Methods of Measuring Pre-slaughter Stress in Farm Animals

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DEFINITION

Stress as defined by Selye (1950) is the action of nervous and emotional stimuli elicited by an animal's environment on the nervous, circulatory, endocrine, respiratory and digestive systems to produce measurable changes in the functional levels of these systems.

PHYSIOLOGY OF STRESS

When an animal is subject to stress the sympathetic nervous system is stimulated which causes the release of the catecholamines epinephrine and norepinephrine from the adrenal medulla. At the same time, as the nervous impulses reach the hypothalamus, they are converted to the neuro-humoral factor corticotropine-releasing factor (CRF) which then stimulates the anterior pituitary to secrete the hormone adrenocorticotropin (ACTH). This in turn reaches the adrenal cortex via the general circulation and causes an increase in glucocorticoid secretion (Siegel, 1971).

These hormones have a number of biochemical and physiological effects on the animal. To summarize some of the more important effects:

A. Effects of Catecholamines:

(1) increased blood sugar, (2) vasoconstriction and rise in blood pressure, (3) increased respiration rate, (4) increased muscle tone, (5) increased nerve sensibility, and (6) increased heart rate.

B. Effects of Glucocorticoids:

(1) lymphatic involution; (2) blood cell changes; (3) changes in the chemistry of the blood such as: (a) ions, (b) cholesterol, (c) nitrogen products and (d) sugar; (4) gastrointestinal ulceration; (5) anti-inflammatory action; and (6) antibody activity.

As a result of these increased activities of the adrenal glands, several changes, termed "adaptive reactions" (Selye, 1950), occur in the animal: (1) glycogen reserves are used up, (2) ATP and creatine phosphate are utilized in tissue respiration, (3) blood is forced out to the capillaries due to the increased heart rate and

muscle tone, (4) oxygen consumption is increased due to the increased tissue respiration.

POST-MORTEM CHANGES

When stressed animals are slaughtered several post-mortem changes occur. A reduced amount of lactic acid is produced by glycolysis due to low levels of glycogen reserves. This results in meat with a higher pH and a greater susceptibility to bacterial spoilage (Hedrick, 1965). Also, stress results in a greater volume of blood pumped out to the capillaries, resulting in a greater tendency for blood to be retained in the meat of such animals, which adversely affects the keeping qualities of the meat. In addition, since there is an increased oxygen uptake just prior to slaughter, oxidative enzymes are more active; oxygen diffusion is reduced and the decreased availability of oxygen acts to convert bright red oxymyoglobin to dark red deoxymyoglobin resulting in a darker cutting meat (Ashmore et al., 1971).

STRESS IN PRE-SLAUGHTER MANAGEMENT

There are a number of areas in pre-slaughter management where farm animals are routinely inadvertently subjected to stress. These include: (1) transportation of animals to slaughter, (2) struggling, fighting and overcrowding of animals in strange holding pens, (3) exposure of animals to acute changes in atmospheric temperature and pressure prior to slaughter, and (5) stress associated with the slaughtering process.

Transportation of animals from the farm to the place of slaughter may cause varying degrees of fatigue. Callow (1936) studied the effects of ante-mortem fatigue in pigs on the quality of cured pork and reported that the physiological condition of the animal at the time of slaughter influenced the subsequent properties of the meat. Pigs slaughtered at the farm were generally unstressed and rested; their muscles had high levels of glycogen. After slaughter the glycogen was converted to lactic acid and the meat acquired a low pH, an open structure, low electrical resistance and good resistance to bacterial spoilage. On the contrary, pigs transported to the slaughterhouse were fatigued, stressed and arrived with lowered muscle glycogen reserves. Following slaughter, muscles from these animals had low levels of lactic acid, a high pH, greater electrical resistance, a closed structure and more susceptibility to bacterial spoilage (Gibbons and Rose, 1950).

