EFFECT OF FEEDING *Portulaca oleracea* EXTRACT ON THE AMELIORATION OF HEAT STRESS IN BROILER

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The Author
ABSTRACT

An experiment was conducted on 21-day old Cobb broilers to study the effect of feeding *Portulaca oleracea* extract on the amelioration of heat stress. The investigations were made on feed intake and growth performance, common blood profile and lymphoid organs status in heat stressed broilers supplied with different types of supplements. In this experiment, 24 broilers were randomly and equally divided into 4 treatment groups (T0, T1, T2, T3) having 3 replications under each with 2 broilers. The T0 group was control and T1, T2 and T3 were the heat stressed only, heat stressed plus ascorbic acid and heat stressed plus *P. oleracea* extract, respectively. The heat stress was induced artificially and the doses of *P. oleracea* extract were equivalent to the recommended dose of ascorbic acid for broiler in terms of total anti-oxidant potentials. Mortality rate and feed intake were recorded on daily basis while the body weight was recorded weekly. At the end of the experiment, all the birds were used for blood sampling and only three birds under each treatment group were used for monitoring the lymphoid organs status. The collected data were analyzed by one-way analysis of variance (ANOVA) using the principles of Completely Randomized Design in SPSS statistical package of version 21.0. Results showed that feeding of *P. oleracea* had slightly increased the body weight in the birds during 2nd week of rearing only and there were no significant effects of feeding *P. oleracea* on any other parameters such feed intake, feed conversion ratio, lymphoid organ status and blood profile. It can be concluded that feeding *P. oleracea* extract has no effect on amelioration of heat stress in broiler.
KESAN PEMAKANAN EKSTRAK *Portulaca oleracea* KE ATAS PENAMBAHBAIKAN TEKANAN HABA TERHADAP AYAM PEDAGING

**ABSTRAK**

Satu eksperimen telah dijalankan pada 24 ayam pedaging Cobb yang berusia 21 hari untuk mengkaji tentang kesan pemakanan ekstrak *Portulaca oleracea* ke atas penambahbaikan tekanan haba dalam ayam pedaging. Kajian dibuat pada kesan pemakanan rawatan yang berbeza terhadap pengambilan makanan dan prestasi pertumbuhan serta profil darah ayam pedaging serta berat organ limfoid ayam yang mengalami tekanan haba. Ayam pedaging telah dibahagikan secara rawak kepada 4 kumpulan rawatan (T0, T1, T2 dan T3) dengan 3 replikasi yang mengandungi 2 ekor ayam pedaging. Kumpulan T0 adalah kawalan manakala T1, T2 dan T3 masing-masing akan bertindak sebagai kumpulan tekanan haba sahaja, tekanan haba ditambah asid askorbik dan tekanan haba ditambah ekstrak *P. oleracea*. Tekanan haba telah dihasilkan secara artificial dan dos ekstrak *P. Oleracea* akan diberikan mengikut dos yang disyorkan bagi asid askorbik untuk ayam pedaging dari segi potensi antioksida. Kadar kematian dan pengambilan makanan telah direkodkan pada setiap hari manakala berat badan direkodkan setiap minggu. Pada akhir eksperimen, semua ayam pedaging telah digunakan untuk pengumpulan sampel darah dan hanya tiga ayam pedaging bagi setiap kumpulan rawatan telah digunakan untuk mengukur organ limfoid. Data yang dikumpulkan dianalisis dengan analisis sehala varians ( ANOVA) menggunakan prinsip-prinsip reka bentuk Rawak Lengkap dalam SPSS pakej statistik versi 21.0. Hasil kajian menunjukkan bahawa potensi kesan pemakanan ekstrak *P. oleracea* telah dilihat pada berat badan badan pada minggu ke-2 semasa tempoh penternakan dan tidak ada kesan penting pemberian makanan*P. oleracea* yang boleh dilihat untuk parameter lain pengambilan makanan seperti nisbah penukaran makanan, organ limfoid dan profil darah. Kesimpulannya, pemberian makanan ekstrak *P. oleracea* tidak mempunyai kesan ke atas penambahbaikan tekanan haba dalam ayam pedaging.
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<tr>
<td>±</td>
<td>Plus minus</td>
</tr>
<tr>
<td>°C</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>%</td>
<td>Percentage</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<td>FPL</td>
<td>Faculty of Sustainable Agriculture</td>
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<td>UMS</td>
<td>Universiti Malaysia Sabah</td>
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<tr>
<td>kg</td>
<td>Kilogram</td>
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<tr>
<td>LSD</td>
<td>Least Significant Difference</td>
</tr>
<tr>
<td>mg/l</td>
<td>Milligram per liter</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
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<tr>
<td>AT</td>
<td>Ambient temperature</td>
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<td>CRD</td>
<td>Completely Randomized Design</td>
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<td>Body weight gain or loss = Final body weight - Initial body weight</td>
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CHAPTER 1

