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RESEARCH ARTICLE

EFFECT OF HEAT STRESS ON PHYSIOLOGICAL AND SOME BLOOD PARAMETERS OF THREE LOCAL CHICKEN AND A BROILER GENOTYPES REASED IN HUMID SUBTROPICAL CLIMATE OF CÔTE D'IVOIRE.

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Abstract

The present study was carried out for evaluating the response of chickens from four genotypes such as naked-neck, frizzle, normal feather and broiler subject to heat stress in order to show the best bird genotype. Thus, for achieving that purpose, the study was conducted in a private farm located in the locality of Tiéplé, precisely in the center of Côte d'Ivoire in the department of Bouaké. The experimental chickens were selected randomly from a group of chickens genotypes identified and were exposed to ambient temperature and warm temperature. Results showed that temperature had a significant effect onto breath rates of broiler and frizzle genotypes, but it had no significant effect on that of the normal and naked-neck genotypes. Moreover, temperature influences significantly the white blood cell and hemoglobin rates of all genotypes ($p < 0.05$) which increase according temperature level. Likewise, a significant effect of temperature onto the MCHC rates of all bird genotypes was observed ($p < 0.05$). This rate increases with increasing of heat stress for all genotypes. However, there is no significant effect of temperature on the calcium and phosphorus rates of all genotypes ($p > 0.05$). Otherwise, temperature does not have any effect on magnesium rate of the naked-neck and broiler genotypes, ($p > 0.05$), while it influences significantly the magnesium rates of normal feathered birds ($p < 0.05$). The results of this work indicated therefore that there was a relationship between heat resistance or tolerance and acclimation of birds which it is in favor to indigenous chicken genotype precisely the naked-neck genotype.

Introduction:-

Modern or traditional poultry breeding is a growing sector in African countries to cope with an increasing demand for animal protein (Fatou, 2012) and they are playing an important role for fighting against poverty and for contributing to food self-sufficiency (Gnandji, 2004). Futhermore, traditional chickens account for about 80% of the poultry livestock in Africa and contribute significantly to global meat production (25-70%) and eggs (12-36%) according Guèye (1998). A report in Côte d'Ivoire showed that 70% of the Ivorian national chicken livestock,

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precisely 33 million are local chickens (MIPRAH, 2007). That showes therefore, the important part of local bird in the economy of Ivorian rural areas as well as other African geographical areas (Bonfoh, 1997). Similarly, in rural areas, poultry products from traditional farms of local chicken livestock are still a source of meat well appreciated, economically and readily available for the consumer.

However, high temperatures recorded during long periods occurs a decrease in their performance (N'dri *et al.*, 2006; Castello, 1990). Indeed, According to these authors, any increase in ambient temperature precisely at temperature above 20 ° C, would lead to a decreasing in food consumption about 1.5% per degree Celsius, as well as a decreasing in growth and often an increasing in mortality rate and an important economic losses (Picard et al., 1993). In fact, ambient heat stress represents one of the major constraints of poultry farming in the tropical zone.
Moreover, during these periods of heat, birds develop abilities for withstanding extreme weather conditions. Thus, they have to make behavioral changes in order to release overheating for controlling their body temperature, which allows the animal to adapt itself to the new environment. Indeed, birds are homeotherms and they must maintain their body temperature almost constant between 40 and 42 ° C, for their survival and for a good functioning of their vital organs (Dayon and Arbelot, 1997). This mechanism is called thermoregulation. The decrease in performance and appetite as well as the energy loss are due to the introduction of thermoregulatory processes.

Nevertheless, heat stress appears when the ambient temperature exceeds the comfort zone which ranges from 20 to 25 ° C and represents the ambient temperature favorable for chickens' growth. This comfort zone would depend on the species, the strain and the genotype (N'dri *et al.*, 2007). Beyond this area, it is difficult for the chicken to lose enough heat by behavioral mechanisms, they become less active, less productive and it reduces food consumption.
Otherwise, the production potential of local chickens is lower than that of exotic birds, but seems to have the advantage of being best suited against stressful environmental conditions such like high temperature (Fotsa *et al.*, 2007).

