrophoresis (34). Oxidised nucleotides and nucleosides can be quantified by HPLC or mass spectrometry.

Protein oxidation leads to malfunctioning of enzymes incapable of performing their cellular tasks. Protein oxidation is estimated in terms of measurement of ‘‘protein carbonyls’’, which are produced by free radical-mediated oxidation of amino acids. A crude homogenate or a microsomal fraction is reacted with dinitrophenylhydrazine, which gives strong absorbance upon reaction with carbonyl groups (35). Increased absorbance at 370 nm is interpreted as an indication of increased protein oxidation.

Lipids are important constituents of the lipid bilayer of the cellular membrane and unsaturated fatty acids in particular are easily oxidised and may initiate chain reactions resulting in further oxidative damage. Polyunsaturated Lipids are more sensitive to oxidation. Lipid oxidation can be assessed by either the malondialdehyde (MDA) or isoprostane assays. Malondialdehyde is a breakdown product of lipids which can be quantified as a measure of lipid hydroperoxides. This is a crude assay known as thiobarbituric acid reactive substances (TBARS) assay which involves derivatisation with thiobarbituric acid, which is measured by spectrophotometer. More refined versions of the TBARS assay measure only the MDATBA2 derivative, by HPLC, or determine MDA directly without derivatisation (36). This assay predicts oxidative damage efficiently and has been shown to have very good correlation with other markers such as isoprostanes, which are the most reliable markers of lipid oxidation (37). Earlier, requirement of mass spectrometry being an expensive equipment limits the isoprostanes determination as a screening marker but nowadays, an ELISA based isoprostane assay has become commercially available. Changes in oxidative stress biomarkers, including superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, glutathione levels, vitamins, lipid peroxidation, nitrite concentration, nonenzymatic glycosylated proteins, and hyperglycemia in diabetes is also reported (38).

4.4. Immunological markers

The immune system is extremely sensitive to stress and therefore, immune variables can be used as indicators of stress. Several aspects of immune function are affected by various stressors, such as exercise, transport, tissue injury, infection etc. The effects of stress on immune function have been widely studied (39-42). Hormones induced in response to stress have effect on immune system of animals either by inhibition or proliferation of immune cells. Hormonal changes in response to stress, including rises in the plasma concentration adrenaline, cortisol, growth hormone and prolactin are known to have immunomodulatory effects. Cortisol levels are known to increase after a stressful event due to the activation of the HPA. It has been observed that cortisol can inhibit the functions of macrophages, mast cells, neutrophils, basophils, and eosinophils. Glucocorticoids and ACTH have been shown to affect the proliferation of B and T cells, cytokine production, and antibody production,

chemotaxis of monocytes and neutrophils and NK cell cytotoxicity (43). Potential immunological markers in response to stress include lecukocyte responses to antigens, salivary IgA, neutrophil/lymphocyte ratio, CD4/CD8 ratio and plasma cytokines.

Strenuous exercise, like exhaustive endurance races in horses may cause detrimental effects on the immune system, by changing the cellular composition of peripheral blood. Exercise induced stress leads to substantial increase in the number of circulating leukocytes (mainly lymphocytes and neutrophils), the magnitude of which is related to both the intensity and duration of exercise. There are also increases in the plasma concentrations of various substances that are known to influence leukocyte functions such as inflammatory cytokine, C-reactive proteins and activated complement fragments. Following recovery from exercise the blood neutrophil count continues to increase and the blood lymphocyte count decreases, it has been suggested that the neutrophil/lymphocyte (N/L) ratio can provide a good measure of exercise stress (44). T-lymphocyte CD4+/CD8+ (helper/ suppressor) ratio, secretory immunoglobulins such as salivary IgA, and *in vitro* mitogen-stimulated lymphocyte proliferation are decreased in overtraining syndrome (45, 46).

Neutrophilia and lymphopenia was also reported in cattle which have been transported (47). They also reported a decrease in antibody responses three days after transport and a decrease in lymphocyte blastogenesis in calves after transport. In a study, a 9 h transportation of young bulls induces a gene expression signature in blood neutrophils that increases their circulating numbers and may enhance their pro-inflammatory and anti-bacterial potential (48).

