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Effect of Various Factors on Hematology and Serum Biochemistry Values of Aseel and Kadaknath Chicken

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A B S T R A C T

The study was conducted to record baseline data for two indigenous breeds of Aseel and Kadaknath and evaluate the effects of Age, sex season and rearing effects on the various hemato-biochemical parameters. For haemoglobin Aseel and Kadaknath recorded values of 11.92 ± 0.21 and 13.26 ± 0.61 for 3 and 6 week and 12.48 ± 0.15 and 12.59 ± 0.20 for 9th and 12th week. Highest value for MCHC was recorded by Aseel male during hot -humid season as 89.07 ± 0.86 in 3rd week and lowest value was recorded as 52.21 ± 0.29 by Aseel male cage in 9th week during winter. The ALT and AST contents were only affected (P<0.05) by season wherein these were noticed higher during winter season. Biochemical profile revealed that cholesterol and creatinine were higher (P<0.05) in Aseel birds during winter season.Total protein level was noticed to be affected (P<0.05) by age and season.

Introduction

India is home to many Indigeneous breeds of fowls that have their own characteristics. Indigeneous birds are known to be hardy and adjust to poor management and feeding. Rural poultry is an important sub sector of poultry production in developing countries (Hussain *et al*, 2018). The present research work was aimed to evaluate the effect of season, age, breed, sex and rearing system in Aseel and Kadaknath under hot-humid and winter season on their hemato-biochemical responses. Yahav and Hurwitz (1996) have described decreased haematocrit values in fattened broilers in connection with a 24−hour exposure to 36 ± 1 °C early in life. Packed cell volume (PCV), haemoglobin (Hb) and total protein values are important haematological parameters, which are of importance in the assessment of health status and disease of birds (Hawkey *et al.*, 1983, 1984). These are good indices of livestock adaptability to prevailing environmental conditions (Kaushish *et al.*, 1976). By evaluating the haematological profile during the seasonal variations, the confusion with disease associated changes can be avoided (Olayemi and Arowolo, 2009). Determination of haematological parameters is a reliable tool

since it gives specific indication of the ongoing events in the body, serving as an aid to diagnosis and health status assessment (Tibbo *et al.*, 2008). Cold temperature, increased oxygen requirement, cardiac output, and blood flow may result in increased pulmonary arterial pressure overload on the right ventricle (Julian *et al.*, 1989).

Materials and Methods

Experimental birds and protocol design

400 eggs of each breed (Aseel peela and Kadaknath) were incubated at Experimental Hatchery Unit of the institute. Day old chicks (sexed by Japanese method of vent sexing) were reared for a period of 12 weeks in two seasons i.e. hot –humid (27.7.2016-24.10.2016) and winter season 15.12.2016-9.3.2017. All the birds were maintained under standard managemental conditions under deep litter/cage system with feed and water *ad libitum*. Birds were offered standard feed.

Equipments

Setter and hatcher (Dayal poultry appliances, Delhi, India), micropipettes (Eppendorf AG, Germany), table top refrigerated microcentrifuge, (3500 Kubota Corporation, Tokyo, Japan), Abacus vet junior hematoanalyser, ELISA reader Spectra max 190.

Glass and plastic wares

Glass wares i.e. beaker, test tubes, cylinders, conical flasks, round bottom flasks etc. used throughout the experiment were procured from Borosil, India. Plastic wares *viz.* flat caps, micro centrifuge tubes (0.2 ml, 1.5 ml and 2 ml) and tips (20 µl, 200 µl and 1ml) were procured from Axygen, USA. Conical centrifuge tubes (15 ml and 50 ml) were purchased from TarsonPvt. Ltd. India.

Chemicals and kits

Abacus reagents for Abacus hematoloanalyzer (diatro cleaner vet 5, diatro rinse, diatro lyse, diatro diluent) and Span diagnostic limited, Surat, India diagnostic kits for biochemistry (glucose, total protein, Creatinine, uric acid, cholesterol, ALT and AST.

Sample analysis

Whole blood (2 ml) collection was collected from jugular vein of randomly selected 50 birds from each breed in each season into sterile vials with and without EDTA as an anticoagulant for haematological and biochemical analysis, respectively. The haematological parameters were estimated manually as well as by Abacus junior vet 5 haemo-analyser (Datron, Ltd.). Blood samples were centrifuged briefly at 1000 rpm for 10 min and supernatant, the serum was decanted and deep frozen (-20^0 C) till analysis. Serum samples were analysed using commercial standard diagnostic kits (Span diagnostic limited, Surat, India) using protocol as mentioned on kits

Statistical analysis

All the generated experimental data was analyzed statistically using SPSS 20.0 (Incl. Chicago) and values were expressed as Mean ± SE. Factorial 2X2X2 and 2X2X2X2 design was used. Values were considered at probability of 5% and 1% level as significant and highly significant, respectively.