Excitement is probably the most common form of pre-slaughter stress encountered by farm animals. In most instances, animals due to be slaughtered are placed in strange environments and subjected to handling to which they are unaccustomed. The influence of excitement on physiological processes and subsequent meat properties is dependent in part on the excitability of individual animals and the duration and severity of the stressor (Hedrick, 1965).

Fighting among pigs in holding pens immediately prior to slaughter has been associated with rapid pH drop post-mortem and decreased water holding capacity of the muscle. The frequency of pigs showing rapid decline in pH and decreased muscle water holding capacity appeared to be related to the level of pre-slaughter stress. Meat

from stressed pigs was paler in color (Wismer-Pedersen, 1959; Wismer-Pedersen and Riemann, 1960).

Environmental temperature was reported by Wismer-Pedersen (1959) and Forrest et al. (1963) to influence the incidence of the pale, soft and watery condition. A higher incidence was observed during periods of high environmental temperature or wide temperature fluctuations immediately pre-slaughter. The incidence was more prevalent during the fall and summer months. Considerable day-to-day variation has been observed in the incidence of the condition within the same slaughter plant (Hedrick, 1965).

Pre-slaughter feed restriction may influence tissue shrinkage. Although the effect of tissue shrinkage on meat palatability has not been fully determined, Zessing et al. (1961) reported that pigs fed maintenance or submaintenance diets for four weeks pre-slaughter had less intramuscular fat in the loin and less back fat than pigs fed a growing and fattening ration during the same period. Cooked roasts and chops from the pigs subjected to dietary restriction were less tender and juicy, had less aroma and flavor, and were less desirable compared to the same cuts from pigs on the higher plane of nutrition. In another study, Jacobs et al. (1973) found that feeding a reduced energy ration to lambs during the last two weeks prior to slaughter had a detrimental effect on slaughter weight, carcass weight, longissimus muscle area, leg conformation score, carcass grade, marbling score, color of lean and yield of both wholesale and retail cuts.

Reduced muscle glycogen stores may be implicated as a cause of some of the problems, but the reduced glycogen may not be a result of feed restriction alone, since this causes an increased adrenal cortical hormone secretion in a normal animal which initiates gluconeogenesis. However, if feed deprived animals are subjected to additional stressors, they are more susceptible to further reductions in muscle glycogen and the effects are then more apparent in the meat (Howard & Lawrie, 1956).

The short-term stress which occurs during the few seconds of shackling, stunning and sticking has hitherto been overlooked by many research workers. Studies carried out in this area at The University of Connecticut revealed that a double rail conveyor system for restraining calves and lambs in higher production operations and a yoke method of restraining these animals in lower production systems were both effective in reducing the short-term stress associated with the slaughtering process (Giger et al., 1976; Westervelt et al., 1976).

MEASURING STRESS

The effects of pre-slaughter stress on farm animals can be measured before, during and after slaughter. The parameters used for the measurement of stress before and during slaughter can be divided into three categories. These are physiological, biochemical and sensory parameters.

The physiological parameters used are: (1) heart rate (electrocardiogram), (2) brain activity (electro-encephalogram), (3) respiration rate, (4) rectal temperature, (5) femoral blood pressure, and (6) bleed-out volume. The biochemical parameters used include: (1) carcass pH, (2) ATP, c-AMP, creatine phosphate and lactic acid concentrations, (3) glycogen levels, (4) levels of glucose-6-phosphate, (5) levels of glucocorticoids, and

capacity, shear force, flavor, juiciness and tenderness.

Four of the most suitable parameters for use in assessing the stress associated with the transportation of animals to slaughter are: muscle glycogen, muscle pH, meat color, and shear force. Glycogen level is measured by the technique of Somoygi, Good and Kramer (1933); muscle pH can be accurately measured by blending 2 grams of the muscle in 20 mls of .01 M iodoacetate pH 7.5 and reading the resulting pH with a combination electrode (Ono et al., 1977). Meat color is best evaluated by a trained taste panel scoring duplicate samples cooked on different days (Lewis et al., 1967). Shear force is measured using a Warner-Bratzler shear device (Lewis et al., 1967).

