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Original Research Paper

AMBIENT TEMPERATURE ASSOCIATED VARIATIONS IN SERUM HORMONES AND INTERRELATED ANALYTES OF BROILER CHICKENS IN ARID TRACT

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Summary: The investigation was carried out to explore the extent of changes in serum hormones and interrelated analytes of broiler chickens during varying ambient temperatures because such changes help to understand the modulations in physiological mechanisms. For this purpose sera were harvested from the broilers when the maximum temperatures were 13-16°C (low); 24-27°C (moderate) and 42-45°C (high). The values of analytes measured at moderate temperature served as control to which values of analytes measured at low and high temperatures were compared.

Higher temperatures resulted in greater variations in hormones and interrelated analytes than the lower temperatures. At 42-45°C, serum corticosterone, growth hormone, glucagon, testosterone, uric acid, creatinine, urea, glucose, cholesterol, triglycerides, free fatty acids, ASAT, ALAT, lipase and amylase increased significantly ($p \leq 0.05$) and thyroxine, triiodothyronine, insulin, gastrin, total proteins, albumin, globulin, LDH, ICDH and pepsinogen decreased significantly ($p \leq 0.05$) from respective control mean values. At low temperatures (13-16°C) serum T_4, T_3, LDH and ICDH were significantly ($p \leq 0.05$) higher; glucose, cholesterol, triglycerides and free fatty acids were significantly ($p \leq 0.05$) lower and corticosterone, growth hormone, insulin, glucagon, gastrin, testosterone, total proteins, albumin, globulin, uric acid, creatinine, urea, ASAT, ALAT, pepsinogen, lipase and amylase showed non significant ($p > 0.05$) changes from respective control values. Serum prolactin, aldosterone, sodium, chloride, calcitonin, alkaline phosphatase, 5' nucleotidase and gamma glutamyl transferase were significantly ($p \leq 0.05$) higher and potassium, C-PTH, calcium, phosphorus and magnesium were significantly ($p \leq 0.05$) lower at 42-45°C temperatures than respective control mean values. At 13-16°C temperature serum aldosterone, sodium, chloride, alkaline phosphatase and 5' nucleotidase showed significant ($p \leq 0.05$) fall while serum potassium marked significant ($p \leq 0.05$) rise as compared to respective control mean values. It was concluded that changes in the ambient temperatures affected the glucose, fat, protein, calcium and sodium metabolisms in the way which helped the birds to survive during adverse conditions. Pattern of changes of interrelated analytes showed the physiological state of the body being governed by endocrine system.

Key words : ambient temperatures; analytes; broilers; hormones; sera

Introduction

Variations in ambient temperatures are too great in arid tract that many a times physiological adjustments in the birds are on the cost of production. Feed intake, feed conversion efficiency and growth rate in broilers are greatly affected due to high ambient temperatures (1, 2, 3). Thermal discomfort may result in improper expression of genetic potential in birds. Thermoregulation is mainly carried out on the expense of water and as a result water intake increases (4). Improper management results in development of slow dehydration. In arid tracts mortality in birds is observed during higher ambience.

Laboratory monitoring to find out effects of ambience can be done by evaluating concentrations of metabolites and electrolytes in blood as these

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parameters reflect the physiological state of the body during changed ambience (5). Heat, cold and dehydration are among the common stressors experienced by broilers. When the pressure from the stressors becomes excessive, or in case of psychological threats, new defense mechanisms are initiated, collectively referred to as stress responses. Determination of these stress responses is essential so that strategies can be made for making congenial ambience in broiler houses for healthier management.

Although many studies have been conducted to evaluate the effect of thermal environment in birds (6, 7) but still dearth of research is there to understand the modulations in physiological mechanisms which can be observed by assessing the changes taking place in controlling system of the body. Endocrine system is one of the regulating systems. Therefore variations in physiological mechanisms can be best understood by endocrine and metabolic responses of broilers which require serum profiling of analytes. This prompted the present investigation to determine serum hormones and interrelated analytes in broilers of arid tract during varying ambient temperatures under natural conditions.

Material and methods

Samples

Blood samples were collected to harvest the sera from 600 broilers (Male, White Leghorn chickens) of 8-9 weeks of age belonging to private poultry farms of arid tracts at the time of slaughter. The birds were maintained under natural conditions with standard feeding and watering practices. Samples (200 each) were collected when the range of maximum ambient temperatures were 13-16°C (low); 24-27°C (moderate) and 42-45°C (high). The clear, non-haemolysed sera were separated from blood samples and stored in a deep freeze at –20°C till analysis.