Unfortunately, local chickens stay very poorly characterized and their genetic potential for production, adaptation to climates and rearing conditions and their resistance to disease remain less studied (Loukou *et al.*, 2009). In addition, Phenotypic characterization of traditional chickens in Côte d'Ivoire has shown significant phenotypic diversity (Loukou *et al.*, 2009, N'dri *et al.*, 2016) due to major genes affecting feather for certain. As well, major and natural genes for heat resistance exist within these chickens.

The general purpose of the present study was to tackle poverty and for contributing at food self-sufficiency. Specifically, this work aims to show out the best genotype resistant to heat generated in our tropics. In fact, the respiratory rate and blood tests were evaluated at the level of four chicken genotypes.

Materials And Methods:-

Study area

The study was conducted in Tiéplé, village located 15 km from the city of Bouaké in the center of Côte d'Ivoire, the capital of the Gbèkè region. Tieple is situated on latitude 07°41'7'' N, longitude 5°01'50'' W. This locality is in a humid tropical climate and is characterized by savanna vegetation with trees. The hygrometry rate oscillates between 50% and 60%, with an ambient temperature varying between 22 ° C and 35 °C.

Biological material

A total of 48 old chickens from 4 genotypes having a body weight ranging from 800g to 1300g were used. These animals were composed by firstly 3 genotypes of local chickens, aged from 12 to 18 months, differing in the way of feather distribution and structure on the body, and secondly by a 45 days old broiler genotype (Cobb 500). In other words, there are 12 "naked-neck" chickens, 12 normally feathered chickens, 12 "frizzle" chickens, and 12 broiler chickens.

Technical material

A 0.01g precision digital scale was used to weigh the animals. Also a thermometer/ hygrometry type with two functions was used for the ambient temperature and humidity measurement. Blood collection equipment was used during this study. They are sampling syringes, sampling tubes used to collect the blood for the various analyzes. Otherwise, a coal burner was used to induce heat in the breeding local. A cooler was also used for sample conservation.

Methods:-

Constitution of groups and treatments

Two groups 1 and 2 with 24 chickens containing all the 4 genotypes which were constituted. Thus, every batch was composed by 6 chickens per genotype, precisely 3 males and 3 females. The two groups (1 and 2) were arranged in two different husbandries, each equipped with an ambient thermometer. The animals of group 1 were subjected to 2 different occurred high temperatures. Indeed the animals were placed at 35 ° C for 2 hours and then at 40 ° C also for 2 hours. Those in batch 2 were placed during 4 hours at ambient temperature. The temperature in this environment ranged from 26 ° C to 28 ° C.

Studied parameters

The physiological parameters in this study were the respiratory rate as measured by the number of gasps per minute. All animals from the two treatments (normal and high ambient temperature) were observed individually to see if they were panting. The gasps were recorded over a period of one minute for each chicken.

Blood parameters

The blood parameters which have been evaluated in this study are the constituents of the blood collected in the tubes. Samples were taken from fasting chickens in oder to avoid postprandial lipemia characterized by a rise in triglyceride-rich lipoproteins after eating which is a result of a lactescence of the blood which can disturb the dosage. The blood samples were taken by puncture of the wing vein using a sterile needle, after having cleared the feathers of the inner side of the wing and after sterilisation of the skin. The blood was collected at a rate of 3 ml and was distributed in different tubes (red and purple caps) at dry state with first anticoagulant (EDTA) and after without anticoagulant. The blood collected in the tubes with anticoagulant (purple cap) was reserved for hematological analyzes and the other (tubes without anticoagulant and having red cap) was used for the analysis of minerals. All samples were stored in a cooler for carrying to the laboratory. All analyzes were done at the medical analysis laboratory of the Pastor Institute of Abidjan, according to the protocol provided by the manufacturer of the various test kits. For hematology, the number of white blood cells (WBC), the hemoglobin content (HC) and the mean corpuscular hemoglobin concentration (MCHC) were studied. Concerning minerals, analyzes were focused on calcium (Ca), magnesium (Mg) and phosphorus (P) levels.