INTRODUCTION

1.1 Introduction

Raising poultry in most South-east Asian countries is characterized by traditional and small-scale systems of farming and operated predominantly by small and low-income farmers. Rather than from large commercial operations, most of the national supplies of poultry meat and eggs in this region are derived from the small and subsistence-type farms. Indonesia, Thailand, Malaysia and the Philippines are the leading countries in egg production in this region, estimated to be about 2748 tonnes (FAOSTAT, 2005). They are also leading in chicken meat production, estimated to be about 3970 thousand tones.

Based on the poultry industry’s development during the last two decades and the need for increased animal protein sources in the hot regions of the world, Richardson (1988) estimated that world production of eggs will reach 51 million tonnes by the year 2000, the greatest increase being in the developing countries and for poultry meat, he estimated that world production will reach 47 million tonnes, with demand in the developing countries reaching 16 million tonnes.

The highest increase was in Asia, Africa and South America, the main warm regions of the world. The most obvious constraint on poultry production in these regions is the climate. High temperature, especially when coupled with high humidity, imposes severe stress on birds and leads to reduced performance. Fortunately, during the past three decades, there has been a great deal of development in housing and housing practice for hot climates, and most modern poultry houses have been properly insulated. Poultry equipment companies have come up with various devices that contribute to lowering house temperatures and reducing heat stress.
Chickens, like all homeothermic animals, maintain a constant body temperature (BT) over a wide range of ambient temperature (AT) (Department for Environment, 2005). In birds, heat loss is limited by feathering and by the lack of sweat glands. The ability of an animal to maintain its BT within the normal range depends on a balance between internally produced heat and the rate of heat dissipation. The amount of internal heat produced by broilers depends on their body weight and feed intake, and the rate of heat dissipation depends on environmental factors, mainly AT, and on the broiler's feather coverage (Cahaner, nd).

Birds are heat stressed if they are facing difficulty in balancing body heat production and body heat loss. Heat stressed can occur at all ages and in all types of poultry such as chicken, duck and quail. So far, there is no clear evidence that gender have effect on heat stress in broiler. Metabolism within the body, which includes maintenance, growth and egg production are produce heat. Level of production, level of feed intake, amount of activity and exercise and improper ventilation are factors that affecting heat production. Birds will respond to the increasing temperature in their body. Birds will try to re-establish their heat balance with the surrounding by changing their normal behavior. Birds tends to move away from other birds, rest to reduce heat generated by activity, reduce feed intake, increase water consumption, start to pant slowly and begin fast panting as the temperature keep rising. Slow panting is a normal activity and can be sustained for extended periods of time.

Exceeded panting activity has consequences on the birds that will affect the production of birds (Department for Environment, 2005). Heat is lost as moisture is evaporated from airways in the birds (Cahaner, nd). However, panting requires muscle activity, requiring energy use and generates some additional heat. Panting activity can increase respiration rate by as much as 10 times the resting rate (Cahaner, nd). When the physiological and behavioural responses to high AT are inadequate, an elevation in BT occurs, causing a decrease in appetite and in actual growth rate. Consequently, the time needed to reach marketing weight is increased, leading to lower efficiency and profitability of poultry meat production (Cahaner and Leenstra, 1992). Moreover, hot conditions also negatively affect the yield and quality of broiler meat, often leading to PSE (pale, soft, exudative) meat (Barbut, 1997; Woelfel et al., 2002).
Studies on the physiological effects of heat stress have yielded several approaches to increasing the thermo tolerance of broilers, in order to minimize mortality and heat-related reduction in productivity. These approaches include acclimation to heat (De-Basilio et al., 2003), feed intake manipulation and feed deprivation (Ait-Boulahsen et al., 1993, 1995) and air velocity (May et al., 2000).

