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## Multiple Environmental Stresses and Broiler Internal Organs Somatic Indices under Controlled Environment

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**Abstract:** The current trial is designed to investigate the effect of multiple environmental stresses (Heat, ammonia exposures and stocking density) on broiler's organs somatic indices (SI) of liver, spleen and bursa fabricious under controlled environment (1-42 DO) during summer. Control birds: Bursa fabricicus (Bf) index showed higher values from 1st -3rd wks. The minimum values for spleen and Bf indices were at 5th week old. Food conversion rate (FCR) was  $1.8 \pm 0.56$ . Indoor ambient temperature ( $T_a$ , °C) is positively correlated with liver index Heat stressed body temperature ( $T_b$ , °C) is increased and be indicative to early stress (1st 4 hours exposure to 41°C) but chronic stress enforced bird acclimation and decreased final B wt/g. Liver and spleen indices were decreased while Bf index increased. The dramatic effects of expected generated ammonia under field can be modulated by allowing recovery period for elimination, where here it accompanied with non significant increased  $T_b$  but decreased final B.wt and SI of liver and spleen except the increased Bf index. Stocking density decreased final B wt and increased  $T_b$  but not affected birds SI. Conclusively persistence of some stresses enforced birds to habituate especially when be mild, at non critical early age (growth easily compensated), beside the controllable indoor climatic conditions in closed house during summer, all could alleviate the impact of intrinsic or extrinsic multiple stresses on broiler performance. The lymph organ (Bf) index was increased post heat and ammonia stress and not badly affected by stocking 17 bird/m<sup>2</sup>.

**Key words:** Environmental stress, somatic indices, liver, spleen, ammonia stress

### INTRODUCTION

Internal organs weights are affected by multiple environmental stresses as thermal stresses (cold or heat) on liver, spleen, gizzard and kidney. Vaccinations on lymph organs (spleen and bursa fabricious). Stocking density, ventilation rates and gaseous pollutants as CO<sub>2</sub> and NH<sub>3</sub> on spleen, liver and bursa fabricious. These effects differed according to age, period of exposure, single or concurrent stresses, the intensity and the environmental management programs (Curtis and Drummond, 1982; Neto *et al.*, 2000; Rodney *et al.*, 1991; Uni *et al.*, 2004; Graczyk *et al.*, 2003; Hair-Bejo *et al.*, 2004) Post Heat stress liver index was ranged 3.56-4.56% at 21 days old while was 2.38-2.68% at 42 days old and spleen index was 0.014-0.136% at 21 days, 0.111-0.168 % at 42 days old (31). Increasing  $T_a$  °C either constant (25-30-35 °C) or cycling increase (25-35°C) resulted in increased liver wt% (somatic indices) (Plavnik and Yahav, 1998), while liver indices were increased with decreased  $T_a$  °C (Buys *et al.*, 1999). Ammonia, NH<sub>3</sub> is generated and emitted within poultry houses by microbial decomposition of birds wastes to the levels that detrimental to broiler performance especially with increased stocking in confined areas and reduced litter thickness with its microclimate which included temperature, moisture content and PH

concentration. NH<sub>3</sub> gas was positively correlated with indoor RH% in broiler houses where increased at 75% vs 45% in closed environment. Its accumulation with varied levels resulted of inadequate ventilation (100, 110 and 125 ppm) and decreased the weights of primary and 2nd lymph organs, spleen and bursa fabricicus as well as affected chicks respiratory system (Heckert *et al.*, 2002; Essa and Mary, 1985; Donker and Beuving, 1989; Rodney *et al.*, 1991; Groot-Koerkamp, 1994; Varel *et al.*, 1997; Takai *et al.*, 1998; Esteves and Angel, 2002; Lacy, 2002; Butcher and Miles, 2003; Tom Tabler, 2003; Carey *et al.*, 2004). The present study is planned to evaluate the degree of controlling broiler environment through summer season on the internal organs somatic indices during and after experimental exposure to multiple environmental stresses heat, ammonia and stocking density.