Statistical Analysis:-

The datas collected were coded into a database created on the Microsoft Office Excel 2010 software. The effects of temperature on the different genotypes were done by using a two-factor variances analysis (ANOVA 2) of the Graph Pad prism software. The 2-fold average comparison was made by the Tukey ANOVA 2 post-test, with a significance level P set at $p = 0.05$ (95%).

Results:-

Physiological parameters

The results of analyzes are recorded in tables 1 to 7. The respiratory rate of the different studied genotypes is presented in table 1. High temperature significantly ($p < 0.05$) affected the respiratory rate of the "frizzle", "broiler" and ‘’normal’’ genotypes. Indeed, at 35 ° C (beginning of the heat stress), no respiratory rate is still present in the "naked-neck" population compared to the other genotypes which respectively present 31.40, 35 and 78.67 panting per minute for the normally feathered, frizzle and broiler birds. Moreover, the heat (at 40°C) increases the breath rate of the all different genotypes. Only, the "naked-neck" genotype in local chickens has low or no respiratory rate. However, the most significantly affected bird was broiler chicken ($p < 0.001$), which ranged from 78.67 to 35 ° C at 94 panting per minute at 40 ° C ambient temperature. There is no significant difference ($P > 0.05$) among respiratory rate for all genotypes at ambient temperature. Animals panting are noticeable only at 35 ° C. Thus, at 35 ° C there is a significant difference in respiratory rate for every genotype. This difference is significant only between broilers and genotypes of local chickens ($p < 0.05$). Concerning local chickens, normal feathered and frizzle birds, have the same respiratory rate (31.40 and 35). Likewise, at 40 ° C, the same trend was observed.

Hematological parameters

The results of the hematological analysis are shown in tables 2 to 7.

White blood cell rate

The results are presented in table 2. The high temperature influences the white blood cell rate of all genotypes. In fact, the rate of white blood cells increases significantly ($p<0.05$) for all studied genotypes. From 35 °C to 40 °C, the increase in broiler white blood cell count observed such as $85.10^3/mm^3$ is higher than those of the "naked-neck", "frizzle" and "Normal" genotypes which are respectively $57.1.10^3/mm^3$; $82.8.10^3$ /$mm^3$and $81,9.10^3$ / mm^3.

At ambient temperature, there is no significant difference in white blood cell rates recorded among the 4 genotypes ($p>0.05$). However, at 35 ° C, there is a significant difference in white blood cell rates stored among the 4 genotypes ($p<0.05$). It was also observed that there is no significant difference ($p >0.05$) between "naked-neck" and "frizzle" chickens. However, this difference is significant between the broiler chickens and the 3 local chicken genotypes. Finally, at the temperature of 40 ° C, the same trend was observed. In fact, the broiler has the lowest rate compared to local chicken strains which are high rates. Nevertheless, "normal feather" chickens had significantly higher rates followed by "naked-neck" and "frizzle" genotypes.

Hemoglobin rates

Table 3 summarizes the results of the evolution of hemoglobin rates of the different chicken genotypes subjected to different ambient temperatures. The high temperature has a significant effect ($p <0.05$) on the hemoglobin rates for all studied genotypes. Indeed, the hemoglobin rates increases significantly for all genotypes. However, broilers had lower hemoglobin rates than local chickens (3.07 vs. 6.83 g / L respectively) at 40 ° C. Hemoglobin rates ranged from 3.067 to 6.133 and 6.833 for broilers due to heat (respectively at 35 ° C and 40 ° C). The hemoglobin rates of local chicken genotypes also increase with increasing ambient temperature. Nevertheless, the increase is higher for the naked-neck genotype compared to other genotypes. Otherwise, at ambient temperature, no significant difference among the 4 chicken genotypes was observed ($p>0.05$). In addition, at the temperature of 35 ° C, there is a significant difference ($p <0.0001$) among these 4 genotypes. However, there is no significant difference ($p >0.05$) between "naked-neck" and "frizzle" chickens. At 40 ° C, there is a significant difference ($p <0.05$) concerning hemoglobin rates of all genotypes. Nonetheless, the difference is so significant only between broilers and indigenous local birds' genotypes ($p <0.05$).