*Portulaca oleracea* is a member of purslane. It is a warm climate species and characterized by freshly leaves and a slightly sour taste, it is high in iron and has appreciable amount of Omega - 3 fatty acids which are more commonly found in seed (Skulski, 2010). *P. oleracea* has been used as herb which clears heat toxin and antibiotic and antifungal effect increases uterine contraction and prevention and treatment of dysentery in Chinese medicine. Not only that, it also used for alleviating pain and swelling. *P. oleracea* has been tested for its contribution to prevent and release kinetic fatigue on male mice since it has ability to anti-antherasis and enhancing immunity. In poultry, research by using *P. oleracea* is done to investigate the effect of Vitamin C and Zinc on broiler performance and immune-competence during heat stress. However, the recommended levels of Vitamin C and Zinc depend on environmental conditions still need further research and manipulation.

### 1.2 Justification of Study

Heat stress is a great problem in poultry industry and will cause on economic loss. As commercial breeding continues to increase the potential growth rate of contemporary broilers, the rate of their internal heat production will also increase because they consume more feed per time unit. Broilers cannot be reared to exhibit high growth rate and large body weight at marketing under hot conditions without using costly housing and cooling systems. Therefore the management and nutritional practices used for alleviation of heat stress are limited as they provide only short-term or costly solutions. Since heat stress can contribute great loss in production process, hence it should be treated as soon as possible.

The purpose of this research is to solve the heat stress problem in broiler using supplement method instead of improving key features of housing since by supplying *P. oleracea* extract is cheaper and easier. *P. oleracea* is weed that planted for a wide variety
of medicinal purposes. Previously, study on benefit of \textit{P. oleracea} on sheep and pigs have been done. The continuous availability of \textit{P. oleracea} as a beneficial weed was such a waste if there is no research on further medical purposes in broiler industry. Hopefully discovery of my research on \textit{P. oleracea} extract will establish new organic alternative to relief heat stress in broiler. It was good if it was using as feed additive that low cost and locally available to increase overall poultry production in Malaysia thus allowing to maintain and further improve of broiler performances in warm and hot conditions.

1.3 Objectives

i. To study the effect of feeding \textit{P. oleracea} extract on feed intake and growth performance in heat stressed broiler.

ii. To study the effect of feeding \textit{P. oleracea} extract on common blood profile and lymphoid organs status in heat stressed broiler.

1.4 Hypothesis

\textbf{H}_0: \textit{Feeding \textit{P. oleracea} extract has no effect on feed intake, growth performance, lymphoid organ status and common blood profile of broilers.}

\textbf{H}_1: \textit{Feeding \textit{P. oleracea} extract has effect on feed intake and at least any one parameters of the growth performance and common blood profile of broilers.}
2.1 Heat Stress

According to Selye (1976), "stress is the nonspecific response of the body to any demand", whereas stressor can be defined as "an agent that produces stress at any time". As temperature rises, the bird will reduce its feed consumption to reduce heat from metabolism to maintain the balance between heat production and heat loss. Research demonstrated that feed consumption is reduced by 5% for every 1°C rise in temperature between 32 – 38 °C (Morêki, 2008). Decreased feed consumption and increased water consumption is the result from heat stress condition. At high temperatures, heat production in birds decreases while heat dissipation increases. When ambient temperatures approach the body temperature, heat was release in the main pathway by respiratory evaporation.