Analysis

To achieve the goal of the study the hormones were determined in 25 randomly selected serum samples each from low, moderate and high ambient temperatures and interrelated analytes were determined in all 600 collected serum samples. The hormones were corticosterone, thyroxine (T_4), triiodothyronine (T_3), prolactin, aldosterone, carboxy-terminal parathyroid hormone (C-PTH), calcitonin, growth hormone, insulin, glucagons, gastrin and testosterone. The serum corticosterone was determined by fluorometric method (8) using a photofluorometer (Systronics, India). Commercial 125_I Radio Immuno Assay (RIA) Kits were used to determine other hormones viz. thyroxine (RADIM), triiodothyronine (RADIM), prolactin(DPC), aldosterone(DPC), carboxy-terminal parathyroid hormone (DiaSorin), calcitonin (DPC), growth hormone(DPC), insulin(DPC), glucagons (DPC), gastrin (DPC) and testosterone (DiaSorin) in Radio Isotope Laboratory, College of Veterinary and Animal Science, Bikaner,India. 125_I Gamma counter (EC, India) was used for counting radioactivity.

Interrelated analytes included metabolites, electrolytes and enzymes. Metabolites analysed were total proteins, albumin, globulin, uric acid, creatinine, urea, glucose, cholesterol, triglycerides; electrolytes were sodium, potassium, chloride, calcium and phosphorus; and enzymes were lactic dehydrogenase (LDH), isocitrate dehydrogenase (ICDH), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase (AKP), 5'-nucleotidase, gamma glutamyl transferase (γ-GT), pepsinogen, lipase and amylase.

Serum isocitrate dehydrogenase, 5'-nucleotidase, pepsinogen, and amylase were determined by the standard spectrophotometric methods (9) by using spectrophotometer (Systronic, India). Serum lipase and free fatty acids were determined by standard titrimetric method (9). Sodium and potassium were determined by using flame photometer of Systronics, India (8) and magnesium by titan yellow method (8). Standard commercial spectrophotometric kits (Glaxo and Wipro) were used to determine remaining analytes viz .total proteins, albumin, globulin, uric acid, creatinine, urea, glucose, cholesterol, triglycerides, chloride, calcium, phosphorus, LDH, ASAT, ALAT, AKP and γ-GT as per manufacturers instructions by using spectrophotometer (Systronics, India). For serum calcium necessary corrections were made (10).

All reported values are means (± SEM). To assess the effect of low and high ambient temperatures on individual serum analytes the respective mean value at 24-27°C was considered as control. Significance of changes was assessed by student 't' test (11).

Results

The results are presented in table 1. and 2 and analytes are grouped according to their relationship with each other.

Table 1: Serum hormones, metabolites and enzymes in the broilers (Mean ± SEM)

Analytes	Maximum ambient temperature		
	13-16°C	24-27°C	42-45°C
Corticosterone, ng/ml[a]	7.9± 0.76	7.3± 0.54	13.1± 0.78*
Thyroxine, ng/ml[a]	18.1±0.6*	14.8±0.5	9.9±0.4*
Triiodothyronine, ng/ml[a]	3.9±0.2*	2.2±0.2	1.4±0.3*
Growth hormone, ng/ml[a]	49± 1.76	52± 2.1	69± 2.8*
Insulin, ng/ml[a]	2.71±0.27	2.56±0.20	1.56±0.41*
Glucagon, ng/ml[a]	1.66±0.31	1.71±0.25	3.1±0.32*
Gastrin, pg/ml[a]	98±9.7	100±7.9	71±6.9*
Testosterone, ng/ml[a]	1.2±0.08	1.4±0.07	2.3±0.06*
Total proteins, g/L[b]	42.0 ±3.0	45.0 ±2.0	32.0±2.0*
Albumin, g/L[b]	21.0±3.0	22.0±2.0	18.0 ±1.0*
Globulin, g/L[b]	21.0±3.0	22.0±2.0	14.0±2.0*
Uric acid, mmol/L[b]	0.312 ± 0.02	0.295 ± 0.025	0.383 ± 0.01*
Creatinine, µmol/L[b]	88.4 ±0.318	79.56 ±0.31	167.0 ±0.31*
Urea, mmol/L[b]	0.649± 0.05	0.599± 0.02	0.865± 0.025*
Glucose, mmol/L[b]	8.17 ±0.43*	9.99±0.39	11.92 ±0.44*
Cholesterol, mmol/L[b]	3.26 ±0.17*	3.88 ±0.15	4.22 ±0.20*
Triglycerides, mmol/L[b]	4.51±0.11*	5.29±0.12	5.75±0.10*
Free fatty acids, mmol/L[b]	10.98±0.34*	12.62±0.30	15.07±0.39*
Lactate dehydrogenase, IU/l[b]	1324.8±88*	1000.5 ±87	811.7 ±78*
Isocitrate dehydrogenase, IU/l[b]	13.3 ±1.0*	9.3 ±0.87	7.13 ±0.94*
Aspartate amino transferase, IU/l[b]	238±7.9	246±10.0	354±9.87*
Alanine amino transferase, IU/l[b]	6.1±0.53	6.9±0.56	10.12±0.8*
Pepsinogen, U/L[b]	2.4±0.1	2.51±0.4	1.2±0.3*
Lipase, U/L[b]	485±13.8	511±14.9	600±15. 2*
Amylase, U/L[b]	500±19.12	523±18.21	556±17.91*