Mean corpuscular hemoglobin concentration (MCHC)

The results concerning the MCHC are shown in table 4. The temperature has a significant effect ($p <0.05$) on the MCHC rates for all genotypes. The rate increases with increasing of heat stress for all genotypes. However, at ambient temperature, no significant difference ($p >0.05$) among the 4 chicken genotypes was observed for the MCHC rate. Also, the same trend was observed at temperatures of 35 ° C and 40 ° C.

Minerals: Calcium (Ca), Phosphorus (P) and Magnesium (Mg)

The results of the calcium rates of the different studied genotypes are shown in table 5. The temperature had no effect ($p>0.05$) on the calcium rates for all the chickens. In addition, no significant difference ($p>0.05$) was observed among the calcium rates of the different chicken genotypes of every treatment. Similarly, hot temperature has no effect on the phosphorus rates of chickens from all genotypes (table 6). No significant difference ($p>0.05$) for phosphorus rates among the different chicken genotypes at the two temperatures used was observed. The results concerning the magnesium rates are registered in table 7. The high temperature did not have a significant effect ($p>0.05$) on the magnesium rates of "naked-neck", "frizzle" and "broiler" genotypes. No significant difference was observed for the magnesium rates concerning every genotype at the different temperatures applied. However, the high temperature influenced the magnesium rates of "normal feathered bird" genotypes. At 40 ° C, the magnesium rates have significantly ($p <0.05$) increased for that genotype (3.31 vs 3.67 mg / l).

Table 1:-Mean of breath rates according to the genotype at ambient temperature and warm temperature (35 ° C and 40 ° C)

PARAMETER	RESPIRATORY RATE (n/min) M±ES		
GENOTYPES	Ambient temperature	35°C	40°C
Naked-neck	0.0 ± 0.0^{1a}	0.0 ± 0.0^{3a}	4.667 ± 4.667^{3a}

Frizzle	0.0 ± 0.0^{1c}	35.00 ± 11.84^{2b}	50.00 ± 16.83^{2a}
Broiler	0.0 ± 0.0^{1c}	78.67 ± 5.925^{1b}	94.00 ± 2.000^{1a}
Normal feathered	0.0 ± 0.0^{1c}	31.40 ± 14.30^{2b}	53.80 ± 14.95^{2a}

NB: M ± ES = Mean ± Standard error; on the same line, the assigned values of the same letter are not significantly different, on the same column the averages affected by the same numbers are not significantly different (* P <0.05, *** P <0.001) (n / min): number of panting per minute

Table 2:-Average of white blood cells by genotype at ambient temperature (AT) and warm temperature (35 ° C and 40 ° C)

PARAMETER	WHITE BLOOD CELLS ($10^3/mm^3$) M±ES		
GENOTYPES	Ambient temperature	35°C	40°C
Naked-neck	118.5 ± 66.3^{1c}	280.3 ± 13.2^{2b}	337.4 ± 30.4^{2a}
Frizzle	129.4 ± 15.9^{1c}	262.1 ± 20.4^{2b}	344.9 ± 27.6^{2a}
Broiler	120.0 ± 9.8^{1c}	194.4 ± 13.6^{3b}	279.4 ± 5.4^{3a}
Normal feathered	217.2 ± 101.4^{1c}	370.1 ± 8.3^{1b}	452.0 ± 8.3^{1a}

NB: M ± ES = Mean ± Standard error; on the same line, the assigned values of the same letter are not significantly different, on the same column the averages affected by the same numbers are not significantly different (* P <0.05, *** P <0.001) (n / min): number of panting per minute

Table 3:-Hemoglobin rates according the genotype at ambient temperature (AT) and warm temperature (35 ° C and 40 ° C).