### MATERIALS AND METHODS

#### Experimental design

**Experimental unit:** The experimental unit was constructed followed the recommended environmental requirements after (Moura *et al.*, 2001; Sainsbury, 2000), floor area was 5x3 m (15m<sup>2</sup>)x3 m height. with conventional controlled ventilation system and 2 exhaustion fans (30x30 cm dimension and 2300m<sup>3</sup>/hour

Table 1: Indoor ambient climatic conditions in control group

Age/week	Ta. °C	RH%
1	32.25±.309	57.43±2.05
2	30.93±.600	61.29±3.85
3	29.61±1.03	58.46±2.92
4	28.96±1.20	57.21±2.49
5	29.25±1.31	60.43±2.93
6	27.86±1.61	62.43±3.38
Mean±SD	29.81±1.555	59.52±.0157

Table 2: Internal birds measures and productivity in control group

Age/wk	Li%	Sp%	Bf%	BW/g	FC	RM.%
1	4	0.75	0.235	136.8	1.4	-
	0.337	0.005	0.017	12.479		
2	4.15	0.01	0.265	305.7	1.9	-
	0.387	0.021	0.07	32.721		
3	2.57	0.075	0.243	640.5	1.9	-
	0.263	0.025	0.02	56.508		
4	2.371	0.118	0.183	1155.6	1.3	1
	0.34	0.054	0.112	69.069		
5	2.14	0.075	0.04	1310.6	2.8	2
	0.085	0.021	0	119.855		
6	2	0.1	0.065	2062.4	1.5	-
	0.424	28	0.007	224.049		
Mean±	2.87	0.092	0.172	935.17	1.8	
SD	0.952	0.016	0.095	718.358	0.557	

capacity) located at 50 cm height from the ground. The plastic curtain partitions were located between each of heat and ammonia and stocked groups and the control group 2).

**Management procedures:** Total 180 baby chicks, mixed sex (Cobb 500) were obtained from the Recent poultry company in Al Dammam and housed under automatically controlled environment with conventional ventilation, indoor ambient temperature started as 34°C then decreased gradually 1°C each 2 days according to (Sainsbury, 2000) but accidentally the air condition failed at 10th, 11th days of brooding that required manual control to keep the required heat, relative humidity and ventilation rate. The indoor relative humidity ranged between 44-55% that in turn needed to be raised to range of 60-70% according to (Aengewanich and Simaraks, 2004). Food and water were available *ad libitum*. Light 23 hours (Smith and Bartlett, 2001) and traditional prophylactic programs were applied through brooding period. They were reared under proper environmental conditions as could as possible till 21 days old then separated as 30 birds for each of heat stress, ammonia exposures and 40 birds for stocking density and the rest kept as control (80 birds).

#### Stress procedures

**Heat exposure:** At 22 days old birds were separated and marked with plastic leg tags and exposed to continuous 41°C for 4 hrs (12.00-04.00 pm daily) till 31 days old by using electric heater 2000 watt placed 50 cm over the ground. The allowed floor was 2.5m<sup>2</sup> (Zakia and Al Ghamdi, 2005).

Table 3: Indoor ambient climatic conditions and birds internal parameters of control group

Climate	Li	Sp	Bf	BW/g	FCR	M.%
Ta.°C	0.886***	-0.471	657	0.943***	0.143	-0.617
	0.01	0.34	0.15	0.01	0.78	0.19
RH%	-0.257	0.000	-0.086	0.429	0.600	0.617
	0.62	1.00	0.87	0.39	0.20	0.19

**Ammonia exposure:** At 22 days 30 of birds were separated and marked with numbered plastic leg tags and complete separation was done by partition and released thick plastic curtain between groups from near roof to ground. Birds were exposed to vaporized diluted ammonia solution of 25 % stock where injected into litter materials (24 hours) followed by continuous checked indoor ammonia levels using Kitagawa pump and its detecting tubes (Mehta and Shangari, 1999; Zakia and Gross, 1995) till reached the approximate desired concentrations 120 ppm through first exposure period (22-26 days) old and 120-150 ppm for the 2nd exposure (31-35 days/24 hours) with complete elimination of ammonia sources during rest period between 27-30 days old. The proper litter management and ventilation were applied for rest period to relief ammonia impact. c).