2.1.1 Impact of Heat Stress on Poultry Production

Heat stress can be described as acute or chronic in poultry production (Morêki, 2008). Short and sudden periods of extremely high temperature will leading to acute heat stress refers to, whereas chronic heat stress is caused by extended periods of elevated temperature. Chronic stress has deleterious effects on birds through reducing feed consumption and increasing water consumption. Due to reduced maintenance requirement by feed consumption is decreases. In broilers, reducing feed consumption will negatively affecting growth rates, feed efficiency and carcass quality (Esmail, 2013), as well as increase the broilers’ time to reach market weight and increase mortality. It has been reported that chronic heat exposure negatively affects fat deposition and meat quality in
broilers, in a breed-dependent manner (Lu et al., 2007). In fact, recent studies demonstrated that heat stress is associated with depression of meat chemical composition and quality in broilers (Dai et al., 2012 and Imik et al., 2012).

Zhang et al., (2012) demonstrated that chronic heat stress decreased the proportion of breast muscle, while increasing the proportion of thigh muscle in broilers. Moreover, the study also showed that protein content was lower and fat deposition higher in birds subjected to heat stress (Lucas, 2013).

Reduced feed intake during heat stress is a highly conserved response among species and presumably represents an attempt to decrease metabolic heat production (Baumgard and Rhoads, 2012). Acidosis may exacerbate intestinal issues (Khafipour et al., 2009) as rumen pH is lowered during the summer months (Mishra et al., 1970).

Continuous panting during heat will cause acid base imbalance in the body due to loss of carbon dioxide (CO₂) and increases the need for the kidney to secrete bicarbonate to maintain a healthy of 20 to 1 ratio of bicarbonate to (CO₂) in the blood. (Sanz-Fernandez, nd)

According to (Ndashe, 2013), results in increased blood pH referred as alkaloidosis. Renal exchange of bicarbonates with chlorine ion is happen when kidney act to restore the acid base balance. This results in increased excretion of bicarbonates in urine and retention of chlorine in plasma and that leads to systemic acidosis. Thus in heat stress initial alkaloidosis changes to systemic acidosis will kills the birds due to acid shock.

2.2 Feeding Strategies
As mentioned earlier, heat stress will reduce feed consumption and resulting in some nutrients becoming insufficient. Thus, minimizing heat stress is a vital part of having a profitable flock. To encourage feed consumption during heat stress various feeding strategies are employed to alleviate heat stress. During hot weather, it is recommended to increases the energy content of the broiler’s diet. The use of supplemental fat is suggested as dietary fat increases palatability of feeds and reduces the amount of heat increment that is produced during its utilization in the body (Moréki, 2008). The reduction in feed consumption in hot weather often creates problem on reduction intake of essential
nutrients such as protein, essential amino acids, minerals and vitamins. Thus, feeding strategies approaches is needed to make correction on bird’s diet.

As for protein feeding strategies, it is recommended that protein content of feed should be increased from 16% to 17-18% to ensure that birds do not suffer nutritional stress of hot weather (Moréki, 2008). He contended that increasing dietary protein content would cover the requirements for isoleucine and typtophan, while methionine and lysine can be supplemented with synthetic compounds that are cheaper than natural sources. However, there is fear that increasing dietary protein might be detrimental to the bird as more heat is produced during its utilization. Therefore, he suggested that improving overall balance of the diet by amino acid supplementation appears to be more effective than increasing protein intake.

Addition in calcium is crucial in layer as for egg shell thickening purpose. Thus, calcium content of the diet should be adjusted so that each bird consumes the right amount per day according to anticipated level of intake (Moréki, 2008). In his journal stated that top dressing feed with oyster shell or large particulate limestone is beneficial and has the added advantage of stimulating feed consumption to promote shell thickening for laying hens group.