PARAMETER	HEMOGLOBIN RATE (g/L) (M±ES)		
GENOTYPES	Ambient temperature	35°C	40°C
Naked-neck	5.20 ± 2.06^{1c}	8.17 ± 0.20^{2b}	9.70 ± 0.74^{1a}
Frizzle	5.50 ± 0.95^{1c}	8.03 ± 0.27^{2b}	8.87 ± 0.22^{1a}
Broiler	3.07±0.12^{1c}	6.13 ± 0.03^{3b}	6.83 ± 0.12^{2a}
Normal feathered	7.73 ± 0.49^{1c}	8.80 ± 0.10^{1b}	9.70 ± 0.10^{1a}

NB: M ± ES = Mean ± Standard error; on the same line, the assigned values of the same letter are not significantly different, on the same column the averages affected by the same numbers are not significantly different (* P <0.05, *** P <0.001) (n / min): number of panting per minute

Table 4:-Mean hemoglobin corpuscular concentration (MCHC) by genotype at ambient temperature and at warm temperature (35 ° C and 40 ° C).

PARAMETER	Mean hemoglobin corpuscular concentration (%) (M±ES)		
GENOTYPES	Ambient temperature	35°C	40°C
Naked-neck	21.90 ± 0.56^{1c}	23.00 ± 0.21^{1b}	24.03 ± 0.23^{1a}
Frizzle	21.83 ± 0.29^{1c}	23.10 ± 0.26^{1b}	27.13 ± 2.68^{1a}
Broiler	22.37 ± 0.45^{1c}	23.07 ± 0.03^{1b}	23.63 ± 0.24^{1a}
Normal feathered	20.10 ± 0.90^{1c}	22.97 ± 0.03^{1b}	24.57 ± 0.68^{1a}

NB: M ± ES = Mean ± Standard error; on the same line, the assigned values of the same letter are not significantly different, on the same column the averages affected by the same numbers are not significantly different (* P <0.05, *** P <0.001) (n / min): number of panting per minute

Table 5:-Calcium (Ca) average of every genotype at ambient temperature and warm temperature (35 ° C and 40 ° C).

PARAMETER	Calcium rates (mg/l) (M±ES)		
GENOTYPES	Ambient temperature	35°C	40°C

Naked-neck	10.93 ± 0.1797^{1a}	11.37 ± 0.2404^{1a}	10.80 ± 0.1732^{1a}
Frizzle	10.80 ± 0.3786^{1a}	12.65 ± 1.950^{1a}	11.57 ± 0.5783^{1a}
Broiler	10.70 ± 0.2309^{1a}	10.78 ± 0.2250^{1a}	10.63 ± 0.7424^{1a}
Normal feathered	13.43 ± 1.562^{1a}	11.93 ± 0.7535^{1a}	11.35 ± 0.2500^{1a}

NB: M ± ES = Mean ± Standard error; on the same line, the assigned values of the same letter are not significantly different, on the same column the averages affected by the same numbers are not significantly different (* $P < 0.05$, *** $P < 0.001$) (n / min): number of panting per minute

Table 6:-Phosphorus (P) average by genotype at ambient temperature and warm temperature (35 ° C and 40 ° C)

PARAMETER	Phosphorus rates (mEq/l) (M±ES)		
GENOTYPES	Ambient temperature	35°C	40°C
Naked-neck	122.55 ± 0.65^{1a}	124.4 ± 18.75^{1a}	126.30 ± 8.37^{1a}
Frizzle	120.3 ± 34.01^{1a}	117.45 ± 1.35^{1a}	121.43 ± 2.42^{1a}
Broiler	123.5 ± 36.10^{1a}	100.0 ± 30.90^{1a}	82.20 ± 43.46^{1a}
Normal feathered	97.70 ± 22.58^{1a}	108.00 ± 3.70^{1a}	115.15 ± 10.15^{1a}

NB: M ± ES = Mean ± Standard error; on the same line, the assigned values of the same letter are not significantly different, on the same column the averages affected by the same numbers are not significantly different (* $P < 0.05$, *** $P < 0.001$) (n / min): number of panting per minute

Table 7:-Magnesium (Mg) average by genotype at ambient temperature and warm temperature (35 ° C and 40 ° C).