Macrophages and lymphocytes in bronchoalveolar fluid from young calves were changed in number and function after short-term transport (49). Neutrophils are also targets of the stress response and are important in lung defense. Neutrophilia along with altered the neutrophil:lymphocyte ratio has also been observed during the weaning stress increasing the proportion of neutrophils (33). Changes in the neutrophil:lymphocyte ratio is thought to be a potential biological indicator of stress and disease susceptibility. It has been reported that corticosteroids decrease the accumulation of neutrophils at a local inflammatory site such as neutrophil accumulation is important in clearance or resistance to bacterial infection. Increased susceptibility to infection as well as severity of disease due to altered immune function in stress response has been demonstrated in many species including humans, cattle and mice (50-52). T-helper 1 (Th1) cells are involved in cell-mediated immunity whereas Th2 cells are involved in antibody production. Inhibition of IL-12 synthesis and an increase in IL-10 production has been reported when peripheral blood leukocytes (PBLs) were treated with catecholamines *in vitro* (53). This shift in cytokine production causes a shift in T-helper (Th) cells from Th1 cells to Th2 cells. A shift away from a Th1 cell mediated response can result in increased susceptibility to viral infections (53).

4.5. Genomic and Proteomic markers

The involvement of genetic factors in neuroendocrine stress responses has been shown by selection studies. Divergent lines for HPA axis functioning have been obtained by genetic selection in poultry, pigs and turkeys (54-57). Considerable differences between breeds of pigs have been observed in cortisol levels both in basal conditions and under stress. To understand multiple responses studies are underway to map genomic areas, identify candidate genes, mutations and underlying genetic pathways responsible for such responses. Genomic regions responsible for phenotypic variation in stress conditions have been detected by using Quantitative trait loci (QTL) approach. The gene encoding corticosteroid-binding globulin (CBG), carrier of cortisol in plasma, had been mapped at chromosome 7 in Meishan and Large White crosses (58). Mutations in this gene have been found to influence cortisol levels, carcass composition and meat quality in pigs.

Phenotypic variation is not only associated with mutations but significant differences are also attributed to alterations in expression levels of clusters of genes. Nowadays, microarrays, serial analysis of gene expression allow the measurement of expression of thousands of genes at the same time in different tissues and provide unique possibilities to expedite large-scale analyses of gene expression. Changes at the transcription level do not always correlate with changes in expression of proteins but study of proteome can provide better understanding of the functioning inside body. Proteomics methodologies have sufficient resolution and sensitivity to identify candidate biomarkers. Proteomics techniques make it easier to identify changes or differences between a stressed and a healthy animal. High-throughput proteomic methods based on matrix-assisted laser desorption/ionization with time-of-flight mass spectrometry (MALDI-TOFMS) analysis of tryptic 2D electrophoresis (2-DE) spot digest and peptide matching with available database have been used to study the protein biomarkers. This technique has been used to find the differential stress responses at protein levels in mandibular lymph nodes and oropharyngeal tonsils of European wild boars (*Sus scrofa*), naturally infected with *Mycobacterium bovis (*59) .

The intergrated approach of combined OMICS has also been used to obtain proteomic, metabonomic, and elemental profiles of bovine serum samples from stressed and control animals before and after a primary viral infection. (60). Differential trends of protein, metabolite, and element profiles were observed following a stress response by multivariate analysis. Proteomics profile analysis in rat liver has also been used to unravel the molecular mechanisms involved in the cellular responses to ionizing radiation (61). Comparison of the serum proteome yielded a new stress biomarker in pigs housed at higher density (62).

5. CONCLUSIONS

Despite our pursuit of understanding the complexity of the physiological processes mediating stress

responses, it is not possible to make generalized predictions concerning the degree of impact of stress on the animal. Although it is easy to measure the variations at physiological and molecular levels in response to presumed stressful events, but it is the animal's perception of these events that dictates its state of well-being. The traditional physiological indictors need to be complemented with the behavioral, immunological and proteomic profiles to ascertain the well being of farm animals. With a better understanding of the basic biology, altered physiological processes and the genes/proteins involved in stress adaptability, it would be possible to devise strategies for animal welfare and optimal production.

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Abbreviations: GH: growth hormone; GnRH: gonadotropin-releasing hormone; CRF: corticotrophin-releasing factor; ACTH:adrenocorticotrophic hormone; HPA: hypothalamo-pituitary-adrenal; LH: leutinizing hormone; BRD: bovine respiratory diseases; BHV-1: bovine herpesvirus-1; CRH: Corticotropic-releasing hormone; ROS: reactive oxygen species; GSH: reduced glutathione; GSSH: oxidized glutathione; HPLC: high-performance liquid chromatography; MDA: malondialdehyde; TBARS: thiobarbituric acid reactive substances; NK: natural killer; Th: T helper; IL: interleukein; PBLs: peripheral blood leukocytes; QTL: Quantitative trait loci; MALDI-TOFMS: matrix-assisted laser desorption/ionization with time-of-flight mass spectrometry; 2-DE: 2 dimensional electrophoresis

Key Words: Stress; Animals; Disease susceptibility; Oxidative stress biomarkers; Genomic and Proteomic markers, Review

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