**Stocking stress:** At 22 days old 17 birds/m<sup>2</sup> were kept till 42 days old Vs 12 birds/m<sup>2</sup> in control group (Maretenchar *et al.*, 1997).

**Measures:** Indoor Ta.°C, RH% were checked daily to keep proper environment till 22 days old (representing the controlled group for all stresses) using digital thermohygrometer. Indoor NH<sub>3</sub> levels were checked daily for control and at exposure to maintain the desired Ta.°C and approximate NH<sub>3</sub> concentration using the kitagawa pump and its detecting tubes. Birds were randomly selected from either control (5 birds weekly) or NH<sub>3</sub> stressed groups (4 birds were sacrificed at 3 occasions, at 22, 26 and 35 days old) but 5 birds for each age at 22, 32 and 42 days old for stocked group then recorded their Tb.°C and weighed individually to record B. wt/g, slaughtered and eviscerated to get internal organs for weighing and estimation of their somatic indices.

## RESULTS AND DISCUSSION

**Control birds group:** Data in Table 1 revealed mean indoor Ta.°C was 29.81±1.55 and RH% was 59.5±2.016 in control room. Results in (Table 2) showed mean Tb.°C ranged from 39.7±0.442 - 41.7±0.234°C at 2nd and 6th wks with mean 41.0±0.791°C. The weekly mean B.wt/g was increased with age while liver and spleen SI were decreased. They showed sudden decrease during 1st and 2nd wks and then maintained approximate near values till 6th wk old, while Bf index showed higher values from 1st -3rd wks then declined gradually from 4th to 6th wk old. The minimum values for spleen and Bf

Table 4: Mean values of internal birds parameters under heat stress

Parameter/Age	22		26		31	
	A	B	A	B	A	B
(X ±SD)						
Tb °C	42.9±0.46	41.7±0.30	42.1±0.64	42± 0.30	42.4±0.52	41.5±1.09
LI %		2.60±.271		2.74±0.351		2.34±.458
Sp %		0.075±.025		0.105±0.003		0.103±.021
Bf %		0.242±.021		0.170±0.112		0.057±.009
B wt /g		721.5±69.40		832.7±70.25		1193.8±146.83

B and A = mean Tb .before and after heat stress

Table 5: Mean values of bird performance parameters under ammonia stress

Ages/day	B wt/g	Tb.°C	Liver%	Spleen%	BF %
22 (non exposed)	741.0	41.5	10.39	2.60	0.075
	57.64	0.254	0.468	0.270	0.025
26 1st exposure	875.0	40.5	9.51	2.75	0.113
	68.57	0.336	0.588	0.358	0.039
31 end of 1st exposure	1256.3	42.000	9.36	2.72	0.135
	66.26	0.540	0.549	0.368	0.007
35 2nd exposure	1429.6	42.600	9.21	1.90	0.085
	177.57	0.577	0.717	0.307	0.017
42 end experiment	1771.14	41.8	9.95	2.08	0.110
	276.92	0.707	1.279	0.149	0.014