Shorthose (1977) assessed the effects of resting sheep after a long (1110 km) journey on concentrations of plasma constituents and post-mortem changes in muscle and meat properties and reported that animals rested for only 18 hours had less glycogen in the longissimus dorsi muscle and liver at slaughter than those rested for 120 hours. The ultimate pH values of the longissimus dorsi (5.94) and the semitendinosus (6.31) muscles of rams rested for 18 hours were greater than those of the same muscles from animals that ate during their 120 hour rest period. Meat color was darker in chops from animals rested for 18 hours than those from animals rested for 120 hours.

The stress associated with the struggling, fighting and overcrowding of animals prior to slaughter can be assessed by analyzing post-mortem pH changes, water holding capacity and color of lean. Huffman et al. (1969) reported the use of a portable pH meter as a convenient and rapid technique for determining muscle pH changes post-mortem on the freshly exposed surface of the semimembranosus muscle. Water holding capacity can be determined using an inverse phase gas chromatograph as described by Coelho, Miltz and Gilbert (1979). In addition to the very subjective visual appraisal of meat color, reflectance analysis can be used to give a more reliable and accurate determination. Using this technique, Ngoka et al. (1982) observed substantially greater redness and increased myoglobin concentration in breasts from birds subjected to normal excitement prior to slaughter and allowed to struggle freely during slaughter.

The most convenient methods for measuring the effects of pre-slaughter stress caused by acute changes in environmental temperature and pressure are by recording physiological parameters such as respiration rate, heart rate and rectal temperature. Respiration rate can be recorded by an apparatus that responds to changes in pressure in the pleural cavity or the trachea. A pneumograph, stethograph or plethysmograph may be used to indicate changes in the circumference of the thorax and thus record respiratory movements. The pneumograph consists of a coil spring inside a rubber tube tied around the thorax. One end of the tube is closed; the other end is connected to an appropriate recording device. The stethograph consists of a cylinder with a rubber diaphragm at each end fastened to a cord around the thorax. The cylinder is connected to a recording device. In both the pneumograph and stethograph, inspiration decreases the pressure in the apparatus, and expiration increases the pressure. Translation of these pressures to a line of pen-recording instrument indicates respiratory movements.

The plethysmograph consists of a rubber bag filled with air, fixed between a metal sheath and the thorax. In this apparatus, inspiration increases the pressure in the bag and expiration decreases the pressure (Frandson, 1975).

Heart rate is usually measured with an electrocardiograph. This is essentially a strong galvanometer whose fluctuations are recorded on sensitized paper to produce the record called the electrocardiogram (ECG). It is a record of the electrical activity of the heart picked up from electrodes attached at parts of the body other than the heart itself. The conventional chest leads have been recorded in birds (Kisch, 1951; Douglas, 1960) while the standard bipolar leads have been used for larger animals.

Rectal temperature can be measured with a copper-constantan thermocouple or with thermistor probes and telethermometers which have been found to operate with more precision (Misson, 1978).

The effects of pre-slaughter dietary stress on farm animals can be best assessed by the grading of the carcass using the USDA meat grading standards and a sensory evaluation of the meat (Romans and Ziegler, 1974).

A number of methods can be used for evaluation of the effects of stress associated with the slaughtering process. The physiological parameters which can be measured are: heart rate, brain activity, respiration rate, rectal temperature, femoral blood pressure and bleed-out volume; the biochemical parameters which can be used are: carcass pH, ATP, c-AMP, creatine phosphate and lactic acid concentrations, glycogen level, glucose-6-phosphate, levels of glucocorticoids and adrenal medullary secretion. Post-mortem parameters which can be used are meat color, cooking loss, water holding capacity, shear force, flavor, juiciness and tenderness.