PARAMETER	Magnesium rates (mg/l) (M±ES)		
GENOTYPES	Ambient temperature	35°C	40°C
Naked-neck	2.98 ± 0.21^{1a}	3.05 ± 0.22^{1a}	2.64 ± 0.09^{1a}
Frizzle	2.59 ± 0.12^{1a}	2.49 ± 0.07^{1a}	2.39 ± 0.28^{1a}
Broiler	2.82 ± 0.22^{1a}	2.55 ± 0.30^{1a}	3.15 ± 1.05^{1a}
Normal feathered	3.31 ± 0.13^{1b}	3.38 ± 0.04^{1b}	3.67 ± 0.13^{1a}

NB: M ± ES = Mean ± Standard error; on the same line, the assigned values of the same letter are not significantly different, on the same column the averages affected by the same numbers are not significantly different (* $P < 0.05$, *** $P < 0.001$) (n / min): number of panting per minute

Discussion:-

The aim of this study was to evaluate the response of 4 chicken genotypes to heat stress by evaluating the respiratory rate of the animals and some hematological parameters. Genotypes comparison concerning the responses at heat stress showed a clear resistance of the "naked neck" bird followed by normal feather bird genotypes and the most stressed were the broilers for the parameter about respiratory rate.

The heat stress response of local chickens compared to that of broilers may depend on the acclimation degree of chickens and strain (Collin *et al.*, 2011; N'dri *et al.*, 2007). These authors found that acclimation had a beneficial effect on survival during heat period or warm temperature. Furthermore, for these same authors, the maximum respiratory rate would be lower in chickens population more acclimated to heat than the other less acclimated. According this, the resistance of local chickens compared to that of broilers could be notice. Indeed, broilers are strains of chickens selected in a temperate climate (around 20 °C) and distributed in our tropics. Moreover, chickens do not sweat, so their mechanism to increase their heat loss in warm environment will consist of spraying water in the airways.

Concerning white blood cell rates, heat stress had a significant effect onto the different genotypes of chickens. Thus, the white blood cell rates increased with increasing of ambient temperature in all genotypes. This increase in the rate is due to immunosuppression caused by exposure to high temperature, making them more susceptible to pathogens, which increases the number of white blood cells. Indeed, white blood cells are responsible of the body's immune defense against external aggression from germs. The high white blood cell rates of local strains compared to broilers showed the high infestation of these local strains. Clearly, local chickens wandering for food are constantly exposed

to infections. Likewise, these findings might suggest that the "normal feather" genotype would be more infested. In addition, the white blood cell rates did not dependent on age and sex (Islam *et al.*, 2004).
The hemoglobin rate is influenced significantly by high heat in all genotypes. This increase in this rate would be due to hypoxia. In fact, the polypnea increases the pulmonary gas exchanges leading the animal to hypoxia state rapidly (Mandonnet *et al.,* 2011), which is responsible of increasing in hemoglobin rates in the red blood cells. However, the hemoglobin rates obtained in this study are lower than those found by Albokhadaim (2012) for indigenous chickens in Arabie Saoudite.

Futhermore, the effect of temperature is significant on the MCHC rates, which increases for all genotypes. The rate of MCHC found in this study ranged from 20.10 to 27.13%. This rate is more inferior than that obtained by Albokhadaim (2012), which obtained values ranging from 28.6 to 34%.

Concerning minerals, the effect of temperature and genotype was not significant onto calcium and phosphorus rates. These results are in accordance with previous study (Albokhadaim, 2012; Talebi *et al.,* 2005). These authors found that the calcium and phosphorus rates were not significantly different in the population of studied animals. However, the work of (Ritchie *et al.*, 1994) is in disagreement with our findings. In their study, the phosphorus rates of local Saudian chickens would be lower than that of broilers. Regarding the magnesium rate, the lack of difference among the rates could be due to the intake of food. In fact, variations of electrolytes concentration (Na, K, Cl, Ca and Mg) in plasma are mainly due to a lack or an excess of food intake (Ritchie *et al..*, 1994; Carison and Bruss, 2008).

Conclusion And Perspectives:-
At the end of our study, we can say that local chicken populations are more resistant to heat stress than broilers. This phenomenon could be due to the natural environment of these genotypes. However, naked-neck bird genotypes showed better resistance to heat stress than other genotypes. In fact, the results showed that an improvement of the zootechnical performances of this genotype will increase the income of the breeders during the warm period.

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