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Effect of Dietary Factors on Induction of Fatty Liver-Hemorrhagic Syndrome and its Diagnosis Methods with Use of Serum and Liver Parameters in Laying Hens

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Abstract: An experiment was conducted to determine the effect of dietary factors on induction of fatty liver hemorrhagic syndrome (FLHS) and its diagnosis methods with use of selected serum enzymes on Hy-line W-36 hens. The experiment was conducted in completely randomized design with 6 treatment groups and 4 replicates each with ten hens from 94 to 106 wk of age. Three 28-d periods used for different dietary factors including:1) Control (C); 2) Low methionine (LM); 3) Low linoleic acid (LLA); 4) High energy (HE); 5) Low methionine, linoleic acid, choline and high energy (LM-LLA-LCH-HE) and 6) Low choline (LCH). Feed intake, body weight, egg production (EP), egg weight, egg quality, serum and liver parameters were measured at the end of each 28-d periods. Overall feed intake was significantly ($P<0.05$) lower for the hens fed HE diets compared to the control. Overall egg weight was significantly ($P<0.05$) lower for the hens fed LM and LM-LLA-LCH-HE compared to the control. Overall Liver weight was significantly ($P<0.05$) higher for the hens fed LLA and LM-LLA-LCH-HE compared to the control. Liver hemorrhage score (LHS) was positively correlated ($P<0.05$) with liver weight. Serum enzyme activities including: aspartate aminoteransferase (AST), alanine aminoteransferase (ALT), lactate dehydrogenase (LDH) were not significantly affected by treatment groups. Although the AST enzyme activity was not significantly different, but it was numerically higher for all dietary treatments except HE diet. The results showed the effectiveness of dietary factors on induction of FLHS in laying hens. AST enzyme activity could be used for diagnosis of FLHS in laying hens.

Key words: Fatty liver-hemorrhagic syndrome, dietary factors, enzymes, liver, laying hens

Introduction

Fatty liver-hemorrhagic syndrome (FLHS) is a metabolic disorders that sporadically affects laying hens (Squires and Leeson, 1988). The first sign of the syndrome is an increase in flock mortality, with birds in full production found dead (Butler, 1976), and often a sudden drop in egg production occurs (Riddell, 1997). Dead hens have large blood clots in the liver and ventral hepatoperitoneal abdominal cavities, and the liver is pale and friable with a color varying from a golden yellow to pale yellow or very pale brown (Fowler, 1996). The diagnosis of FLHS is usually made from findings at necropsy (Grimes *et al*., 1991). However, no definitive diagnosis criteria for FLHS have been outlined for use in live birds. Measurement of plasma enzyme activities indicative of liver damage in birds might help in the diagnosis of FLHS. In experimentally induced liver damage in birds, plasma aspartate aminotransferase (AST) activity was found to be the most sensitive indicator liver damage, followed by plasma alanine aminoteransferase (ALT) and lactate dehydrogenase (LDH) activities (Lumeij, 1997).The purpose of this study was to investigate whether selected serum enzyme activities, which are indicative of liver damage, can be used as diagnosis tools for FLHS in live laying hens.

Materials and Methods

Birds and diets: Two hundered and fourty Hy-line W-36 hens were used in this experiment, which were 94 weeks of age, and continued for three 28-d periods. Ten hens were grouped house and shared a common feed trough between them, forming one experimental unit. There were 4 experimental units for each of the 6 treatment groups. Diets were formulated to meet the nutrient requirements for poultry (NRC, 1994) (Table 1). The diets were:1) Control (C); 2)Control with low methionine (LM); 3) Control with low linoleic acid (LLA); 4) Control with high energy (3000 Kcal/kg) (HE); 5) Control with low methionine, linoleic acid, choline and high energy (LM-LLA-LCH-HE) and 6) Control with low choline (LCH).