A number of advances have been made in the field of biotelemetry over the last two decades which have resulted in a wide range of more accurate equipment becoming available for the measurement of the physiological reactions of animals to stressors. Grant, Thompson and Corner (1971) reported that a single channel radio transmitter built commercially to telemeter electrocardiographs (ECG), electromyographs (EMG) or electroencephalographs (EEG) had been modified to transmit both ECG and respiration with a negligible increase in weight by adding a 20,000 ohm resister between the a-c signal output and the positive terminal of the battery. The respiration is recorded as a modulation of the ECG trace. New multi-channel transmitters are now available.

Radiotelemetry has been particularly useful in overcoming the problems associated with handling because after the transmitters have been implanted or attached to the animal there is little subsequent interference. For example, Duncan and Filshie (1979) used radio telemetry to record heart rate and body temperature of domestic fowl.

Electroencephalogram is the recording of the electrical activity of the brain of an animal. Like ECG, it may be recorded by either a bipolar or a unipolar technique. Interpretation is based on the amplitude and pattern of fast wave signals (Newhook et al., 1982).

Femoral blood pressure is usually measured by inserting a tube-cannula into the femoral artery of one hind leg

of the animal. It is connected to a pressurized vessel or electronic transducer which in turn is connected to an electronic amplifier and recorded. Pressure changes are then permanently recorded as a pen is caused to move on graph paper with changes in the transducer created by blood pressure changes. Blood pressure is conventionally measured in millimeters of mercury (mm Hg) (Frandson, 1975).

Bleed-out volume is determined by collecting all blood directly into graduated cylinders when the animal is exsanguinated (Westervelt et al., 1976).

ATP, creatine phosphate and lactic acid are assayed according to the procedures of Bergmeyer (1963), while the competitive protein binding technique (Gilman, 1970) can be used to assay for c-AMP. Glucose-6-phosphate can be determined according to the procedures of Schmidt et al. (1972). Glucocorticoid levels have been determined by the procedure of Martin and Martin (1968); however, the competitive protein binding technique has been found to give more accurate results (Buckland, Belgrave and Lague, 1968). Analysis for epinephrine and norepinephrine can be done by the photo-fluorometric method. More recently radio-immunoassays have been develooed for many of these analyses.

Color of lean, tenderness, juiciness and flavor can be best evaluated by a trained taste panel. Cooking loss is determined as the difference between the raw and cooked weights of a unit volume of meat. Shear force is measured using a Warner-Bratzler shear device (Lewis et al., 1967).

Because of the large number of methods available for measuring stress during slaughter, it is very probable that one will need to select a limited number of methods based on the resources available. In determining what stress indices should be studied, one should assess the versatility, repeatability, cost and reliability of the different methods. Of the methods described, heart rate, respiration rate, rectal temperature and carcass pH have been found to best fit the criteria (Westervelt et al., 1976).

It is always very difficult to study the effects of specific stressors on a live animal without introducing artifacts due to the investigative technique (Alder, 1976). This is particularly so in the measurement of such parameters as respiration rate, heart rate and glucocorticoid levels where recording instruments have to be attached to the animals. Will it ever be certain beyond a reasonable doubt that the experimental manipulation necessary to obtain the needed samples or observations are not themselves so stressful as to compromise the results? As mentioned earlier, the advent of radiotelemetry has been particularly useful in overcoming the problems associated with recording physiological data from unrestrained animals.

Although biotelemetry has undoubtedly improved the accuracy of stress measurements, there are a number of other possible sources of error. There is some evidence to suggest that the peak concentration of glucocorticoids which occurs in animals during a daily cycle represents the maximum secretory rate for that animal (Perry, 1973). Thus, if an animal is stressed at this time there will be no response in terms of an increase in the level of glucocorticoid.

In addition to variability within individuals, there is also variability between individuals due to such factors as age, sex and breed. Westervelt et al. (1976) found that wethers had a significantly ($p < .01$) lower mean heart rate than ewes or rams while Dorsets had a significantly ($p < .05$) higher mean heart rate than Suffolks, Southdowns or Shropshires.

Nevertheless, if the researcher is cognizant of the potential sources of error and makes allowances for them in his experimental design and statistical analysis, all the methods described can be used with a good degree of accuracy as indices of stress before and during slaughter.

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