Superscript 'a' = number of broilers at each ambience was 25
Superscript 'b' = number of broilers in each ambience was 200
*significant ($p \leq 0.05$) difference in comparison with respective control mean value
Mean values of each analyte at low (13-16°C) and high temperatures (42-45°C) were compared to respective values at control temperature 24-27°C.

At 42-45°C, serum corticosterone, growth hormone, glucagons, testosterone, uric acid, creatinine, urea, glucose, cholesterol, triglycerides, free fatty acids, ASAT, ALAT, lipase and amylase increased significantly ($p \leq 0.05$) and thyroxine, triiodothyronine, insulin, gastrin, total proteins, albumin, globulin, LDH, ICDH and pepsinogen decreased significantly ($p \leq 0.05$) in comparison with respective control mean values.

At low temperatures (13-16°C) serum T4, T3, LDH and ICDH were significantly ($p \leq 0.05$) higher; glucose, cholesterol, triglycerides and free fatty acids were significantly ($p \leq 0.05$) lower and corticosterone, growth hormone, insulin, glucagons, gastrin, testosterone, total proteins, albumin, globulin, uric acid, creatinine, urea, ASAT, ALAT, pepsinogen, lipase and amylase showed non significant ($p > 0.05$) changes in comparison with respective control mean values.

Serum prolactin, aldosterone, sodium, chloride, calcitonin, alkaline phosphatase, 5' nucleotidase and γ-GT were significantly ($p \leq 0.05$) higher and potassium, C-PTH, calcium, phosphorus and magnesium were significantly ($p \leq 0.05$) lower at 42-45°C temperatures than respective control mean values. At 13-16°C temperature serum aldosterone, sodium, chloride, alkaline phosphatase and 5' nucleotidase showed significant ($p \leq 0.05$) fall while serum potassium marked significant ($p \leq 0.05$) rise as compared to respective control mean values. Other analytes of table 2 showed non significant ($p > 0.05$) differences from respective control values.

Table 2: Serum hormones, electrolytes and enzymes in broilers (Mean ± SEM)

Analytes	Maximum ambient temperature		
	13-16°C	24-27°C	42-45°C
Prolactin, ng/ml[a]	56.3±4.32	60.9±4.41	84.0±5.53*
Aldosterone, pg/ml[a]	44.2±5.0*	59.1±4.3	99.8±8.1*
Sodium, mmol/L[b]	120.4±3.8*	134.4±4.5	149.9±3.2*
Potassium, mmol/L[b]	6.9±0.22*	6.1±0.25	5.1±0.3*
Chloride, mmol/L[b]	90.2±4.8*	105.6±5.3	120.6±4.2*
C-PTH, ng/ml[a]	2.4±0.2	2.3±0.1	1.9 ±0.1*
Calcitonin, ng/ml[a]	1.03±0.2	1.1±0.2	2.0±0.2*
Calcium, mmol/L[b]	2.45 ±0.14	2.33 ±0.09	1.77±0.12*
Phosphorus, mmol/L[b]	1.59 ±0.06	1.51±0.09	1.25±0.09*
Magnesium, mmol/L[b]	0.86±0.14	0.82±0.08	0.53±0.04*
Alkaline Phosphatase, U/L[b]	430.1±8.2*	473.5±9.0	513.2±9.3*
5'-Nucleotidase, m-U/L[b]	5.0±0.21*	7.3±0.32	15.1±1.22*
Gamma Glutamyl Transferase, U/L[b]	17.0±1.3	18.23±2.2	26.1±2.3*

Superscript 'a' = number of broilers at each ambience was 25
Superscript 'b' = number of broilers in each ambience was 200
*significant (p≤0.05) difference in comparison with respective control mean value
Mean values of each analyte at low (13-16°C) and high temperatures (42-45°C) were compared to respective values at control temperature 24-27°C.
C-PTH= Carboxy terminal parathyroid hormone

Discussion

Pattern of changes of interrelated analytes showed the physiological state of the body being governed by endocrine system. During hot ambience all the parameters studied showed significant (p ≤0.05) changes while during cold ambience only few parameters showed significant (p ≤0.05) differences.