Sample collection: Egg production was recorded daily and expressed monthly as eggs produced per hen per day. On the 2 d of each 28-d period, eggs were collected to measure egg weight, shell weight and shell thickness. Body weights were determined by weighting birds individually, at the start, and at the end of each 28-d period. Feed intake was determined at the end of each 28-d period. A three ml blood sample was taken from a wing vein artery at the end of each 28-d period from 2 birds of each treatment groups. The samples were

Table 1: Diet composition (%)

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Corn	64.91	64.82	-----	57.1	-----	64.91
Wheat	-----	-----	69.39	-----	61.54	-----
Soybean meal	21.57	21.72	15.51	23.03	18.09	21.57
Dicalcium phosphate	1	1	1.02	1.01	1.02	1
Vegetable oil	1.65	1.69	-----	7.99	-----	1.65
Tallow	-----	-----	3	-----	8.59	-----
Calcium carbonate	7.9	7.9	7.88	7.89	7.87	7.9
Oyster shell	2	2	2	2	2	2
Salt	0.37	0.37	0.34	0.37	0.34	0.37
DL-methionine	0.1	-----	0.14	0.11	-----	0.1
Lysine HCl	-----	-----	0.22	-----	0.04	-----
Vitamin premix	0.25	0.25	0.25	0.25	0.25*	0.25*
Mineral premix	0.25	0.25	0.25	0.25	0.25	0.25
Enzyme**	-----	-----	0.05	-----	0.05	-----
Calculated analysis						
ME (Kcal/Kg)	2750	2750	2750	3000	3000	2750
CP (%)	14.59	14.59	14.59	14.59	14.59	14.59
Ca (%)	3.89	3.89	3.89	3.89	3.89	3.89
AP (%)	0.29	0.29	0.29	0.29	0.29	0.29
Methionine (%)	0.34	0.24	0.34	0.34	0.2	0.34
Lysine (%)	0.72	0.72	0.72	0.72	0.72	0.72

*Vitamin premix without choline; **Vitazyme x

Table 2: Treatment effects on feed intake and body weight (Mean ± SE)

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Feed intake(g)						
Period 1	104.1±1.2	94.1±1.9	100.4±0.8	96.6±4.1	102.4±6.7	101.5±2.6
Period 2	100.8±3.5	99±2.4	109.2±4.1	92.4±5	101.6±4.4	102.7±5.9
Period 3	100 ± 3.1^{bc}	92.7 ± 5.8^{ab}	108.4 ± 4.3^{c}	85.8 ± 3.5^{a}	89.1 ± 4.7^{ab}	106.7 ± 4.5^{c}
Average	101.6 ± 1.9^{bc}	95.3 ± 3.1^{ab}	106 ± 2.3^{c}	91.6 ± 2.9^{a}	97.7 ± 3.9^{abc}	103.6 ± 1.5^{bc}
Body weight(g)						
Initial	1818±69	1684±43	1692±22	1704±12	1720±46	1649±19
Week 4	1851 ± 67^{c}	1617 ± 28^{a}	1798 ± 33^{bc}	1715 ± 39^{abc}	1727 ± 58^{abc}	1628 ± 66^{ab}
Week 8	1930±64	1652±37	1900±31	1761±42	1873±131	1701±84
Final	1875±106	1644±76	1888±44	1735±57	1704±91	1683±89
Average	1868±75	1649±31	1818±29	1729±32	1756±78	1665±61

a-c Means within a row with no common superscript different significantly ($P<0.05$)

centrifuged and serum collected. Duplicate aliquots from each sample were prepared for enzymes activities analysis. The activities of AST, ALT and LDH were determined with commercial kits. Two birds of each treatment groups were humanly killed on d 28 of the experiment and the livers were carefully removed, weighed, and scored for liver hemorrhage by assigning a score from 0 to 3 with 0 indicating no hemorrhages; 1, up to 10 subcapsular petechial or ecchymotic hemorrhages; 2, more than 10 subcapsular petechial or ecchymotic hemorrhages; and 3, massive liver hemorrhage (Diaz *et al.*, 1999). Liver samples from hens were taken for the determination of dry matter and fat content. The dry matter content was determined by oven-drying a preweighed sample. The lipid cotent was determined by extracting a grouped sample of oven-dried liver in a soxhlet type extractor for 24 hr with petroleum ether with a boiling point of 60-70^{0C} (AOAC, 1984).

The experiment was a completely randomized design, and the experimental unit was the replicate consisting of five adjacently caged birds fed as one group. Data were analyzed using the general linear model procedure of SAS software (1992). Mean values were compared by a multiple range test (Duncan). The level of significance was $p<0.05$.