indices were at 5th wk old (0.075±0.21 and 0.040±0.000 respectively). Final means of SI were 2.874±0.952, 0.092±0.016 and 0.017±0.095 for liver, spleen and Bf respectively). The results partially matched that of (Silversides *et al.*, 1997) where recorded mean liver SI was 3.56-4.56 and 2.38-2.68 between 21-42 days old while spleen indices were ranged 0.014-0.136 and 0.111-0.168 at 21 and 42 days old respectively, the current liver index was within normal level so coincided with (Cheema *et al.*, 2003; Petek, 2000) who reported mean liver index for free feed control birds was 2.53 between 1-40 days old. FCR for current control birds was ranged 1.3-2.8 at 4th and 5th wks with mean 1.8±0.56, this result was in agreement with (Coles *et al.*, 1999) who reported mean FCR for Cobb x Ross breed at 1,3, 6 weeks was 1.55, 1.37 and 1.88.) as well as (Petek, 2000) where recorded FCR ranged 1.18 to 2.03 through 1-40 days old. The sudden increase of current birds FCR at 5th week might be attributed to the sudden decrease of B wt despite all levels were within recommended for Cobb 500 breed. The mortality number was 2, 1 at 4th and 5th weeks only. On regarding the effect of indoor Ta°C on internal organs indices results in Table 3 only positive correlation was noticed with liver index (P=0.01).

**Heat stress:** Data in Table 4, indicated the highest Tb.°C 42.9°C±0.46 was post 1st four hours exposure then decreased at 5th and 10th days of stress. The Tb.°C after stress was significantly increased than before (P=0.001), the result was coincided with (Zhou and Yamamoto, 1997) when exposed bird to 35°C and noticed increased Tb.°C. At 26 days, the Tb.°C didn't significantly increased vs before stress, this might be

attributed to possible acclimation as clarified by (De Basilio *et al.*, 2003) when exposed birds to 38-40 °C for 5 days/24 hours and noticed decreased their Tb. At 31 days old Tb °C increased significantly vs before stress (p= 0.024), this was expected finding because birds at this age with fast growth, more heat production, their body temperatures tend to rise (Mehta and Shangari, 1999). Continuity of stress in same pattern and using the controlled ventilation could enhance birds thermoregulation and acclimation as shown by decreased Tb.°C through next ages. The age related stress response was illustrated by (Aengewanich and Simarak, 2004) when exposed broiler at 28 days old to 33°C±1 under controlled environment and recorded increased Tb.°C. The mean B wt/g at 1st, 5th and at the last days old of stress were 721.5g±69.40, 832.7g±70.25 and 1193.8g±146.83 respectively. Liver index was higher than spleen and Bf and all organs had the lowest indices values at the last day of stress This decrease considered as a negative effect of heat stress on liver which supposed to be increased with age as a relative weight percent, the result was confirmed via recorded moderate and severe histopathological changes on these livers by (Zakia and Al Ghamdi, 2005). Bf index showed the lowest value at last day of stress, this might be a complicated response with the normal growth regressive pattern of the bursa with age as regarded by (Pettit-Riley *et al.*, 1983) and its involution with vaccination programs as recorded by (Siegel, 1971).

**Ammonia stress:** Data recorded in (Table 5) revealed that, final B wt at 42 days old was 1771.14±276.92 g, highest Tb, was 42.6°C±0.577 at 35 days (end 2nd exposure) while the lowest was 40.5°C± 0.336 at 26 days old (end of 1st exposure). Liver index was higher than spleen and Bf indices from 22-42 days old, where liver index ranged from 9.21±0.717 at 35 days to 9.95±1.279 at 42 days, spleen index was 1.90±0.307 at 35 days to 2.72±0.368 at 31 days, Bf was 0.085±0.017 at 35 days to 0.135±0.07 at 31 days. while at 42 days old non significant decreased body wt was for stressed vs control (1771.14g±276.92 vs 2261.0±125.87g (P=0.121). On comparing stressed with control group (Table 6), a significant increased Tb was noticed. vs control (42.6°C±0.577 vs 41.6°C±0.284, P=0.003) at 35 days