Results and Discussion

Hematology (Table 1 and 2)

Total red blood cell count (TRBC, 10^6 /µl)

TRBC count was significantly affected by breed. Kadaknath recorded higher values than

Aseel which is in agreement with finding of (Pandian *et al.,* 2012) who reported values of 2.96 and 2.82 for Kadaknath and Aseel. RBC count was higher in hot-humid than in winter Decreased erythropoiesis due to stress low feed consumption reduces the number of circulating erythrocyte (Donkoh, 1989).

Haemoglobin (g/dl)

Significant difference was observed in haemoglobin between the two seasons. Pampori and Iqbal (2007) reported haemoglobin of female and male adult native chickens of Kashmir as 11.32 ± 0.19g% and 13.21 ± 0.39 g%. Tyagi *et al.,* (2007) reported that haemoglobin of broiler chicks at 6^{th} and 8^{th} weeks of age at 23°C were 7.63 and 7.70 g/dl. Lower hemoglobin levels in winter in present study are in agreement to the finding of Pandian *et al.,* (2012).

Packed cell volume (PCV %)

Older birds (9 and 12 weeks) recorded higher values than younger age groups which is in agreement with Islam *et al.,* (2004). Addas *et al* (2012) reported PCV values for days 60, 74 and 104 age groups as (18.60±0.76%), (22.30±0.76%) and (21.20±0.76%) respectively. Pandian *et al.,* (2012) reported PCV of Kadakanath as (25.16±1.53), Nicobari (28.33±1.14) and Aseel (30.16±1.81) during Hot-humid (38°C). Tibetan chickens reared at high altitude had higher haematocrit than chickens reared at low altitude.

Mean cell volume (MCV,fl)

Results of this experiment are in agreement with the finding of Panigrahy *et al.,* (2017) that MCV and MCH were significantly ($p<0.05$) higher in females and Effect of season was observed in MCV and MCH but in contrary to reporting of Kundu *et al.,* (2015) who found MCV values in males were significantly higher than that in females. Zhang *et al.,* (2007) studied blood Characteristics for High Altitude Adaptation in Tibetan Chickens and reported MCV values of 139.7 ± 2.29 μ m^3 in Tibetan chicken.

Mean corpuscular haemoglobin (MCH, pg).

MCH reduced in 6 week old chicken which is in agreement with findings of Islam *et al.,* (2004) who studied haematological parameters of native chicken of Bangladesh and reported that MCH decreases gradually with the advancement of age. Ladokun *et al.,* (2008) found no significant difference between MCH values in Nigerian chickens and normal feathered chicken and reported their values as 27.20 fl and 28.90 fl respectively.

Mean corpuscular haemoglobin concentration (MCHC, g/dl)

Rearing system significantly affected MCHC values. Addas *et al.,* (2012) reported significant ($P<0.01$) effect of management system for MCHC intensively kept chickens produced higher values (17.52±0.30 g/dL) than the semi - intensively kept chickens (15.82±0.30 g/dL). But in contrast to the above, birds in deep litter produced higher values as compared to birds in cage which may be a result of higher metabolic activity due to physical movement.

WBC count (WBC, 10^9/l)

Total white blood cell (WBC) counts and antibody production were significantly inhibited in hens in the heat stress group Mashaly *et al., (*2004). Higher WBC count in winter was obtained as compared to hot-humid season which agrees with findings of

Heller *et al.,* (1979) who found that high environmental temperatures (44.4 to 47.8°C) suppress circulation of white blood cells (WBC). Datta *et al.,* (1996) noticed similar increase in the amount of leucocytes in blood of ducks that were exposed to higher environmental temperature.

Serum Biochemistry (Table 3 and 4)

Cholesterol (mg/dl)

Present findings are in agreement with Panigrahy *et al.,* (2017) who reported that no significant ($p>0.05$) difference was observed in total cholesterol concentration between male and female in both seasons. During winter, cholesterol level was significantly higher than hot humid during 9 and 12 weeks. Our findings are consistent with the report by Zulkifli *et al.,* (1999) that stated serum concentration of cholesterol declined in response to heat stress.