Feeding supplements such as minerals and vitamins are beneficial for heat stressed birds. Mineral and vitamin is needed as supplement during heat stress when the chicken is not able to synthesize enough vitamin C to meet physiological demands. As heat stressed birds are experiencing imbalances in acid-base balance, therefore, inclusion of various compounds in the diet or water is a common practice to alleviate the adverse effects of heat stress (Moréki, 2008). He claimed that common compounds supplied to birds are including sodium bicarbonate (NaHCO₃), potassium chloride (KCl), calcium chloride (CaCl₂), ammonium chloride (NH₄Cl) and vitamin C (ascorbic acid). He came out with statement that ascorbic acid and NaHCO₃ appear to be the most popular electrolytes used and vitamin C needed is increase as the stressors increased. He added that chickens require vitamin C for amino acid, mineral metabolism and synthesis of hormones. He stated that supplementation of vitamin C in drinking water at 40 milligrams per bird per day is reported to give beneficial effects in broilers. He also contended that aspirin in soluble liquid form can be used for its antipyretic or commonly referred as cooling effect at the rate of 0.3 grams per litre of water.
2.3 Portulaca oleracea

Common purslane (Portulaca oleracea L.), a member of the Portulacaceae family, is one of 25 genera of succulent herbs and shrubs in this family (Mitich, 1997). *P. oleracea* is widely distributed around the world and can be found from sea level to 3835m above sea level (Mitich, 1997). *P. oleracea* has been described in detail by several authors (Holm et al., 1977; Matthews et al., 1993; Mitich, 1997; Rydberg and Howe, 1932). In general, purslane is described as a succulent (water content of over 90%) with predominately prostrate growth and reproducing by stem fragments as well as by seed. Stems are glabrous, reddish in color, and branch radially from the central axis forming a mat up to 60 cm in diameter and roots consist of a long thick taproot as well as many fibrous lateral roots. Leaves are broad and rounded at the tips. Yellow flowers open on hot sunny mornings, can self-pollinate, and are not apomictic (Zimmerman, 1969). It has a cosmopolitan distribution in Africa, China, India, Australia, Middle East, Europe and United States (Chan et al., 2000; Oran & Al-Eisawi, 1998 and Mitich, 1997).

Several reports in the literature claim that *P. oleracea* contains several biologically active compounds and it is a source of many nutrients. Some of the reported biologically active compounds has been described in detail by several authors (Ezekwe et al., 1999; Garti et al., 1999; Hussein, 1985; Mohamed and Hussein, 1994 and Simopoulos et al., 1992). In general, biologically active compounds found in *P. oleracea* include organic acids (free oxalic acids in traces only, cinnamic acids, caffeic acid, malic acids and citric acids), alkaloids, coumarins, flavonoids, cardiac glycosides, anthraquinone glycosides, alanine, catechol, saponins and tannins. *P. oleracea* reported to contain also other chemical constituents, including urea, calcium, iron, phosphorous, manganese, copper and fatty acids, especially omega-3 fatty acids whose concentration in *P. oleracea* is the highest found in leafy vegetables. Moreover, the whole plant contains large amounts of 1-norepinephrine (0.25% in fresh herb), soluble carbohydrates, fructose/fructane, vitamins, A, B₁, B₂ and it is rich in ascorbic acid (Garti et al., 1999).

According to Liu et al., (2000), recent research demonstrates that purslane has better nutritional quality than the major cultivated vegetables, with higher beta-carotene, ascorbic acid, and alpha-linolenic acid. Additionally, purslane has been described as a power food because of its high nutritive and antioxidant properties by (Simopoulos et al.,
(1995). (Oliveira et al., 2009) finding shows that different varieties, harvesting times, and environmental conditions can contribute to purslane’s nutritional composition and benefits. Kamal et al (2012) had reported the antioxidant activity of *P. oleracea* over the different growth stages. The concentration of calcium (Ca), magnesium (Mg), potassium (K), iron (Fe) and zinc (Zn) increased with plant maturity thus it was concluded that mature plants of *P. oleracea* had higher total phenol content and antioxidant activities than plants at immature stages.

There was study done to evaluate the effect of vitamin C and zinc performance and immune-competence during heat stress that had been done by (Motasem, 2012). The results indicated that the immune response of broilers can be influenced under heat stress conditions by combination of different levels of vitamin C and zinc in the diet. The results also support the statement that supplement of vitamin C and zinc in the broiler diets can reduce the adverse effects of heat stress. Since *P. oleracea* contain both vitamin C and zinc, so it may be help broiler to alleviate heat stress in form of extract and supply in the water.