Results and Discussion

Overall feed intake was significantly ($p<0.05$) lower in the hens fed treatment 4 compared to the control (Table 2). This result was consistent with that of Harms *et al.* (2000), who reported that hens fed a high energy diet consumed less feed than hens fed a control diet. Body weight was lower for all treatment groups throughout the

Table 3: Treatment effects on egg parameters (Mean ± SE)

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Egg production(%) (eggs/h/d)						
Period 1	74.6±3	68.4±2.8	64.7±2.8	71.2±3.4	74±1.4	74.8±0.8
Period 2	67.7±1.6	63.6±3.6	62.6±4.7	65.6±4.6	63.9±4.5	69.8±3.3
Period 3	61.7±3.5	57.9±1.8	61.9±9.1	63.6±3.4	59.3±3.5	64.5±5.8
Average	68±2.4	63.3±2.4	63.1±5.5	66.8±3.4	65.7±2.9	69.7±2.8
Egg weight (gr)						
Period 1	64.2±1.8	58±2.9	66±1.3	64.5±2.1	61.4±1.5	63.4±1
Period 2	65.6±1.6	62.1±2.1	67.1±2.3	64.7±2.5	64.4±1.6	64.8±1.3
Period 3	66.4±1.9^{c}	57.8±1.9^{a}	63.7±1.2^{bc}	67.2±1.1^{c}	58.3±2^{ab}	61.8±2.7^{abc}
Average	65.4±1.3^{c}	59.3±1.4^{a}	65.6±0.9^{c}	65.5±1.2^{c}	61.3±1.4^{ab}	63.3±1.1^{bc}
Shell weigh t(g)						
Period 1	7.57±0.2	8.55±0.63	7.78±0.18	7.79±0.15	7.67±0.3	7.68±0.37
Period 2	8.67±0.3^{c}	7.26±0.19^{a}	8.27±0.27^{bc}	8.06±0.29^{abc}	7.74±0.35^{ab}	7.7±0.16^{ab}
Period 3	7.82±0.25	8.07±0.51	7.22±0.31	7.68±0.21	7.47±0.35	7.9±0.41
Average	8.02±0.13	7.96±0.34	7.76±0.07	7.84±0.17	7.63±0.23	7.76±0.28
Shell thickness (0.01mm)						
Period 1	0.38±0.01	0.38±0.01	0.38±0.02	0.38±0.01	0.38±0.01	0.36±0.01
Period 2	0.47±0.02	0.45±0.02	0.45±0.02	0.44±0.02	0.41±0.01	0.48±0.02
Period 3	0.4±0.01	0.41±0.02	0.42±0.02	0.43±0.02	0.43±0.02	0.41±0.03
Average	0.42±0.01	0.41±0.02	0.42±0.02	0.42±0.02	0.41±0.01	0.42±0.02

a-c Means within a row with no common superscript different significantly (P<0.05)

Table 4: Treatment effects on hepatic parameters (Mean ± SE)

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Total liver weight (g)						
Period 1	39.6±5.3	42.7±4.7	51.3±24.4	42.7±7.7	40.1±0.5	53.4±8.6
Period 2	45.8±5.1	54.3±2.4	57.3±1.1	43±6.5	70.1±31.8	43.1±2.9
Period 3	36.8±8.6	47.2±3.8	74.5±16	45.2±5.2	86.2±28.3	46.8±1.2
Average	40.7±2.8^{a}	48.1±1.1^{a}	61±2.4^{b}	43.5±6.5^{a}	65.5±1.3^{b}	47.8±4.2^{a}
Liver dry matter (%)						
Period 1	62.9±3.5	66.7±5.2	55.5±13.2	68.3±3.1	62.3±2.7	47.9±9.9
Period 2	61.6±1.3	51.5±2.2	59.5±0.3	66.9±4.9	54.3±16.9	63.9±4.3
Period 3	67.3±4.3	54.9±3.6	50.5±4.6	66.1±0.4	58.2±8.6	69.8±4.1
Average	64±0.7	57.7±2.2	55.1±2.8	67.1±2.8	58.2±3.7	60.5±0.5
Liver fat (%)						
Period 1	18.8±4.5	28.4±7.1	19.5±8.8	22.9±2.4	16.3±1.8	10.7±1.7
Period 2	27.5±5.9	19.8±1.8	24.4±3.2	33.3±3.7	19.4±6.5	21.5±2.9
Period 3	27.4±9.5	20.5±2.6	23.3±1.4	26.7±8	25.4±0.8	32.8±11
Average	24.6±6.6	22.9±3.8	22.4±3.5	27.6±0.6	20.4±3	21.7±4.1
Hemorrhage score						
Period 1	0.5±0.5	0.5±0.5	0.5±0.5	0.5±0.5	1±0	1±0
Period 2	0.5±0.5	1±0	0.5±0.5	0±0	2±1	0±0
Period 3	0.5±0.5	0.5±0.5	1.5±0.5	0.5±0.5	2±1	1±1
Average	0.5±0.5	0.67±0	0.84±0.17	0.34±0.34	1.67±0.67	0.67±0.34