Glucose (mg/dl)

Sex influenced glucose levels for 3 and 6 week old birds. Aseel female in litter during hot-humid had higher glucose levels than male. Blood glucose concentration increase may result from various stressors including heat stress (Borges *et al.,* 2004). Our findings are consistent with the above report. Season affected glucose levels for age groups 3 and 6 weeks with winter recording lower values. Khan *et al.,* (2002) reported that glucose levels increased in birds exposed to heat as compared to birds to birds in 28-32 ^{o}C.

Table.1 Influence of season, breed, and sex and their interaction on haematology for age groups 3 and 6 week

Effect	TRBC (10^6/µl)	Hgb (g/dl)	PCV(%)	MCV(fl)	MCH(pg)	MCHC(g/dl)	WBC(10^9/l)
Age effect							
3 week	1.85±0.03	13.31^{a}±0.30	18.29±0.35	102.45^{a}±0.32	70.33^{a}±1.13	69.37^{a}±1.10	33.44±10.20
6 week	1.88±0.02	11.87^{b}±0.57	18.68±0.22	99.79^{b}±0.45	59.72^{b}±0.60	58.94^{b}±0.52	20.23±0.16
P –value	0.485	0.025	0.346	0.000	0.000	0.000	0.196
Sex effect							
Female	1.87±0.03	12.39±0.25	18.94±0.28	102.61^{a}±0.33	65.01±0.93	63.71±0.91	21.20±0.15
Male	1.86±0.03	12.78±0.60	18.04±0.30	99.63^{b}±0.44	65.04±1.03	64.60±0.95	32.47±10.20
P- value	0.837	0.550	0.29	0.000	0.983	0.503	0.270
Breed effect							
Aseel	1.76^{b}±0.02	11.92^{b}±0.21	17.94^{b}±0.23	102.74^{a}±0.40	66.21±0.93	64.49±0.95	31.98±10.20
Kadaknath	1.98^{a}±0.03	13.26^{a}±0.61	19.03^{a}±0.33	99.50^{b}±0.37	63.84±1.02	63.83±0.91	21.69±0.19
P- value	0.000	0.039	0.008	0.000	0.086	0.618	0.314
Season effect							
Hot -humid	1.83±0.03	14.26^{a}±0.61	18.59±0.33	102.55^{a}±0.49	73.77^{a}±1.05	72.29^{a}±1.02	21.70±0.25
Winter	1.91±0.02	10.92^{b}±0.14	18.39±0.24	99.69^{b}±0.24	56.28^{b}±0.23	56.02^{b}±0.21	31.97±10.20
P- value	0.056	0.000	0.625	0.000	0.000	0.000	0.315

Means within columns bearing different superscripts differ significantly ($p<0.05$)

Table.2 Influence of season, breed, age, sex and rearing system and their interaction on hematology for age groups 9 and 12 week

Effect	TRBC (10^6/µl)	Hgb (g/dl)	PCV(%)	MCV(fl)	MCH(pg)	MCHC(g/dl)	WBC(10^9/l)
Age effect							
9th week	2.40±0.12	12.40±0.19	21.96±0.42	99.57^a±0.42	57.21±0.64	59.16±0.64	17.22±0.12
12th week	2.65±0.09	12.59±0.17	21.96±0.42	97.48^b±0.40	58.00±0.57	61.12±0.60	17.59±0.24
P- value	0.089	0.659	0.182	0.000	0.354	0.028	0.166
Sex effect							
Female	2.60±0.13	12.57±0.16	21.11±0.26	99.18^a±0.26	58.23±0.61	60.48±0.61	17.38±0.19
Male	2.45±0.06	12.50±0.20	22.00±0.54	97.87^b±0.50	56.99±0.50	60.40±0.68	17.44±0.19
P-value	0.297	0.744	0.142	0.024	0.149	0.432	0.835
Breed effect							
Aseel	2.50±0.11	12.48±0.15	21.14±0.25	100.08^a±0.49	58.49^a±0.68	60.86±0.76	17.46±0.19
Kadaknath	2.55±0.10	12.59±0.20	22.00±0.54	96.97^b±0.27	56.73^b±0.52	60.00±0.52	17.36±0.18
P-value	0.767	0.624	0.137	0.000	0.040	0.807	0.699
Season effect							
Hot -humid	2.66± 0.16	13.04^a± 0.23	22.52^a±0.55	97.07^b±0.51	60.33^a±0.86	65.36^a±0.77	15.28^b±0.12
Winter	2.41± 0.02	11.84^b±0.10	20.32^b±0.21	100.36^a±0.18	55.14^b±0.20	55.50^b±0.21	19.56^a±0.13
P-value	0.122	0.000	0.000	0.000	0.000	0.000	0.000
Rearing System effect							
Cage	2.35^b±0.03	11.57^b±0.14	21.75±0.24	9950^a±0.45	55.00^b±0.36	57.32^b±0.32	17.50±0.18
Litter	2.70^a±0.14	13.50^a±0.19	21.37±0.55	97.55^b±0.35	60.21^a±0.74	62.95^a±0.81	17.32±0.19
P-value	0.017	0.000	0.531	0.001	0.000	0.000	0.482