### 2.3.1 *P. oleracea* extract

According to Mohammed (2010), *P. oleracea* extract can be prepared mainly by two way which is preparation of ethanolic extract and preparation of aqueous extract. Preparation of ethanolic extract needed step such as air-dried leaves of the plant were crushed to coarse powder and extracted exhaustively in a Soxhlet with 95% ethanol. The extract was concentrated under reduced pressure to yield a viscous mass. The ethanolic extract was kept in airtight containers in a deep freeze maintained at 40 °C until further use.

There are optional ways to prepare the aqueous extract of *P. oleracea*. The collected leaves of *P. oleracea* was air-dried and powdered. Next, dissolving air-dried leaves powder in distilled water using a magnetic stirrer. Then, it was filtered and evaporated to dryness under reduced pressure. An aqueous suspension, which is the form customarily, used in folk medicine, was prepared to facilitate easy handling (Mohammed, 2010). Then, the drug solutions were prepared freshly each time and administered intragastrically with the restricted dosage schedule for the drug was once a day.

The extract was prepared according to the method described by (Youssef and Mokhtar, 2014) with slight modification as follows; 10g of fine dried powder of purslane
was stirred with 250 ml of ethanol at 100 rpm on orbital shaker for 1 h at room temperature 35°C and filtered through filter paper. The residue was then re-extracted with 250 ml of ethanol. The ethanol extracts were combined and then evaporated off under reduced pressure using a rotary evaporator to produce the concentrated extract that readily use for broiler.

2.4 Blood Collection Technique

The blood collection technique is refers to the manual that provided by (Harris, n.d). First of all, we need to know the maximum blood to be draws from chicken. General rule of thumb indicates the rule for amount of maximum blood volume that may collect from the avian patient. He also added that his amount needs to be no more than 1% of total body weight or approximately 10% of blood volume in a normal bird.

Next is selection of appropriate blood collection tools. A 22-27 gauge needle is appropriate for most avian patients. The smaller needle size such as 27-gauge may be used to reduce trauma, it also increases the risk of hemolysis after venipuncture. A micro-blood collection tube with anticoagulant should be used to preserve the collected sample. The syringe used should not be rinsed with anti-coagulant to prevent dilution errors introduced in the sample (Harris, n.d). Harris (n.d), in his manual state that basilic vein is the most preferred part to do venipuncture in chicken. This basilic or wing vein is located over the medial surface of the proximal ulna, and may be convenient in some species. Venipuncture can be done easily by exposing the vein to view by plucking a few feathers from the ventral surface of the humeral region of the wing, puncture the vein of the right wing and collect blood.
CHAPTER 3
METHODOLOGY

3.1 Location and Duration of the Study
Experiment was conducted at the Faculty of Sustainable Agriculture, located at Jalan Sungai Batang, Batu 10, Sandakan, Sabah. The experiment was take place at intensive chicken house and laboratory of Faculty of Sustainable Agriculture. Duration of study was October 2015 until November 2015.

3.2 Materials
For field materials, this experiment required automatic waterer, manger or feeder that provided in the cage, weighting balance, 24 Ross Cobb broilers, bulb, plastic cover, 1ml and 5ml syringe, 12 experiment pens and 27 gauge needle and micro blood collection tube (Lithium Heparin and Potassium EDTA) at the end of experiment for blood collection. While for lab materials oven, leaf grinder, electronic weighing balance, 250ml conical flask, orbital shaker, rotary evaporator, magnetic stirrer, scissor, automated blood counting machine and filter paper was required to do this experiment.

3.3 Methodology

3.3.1 Preparation of P. oleracea extract
The extract was prepared according to the method described by (Youssef and Mokhtar, 2014) with slight modification as follows; 10g of fine dried powder of purslane was stirred with 250 ml of ethanol at 100 rpm on orbital shaker for 1 h at room temperature 35°C and filtered through filter paper. The residue was then re-extracted with 250 ml of ethanol. The ethanol extracts was combined and then evaporated off under reduced pressure using
REFERENCES


Cahaner, A. (n.d.). Being featherless (homozygous sc/sc) provides fast-growing high-yield broilers with genetic adaptation to hot conditions.