Table 6: Effect of ammonia stress on bird's internal parameters and productivity

Age/ days	Parameters Mean diff. ± SD		Ammonia stress	T. test
	Control			
35	B wt/g	1310.6±119.855	1429.6±177.57	1.399 0.162 2.998***
	Tb. °C	41.6±0.284	42.6±0.577	0.003 0.926 0.355
	Li %	2.140±0.085	1.90±0.307	0.953 0.340 1.879*
	Sp %	0.075±0.021	0.085±0.017	0.060 1.549 0.121
	Bf	0.040±0.000	0.072±0.026	0.000 1.000 1.000
42	B wt	2062.4±224.049	1771.14±276.92	0.000 1.000 1.000
	Ta. °C	41.8±0.141	41.8±0.707	0.000 1.000 1.000
	Li%	2.00±0.424	2.08±0.148	0.408 0.683 0.000
	Sp%	0.1000±0.028	0.110±0.014	0.000 1.000
	Bf%	0.065±0.007	0.070±0.028	1.000

Table 7: Internal birds and productive parameters under stocking stress

Age/days	B wt/g	Tb. °C	Liver %	Spleen%	BF %
22	707.6	41.5	2.6	0.075	0.243
	69.25	337	0.27	0.025	0.021
32	1223	42.2	2.24	0.09	0.085
	107.26	0.544	0.111	0.018	0.037
42	1701.2	41.2	1.8	0.065	0.045
	213.75	0.295	0.212	0.007	0.007

Table 8: Effect of stocking density on bird's internal parameters at 42 days old

Parameters Means diff ±SD	Stocking stress		T-value
	Control		
B wt/g	2062.4±222.43	1701.2±213.75	2.949*** 0.01 3.261***
Tb. °C	41.7±0.234	41.2±.295	0.01 0.775 0.43
Li%	2.00±0.424	1.800±.212	1.549 0.12 1.549
Sp%	0.100±0.028	0.065±.007	0.12 1.549 0.12
Bf.%	0.065±0.007	0.045±0.007	0.12

old. Bf index was significantly increased vs control (P=0.06). No significant differences were noticed for rest of parameters vs control. These results confirmed the importance of recovery period of ammonia stress before marketing to ensure approximate organs weight desired for consumers specially liver. As well as the birds ability to restore its organs performance, which in turn draw poultry men attention to proper environmental management keeping non stressful limits of indoor gases (ammonia), but also can not exclude the expected histopathological changes may occur as figured out by (Zakia and Al GHamdi, 2006) where these livers showed moderate reversible histological changes post 1st exposure only, spleen revealed it post two exposures,

while Bf was moderately and severely affected at 26, 35 and 42 days old. The effect of ammonia gas on birds organs weight were previously reported on individual organs, where (Siegel, 1971) reported decreased bursa weight in broilers exposed to increased concentrations of NH3 during their vaccination against infectious bronchitis (IB) at 8 week old, while bursa index didn't affect by 25-50 ppm of NH3 exposure, meanwhile, increased mortality post exposure to 75 ppm NH3 as 13.9% vs 5.8% for non exposed was documented by (Cavney *et al.*, 1981; Miles *et al.*, 2004).

**Stocking stress:** Data in Table 7 indicated final B wt was 1701.2g±213.75, highest Tb. was at 31 days (42.2°C±0.544). Liver index was higher than spleen and Bf and so at 22 days old (2.60±2.270), spleen higher index was at 32 days (0.090 ± 0.018) and Bf higher value was at 22 days (0.243±0.021). Results in Table 8 revealed significant negative stress impact (P=0.001) on final B wt vs control where it was 1701.2 g±213.75 gm vs 2062.4±222.43gm. A significant difference (P=0.01) was between stressed and control Tb. °C at 42 days old but non significant differences were noticed for organs SI at 42 days old despite decreased their mean indices vs control. The result was confirmed by (Ahmed and Gross, 1995; Feddes *et al.*, 2002) where they reported increased stocking density 0.07m<sup>2</sup>/bird decreased B wt at 4 week old vs 0.11m<sup>2</sup>/bird and increased indoor Ta. °C within bird microclimate and decreased air flow, so decreased heat loss channels which in turn altered birds internal environment and decreased spleen and Bf weights % when stocked at 10,15 and 20 birds /m<sup>2</sup>.

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