Means within columns bearing different superscripts differ significantly ($p<0.05$)

Table.3 Influence of season, breed, and sex and their interaction on biochemistry for age groups 3 and 6 week

Effect	Cholesterol (mg/dl)	Glucose (mg/dl)	Total Protein (g/dl)	Uric Acid (mg/dl)	Creatinine (mg/dl)	ALT (IU/L)	AST(IU/L)
Age effect							
3 week	61.18^b±0.84	279.64^b±1.47	3.94±0.08	4.58^b±0.04	0.81±0.01	4.74^a±0.10	143.16±3.32
6 week	79.94^a±1.61	325.30^a±3.04	3.71±0.03	5.16^a±0.06	0.82±0.01	4.25^b±0.13	139.09±1.56
P- value	0.000	0.000	0.008	0.000	0.329	0.003	0.269
Sex -effect							
Female	68.93±1.47	299.31±2.54	3.83±0.07	4.91±0.06	0.82±0.01	4.40±0.12	142.55±2.85
Male	72.19±1.49	305.63±3.38	3.82±0.06	4.82±0.05	0.82±0.01	4.58±0.11	139.70±2.32
P –value	0.121	0.136	0.843	0.249	0.906	0.281	0.438
Breed effect							
Aseel	75.28^a±1.60	315.43^a±3.26	3.81±0.05	4.90±0.05	0.85^a±0.01	4.04^b±0.12	144.22±2.66
Kadaknath	65.85^b±1.25	289.51^b±2.28	3.84±0.08	4.84±0.06	0.78^b±0.01	4.95^a±0.10	138.03±2.52
P –value	0.000	0.000	0.716	0.489	0.000	0.000	0.092
Season effect							
Hot -humid	70.91±1.33	314.02^a±3.63	4.04^a±0.06	4.50^b±0.04	0.77^b±0.01	4.80^a±0.10	155.14^a±2.18
Winter	70.21±1.62	290.92^b±1.77	3.61^b±0.06	5.24^a±0.05	0.87^a±0.01	4.19^b±0.13	127.11^b±2.51
P –value	0.739	0.000	0.000	0.000	0.000	0.000	0.000

Means within columns bearing different superscripts differ significantly ($p<0.05$)

Table.4 Influence of season, breed, age, sex and rearing system and their interaction on biochemistry 9 and 12 weeks for age groups 9th and 12th week

Effect	Cholesterol (mg/dl)	Glucose (mg/dl)	Total Protein (g/dl)	Uric Acid (mg/dl)	Creatinine (mg/dl)	ALT (IU/L)	AST(IU/L)
Age effect							
9th week	104.77±1.97	364.20^{b}±1.51	2.98^{b}±0.10	5.25^{a}±0.09	0.81^{a}±0.01	3.16±0.18	119.30^{b}±1.70
12th week	104.32±1.74	379.60^{a}±4.88	4.20^{a}±0.03	4.05^{b}±0.04	0.71^{b}±0.04	6.13±0.22	155.12^{a}±3.72
P- value	0.862	0.003	0.000	0.000	0.008	0.202	0.003
Sex effect							
Female	106.04±1.85	372.57±3.78	3.59±0.07	4.62±0.08	0.77±0.03	4.74±0.20	134.54±2.83
Male	103.05±1.85	371.22±3.50	3.59±0.10	4.67±0.08	0.75±0.02	4.54±0.26	139.87±3.56
P-value	0.253	0.794	0.975	0.637	0.431	0.542	0.241
Breed effect							
Aseel	108.93^{a}±1.77	366.37^{b}±2.75	3.49±0.07	4.46^{b}±0.07	0.74±0.01	4.55±0.19	132.19±2.41
Kadaknath	100.16^{b}±1.89	377.24^{a}±.33	3.69±0.10	4.84^{a}±0.08	0.79±0.04	4.74±0.27	142.23±3.83
P-value	0.001	0.032	0.8	0.001	0.167	0.564	0.27
Season effect							
Hot -humid	92.31^{b}±1.37	410.11±3.25	3.85^{a}±0.02	4.14^{b}±0.03	0.68^{b}±0.03	3.16^{b}±0.18	119.30^{b}±1.70
Winter	116.78^{a}±1.94	333.68±1.95	3.33^{b}±0.11	5.15^{a}±0.10	0.84^{a}±0.03	6.13^{a}±0.22	155.12^{a}±3.72
P-value	00.000	0.300	0.000	0.000	0.000	0.000	0.000
Rearing system effect							
Cage	104.45±1.81	370.10±4.09	3.71^{a}±0.10	4.38^{b}±0.08	0.79±0.04	4.86±0.22	139.38±3.63
Litter	104.64±1.89	373.70±3.14	3.47^{b}±0.06	4.92^{a}±0.08	0.74±0.01	4.43±0.24	135.03±2.74
P-value	0.941	0.485	0.04	0.000	0.205	0.197	0.339