High ambient temperature

A significant increase in serum corticosterone at high ambient temperature (12) indicated that birds were stressed as was observed in mammals for cortisol (13). It was accompanied by increased concentrations of energy nutrients in sera like glucose, cholesterol, triglycerides and free fatty acids which substantiated the significance of corticosterone in meeting the energy crisis during stress. Increased concentrations of glucose, ASAT and ALAT indicated stimulation of gluconeogenetic process (6). Decreased levels of total serum proteins during hot ambience (14) along with increased creatinine, urea and uric acid levels also confirmed the role of corticosterone as proteolytic hormone. Increased serum lipase could result due to elevated levels of corticosterone as in mammals (15). Serum amylase activity should be interpreted in terms of higher urea and uric acid concentrations.

Reduction in serum T_4 and T_3 was probably to lower metabolic rate for thermoregulation (16) and to prevent hyperthermia. Low food intake during hot ambience was also correlated with circulating levels of thyroid hormones (17). Decreased levels of LDH and ICDH, the enzymes of tricarboxylic acid cycle (TCA) cycle were followed by an increase in serum concentrations of glucose, cholesterol, triglycerides and free fatty acids. Hepatic glycogenolysis and hyperglycaemia were reported in birds exposed to 48°C temperature(18).

Serum calcitonin level in the present study was higher than that in female birds (19) indicating the influence of testosterone on calcitonin (20). It was also higher than that in man, dog, pig, camel and cow (19,21 and 22). Calcium and calcitonin relation (23) in birds is well established but no literature could be traced on calcitonin level during higher environmental temperature. At 42-46°C ambient temperatures, lowering of calcium, phosphorus and magnesium could be attributed to increased level of calcitonin and lowered C-PTH. Hypocalcemic, hypophosphoric and hypomagnaesemic effects of calcitonin (24) could be due to stimulation of the calcium

regulating axis by higher temperature (7). Low phosphorus during hot ambience could be related to low thyroid activity (25) and calcium metabolism. Low levels of calcium during hot ambience in the presence of higher calcitonin levels substantiated the significance of calcitonin as an emergency hormone to protect against hypercalcaemia (20).

The enzyme 5' nucleotidase and AKP increased during hot ambience. The determination of 5' nucleotidase is important in cases where serum alkaline phosphatase in increased. Higher glucocorticoid secretion is related with increased alkaline phosphatase activity (26). Increase in γ-GT could be attributed towards liver stimulation at higher temperatures. Serum γ-GT is possibly derived from high molecular weight fragments of liver that also contains AKP and 5' nucleotidase. Therefore AKP determinations should also be followed by γ-GT and 5' nucleotidase estimations.

Higher ambience induces dual stress, one of higher temperature and second of dehydration. Higher temperature stimulates adrenocorticotrophic hormone (ACTH) release which then regulates the secretion of aldosterone (27). Increased aldosterone level then helps in maintaining water balance. The results of aldosterone and sodium in present study indicated towards efficient handling of salt and water by the birds during conditions of stress. Higher aldosterone helped the birds to retain sodium in the body with increased sodium absorption from lower intestines (28) along with water reabsorption and decreasing potassium levels. Increased sodium and chloride during hot condition was not only due to haemoconcentration but also be due to increased aldosterone. Increased concentration of prolactin during hot ambience supported the earlier reportings that it is involved in important physiological functions (29) like avian salt and water balance (30).

Increased glucagon, corticosterone and growth hormone probably were responsible for increase in blood glucose. Increase in free fatty acids was probably due to lipolytic effect of growth hormone and glucagon. Glucagon served as stress hormone (31). During hot ambience secretion of enzyme pepsinogen decreased because of decrease in gastrin secretion.