a-b Means within a row with no common superscript different significantly (P<0.05)

experiment compared to the control but this difference was not significant (Table 2). Decrease of body weight was expected with due to decrease of feed intake and also induction of insufficient nutrients on the experiment. Body weight is a predisposing factor for affected FLHS in laying hens. Egg production was not significantly different among treatments, suggesting that overall egg production was not affected by treatments (Table 3). In the other words, nutrients deficiency including: methionine and choline can not be causes of large different of egg production among treatments. Overall egg weight was significantly ($p<0.05$) lower for 2th and 5th treatments compared to the control (Table 3). Reducing dietary methionine with or without choline caused decrease of egg weight. This result was in agreement with the previous report that egg weight was

Table 5: Partial correlation coefficients

	Liver fat	Liver dry matter percent	Hemorrhage score	Liver wet weight
Liver wet weight	NS	NS	0.318*	
Hemorrhage score	NS	NS		0.318*
Liver dry matter percent	0.633**		NS	NS
Liver fat		0.633**	NS	NS

NS = not significant; * P<0.05; ** P<0.01

Table 6: Treatment effects on serum enzyme activity (Mean ± SE)

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
AST (IU/L)						
Period 1	82.5±1.5	172.5±58	183±87	114±9	133.5±7	108±6
Period 2	160±60	289±157	197±65	170±0	160±60	354±92
Period 3	388±18	525±175	366.5±41	285±25	425±35	280±0
Average	210.2±26	328.9±130	248.9±21	189.7±5	238.5±34	247.4±28
ALT (IU/L)						
Period 1	78±12	108±36	34.5±19	156±96	144±18	87±27
Period 2	74±4	202±2	120±80	180±0	74±4	122±82
Period 3	170±120	161±4	150±100	104±0	63±7	245±39
Average	107.3±42	157±2	101.5±53	146.7±32	93.7±9	151.3±49
LDH (B-BU/L)						
Period 1	1992±228	2193±177	2370±870	1644±24	1740±12	1737±39
Period 2	$1453±44^{bc}$	$833±3^{ab}$	$1476±86^{bc}$	$1943±31^{c}$	$499±47^{a}$	$1388±80^{bc}$
Period 3	796±208	698±230	357±68	629±237	514±14	674±330
Average	1413.7±4	1241.3±18	1401±296	1405.3±97	917.7±15	1266.3±149

a-c Means within a row with no common superscript different significantly (P<0.05)

significantly affected by methionine and choline (Keshavarz, 2003). Overall eggshell quality was not affected by treatments (Table 3). Overall liver weight was significantly ($p<0.05$) higher for the hens fed 3th and 5th treatments compared to the control (Table 4). Reducing dietary linoleic acid caused increase of liver weight. This is in support with results obtained by Hopkins and Nesheim, (1967). In this experiment linoleic acid deficiency is used as a dietary factor on induction of FLHS that caused increase of liver weight. Liver hemorrhage score (LHS) was not significantly higher for all treatment groups (except treatment 4) compared to the control (Table 4). Liver dry matter and liver percent fat were not affected by treatment groups (Table 4). LHS was positively correlated with liver weight ($p<0.05$) (Table 5).This is in support with result obtained by Schumann *et al*. (2003). Overall serum AST activity was numerically higher for all treatment groups (except treatment 4) compared to the control (Table 6). Normal birds had been reported serum AST activity up to 230 IU/L (Coles, 1986). On this basis, serum AST activity was higher for the hens of all treatment groups compared to the normal birds (control). The results showed that increase of serum AST activity, liver weight and LHS can be indicative of FLHS. Also the use of AST enzyme activity can be helpful for diagnosis of FLHS in laying hens.

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