Means within columns bearing different superscripts differ significantly ($p<0.05$)

Total Protein (g/dl)

Total protein values were remained unaffected by sex in our study which is in contrary to Abdi-Hachesoo (2013) who reported total protein concentration for 18 week old adults as 3.87 g/dl in males and 5.22 g/dl in females, respectively. In the present study, total protein values were more in older birds which in agreement to Schmidt *et al.*, (2007). The total protein values were ranging between 2.66±0.05 to 5.30±0.12 in both the seasons. In hot –humid season, the values were higher (P<0.05) than in winter season, it may be due to Hot -humid stress which is in agreement with the result of Aarif *et al* (2014).

Uric Acid (mg/dl)

Uric acid value for Aseel male reported by Kumar and Kumbhkar *et al.*, (2015) as 5.23±0.15 mg/dL is falling in range of 3.33±0.03-6.47±0.06 reported during present study. Uric acid for male and female was 4.67±0.08 and 4.62±0.08 which is lower than values reported by Pampori and Iqbal (2007) as 6.31±0.33 and 6.21±0.40 for male and female in Kashmiri chicken, respectively.

Uric acid values were significantly affected by breed in age groups 9 and 12 weeks with Kadaknath recording higher values. Uric acid values for adult Aseel (4.46±0.07) and Kadaknath (4.84±0.08) reported during

present experiment are comparable to range (4.16-4.63 mg/dL) determined by Bhatti *et al.,* (2001) for *Desi* and Naked Neck hens. Age and diet may influence the concentration of blood uric in birds.

Creatinine (mg/dl)

Researchers have used creatinine as an indicator of muscle metabolism but this remains to be confirmed (Bowes *et al.,* 1989). Creatinine value recorded for 9 and 12 weeks was 0.79±0.04 in Kadaknath and 0.74±0.01 mg/dl in Aseel which is lower than values of 0.95 mg/dl reported by Ladokun *et al.,* (2008) for Nigerian naked neck. Significant difference was observed between values of 9th week and 12th week. This is similar to a study (Polat *et al.,* 2011) in which creatinine levels varied significantly in broiler chickens due to diet.

ALT and AST (IU/L)

The values of ALT and AST in chicken are 5-15 U/L and 174.8 U/L, respectively (Kaneko, 2008).The ALT and AST enzyme concentration were significantly ($p<0.05$) higher in Hot -humid than winter. Reports of present study are consistent with Panigrahy *et al.,* (2017) who reported no significant ($p>0.05$) differences were observed in ALT and AST enzyme concentration between male and female. Abdi-Hachesoo *et al.,* (2013) observed ALT as 7.80 ± 1.62 in male and 7.20 ± 1.47 in females of indigenous birds.

From this study it may be concluded that interactive effects of age, sex, season and rearing system may affect hemato-biochemical parameters in birds. Age wise baseline hemato-biochemical data for Aseel and Kadaknath for hot-humid and winter season under cage and deep litter rearing system have been recorded.

Statement of Animal Rights

The study was carried out as per the guidelines and approval of institute animal ethical committee (IAEC) and committee for the purpose of control and supervision of experiments on animals (CPCSEA).

Conflict of interest

The authors declare that they have no competing interests.

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