Low ambient temperature:

Higher serum T_4 and T_3 during cold ambience could be due to modulation of thyroid activity by central hypothalamic-hypophyseal axis (32). Cold exposure in birds produces non-thermogenic shivering causing lowered glucose (31). Serum LDH and ICDH increased probably to increase the heat production. Significantly lower levels of aldosterone during moderate and low temperatures than hot could be due to low requirement of water for heat dissipation which increases blood volume resulting in a decline in aldosterone levels. During low ambient temperatures insulin concentration was higher with comparatively lower glucose, cholesterol, triglycerides and free fatty acids as it stimulates lipogenesis and inhibits the release of glycerol and free fatty acids in adipose tissue and stimulates conversion of glucose to fat. This was useful during cold ambience in increasing the peripheral utilisation of glucose for thermogenesis.

It was concluded that modulation of physiological mechanisms was for survival of birds during hot and cold ambience. Metabolic changes showed the endocrine regulation. An increase in corticosterone at higher temperatures denoted stress. Metabolic responses were reflected by the levels of proteins, cholesterol, glucose and fatty acids. Modulation in thyroid activity was a part of metabolic strategy for thermoregulation in varying ambience. Relationship of calcitonin with calcium, phosphorus and magnesium showed the variations in mineral metabolism. The study showed that calcium kinetics are not only important in hens but bears equal functional role in broiler chickens. It was clear that prolactin and aldosterone were important for salt balance. Serum enzymes levels indicated that their values require careful interpretation as changes may not necessarily be related with a pathology. Every laboratory should set the normal values of parameters of various types of birds found in the areas during different seasons.

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POVEZAVA MED TEMPERATURO OKOLJA TER VARIIRANJEM RAVNI HORMONOV IN POVEZANIH ANALITOV V SERUMU PIŠČANCEV IZ PUŠČAVSKIH PODROČIJ

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Povzetek: Za natančnejše razumevanje fizioloških mehanizmov smo raziskali razpon, v katerem se lahko spremenijo hormoni in povezani analiti v serumu brojlerskih piščancev ob spremembah temperature okolja. Zbrali smo kri piščancev, ko so bile temperature med 13 in16 °C (nizke), 24-27 °C (srednje) in 42-45 °C (visoke). Vrednosti analitov, ki smo jih izmerili v serumu, pridobljenem pri srednji temperaturi, smo jemali za kontrolne vrednosti, s katerimi smo nato primerjali rezultate iz obdobja nizkih in visokih temperatur.

Višje temperature okolja so povzročile večje variacije v analiziranih parametrih kot nižje. Pri 42-45 °C so se serumski kortikosteron, rastni hormon, glukagon, testosteron, sečna kislina, kreatinin, ureja, glukoza, holesterol, trigliceridi, proste maščobne kisline, ASAT, ALAT, lipaza in amilaza statistično značilno povečali ($p \leq 0,05$). Tiroksin, trijodtironin, inzulin, gastrin, skupne beljakovine, albumini, globulini, LDH, ICDH in pepsinogen pa so se statistično značilno zmanjšali ($p \leq 0,05$) glede na kontrolne srednje vrednosti. Pri nizkih temperaturah okolja (13-16 °C) so bile vrednosti serumskih T4, T3, LDH in ICDH občutno višje ($p \leq 0,05$), vrednosti glukoze, holesterola, trigliceridov in prostih maščobnih kislin bistveno nižje ($p \leq 0,05$), vrednosti kortikosterona, rastnega hormona, inzulina, glukagona, gastrina, testosterona, skupni beljakovin, albuminov, globulinov, sečne kisline, kreatinina, uree, ASAT, ALAT, pepsinogena, lipaze in amilaze pa se od srednjih vrednosti niso očitno razlikovale ($p > 0.05$). Nadalje so bili pri visokih temperaturah okolja povišani serumski prolaktin, aldosteron, natrij, klorid, kalcitonin, alkalna fosfataza, 5'nukleotidaza in gama glutamil transferaza, medtem ko so bile vrednosti kalija, C-PTH, kalcija, fosforja in magnezija očitno nižje. Pri nizkih temperaturah okolice so zelo padle serumske vrednosti aldosterona, natrija, klorida, alkalne fosfataze in 5'nukleotidaze, serumski kalij pa se je statistično značilno povečal ($p \leq 0,05$) v primerjavi z vrednostmi pri srednjih temperaturah.

Ugotavljamo, da temperatura okolja vpliva na presnovo glukoze, maščob, beljakovin, kalcija in natrija, in sicer tako, da omogoča pticam preživeti zelo raznolike okoljske razmere. Vzorec sprememb medsebojno odvisnih analitov kaže na endokrino uravnavanje fiziološkega stanja organizma.

Ključne besede: temperatura okolja; analiti; serum; brojlerji; hormoni