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Appraisal of Hematological Indices and Humoral Immunity in Commercial Laying Hens Fed Rations Consisting Cottonseed Meal and Sodium Bentonite

Research Article

A. Gilani[1*], H. Kermanshahi[1], A. Golian[1], A. Tahmasbi[1] and M. Aami Azghadi[1]

[1] Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

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*Correspondence E-mail: gilani.ali@stu-um.ac.ir

ABSTRACT

The objective of this study was to evaluate the effects of cottonseed meal (CSM) treated with sodium bentonite (SB) on certain blood components and immune response of laying hens. A 3 × 3 factorial arrangement with 3 levels of SB (0, 10, and 20 g/kg of diet) and 3 levels of CSM (0, 100 and 200 g/kg of diet) were used within 9 dietary treatments of 4 replicates each. Nine mash diets were fed to 288 commercial Hy-Line W-36 hens from 51-63 weeks of age. Blood samples were collected at the end of trial to evaluate the blood constituents. The SB, CSM, or their interaction (SB×CSM) did not have any adverse effect on blood cells and activity of serum enzymes. Sheep red blood cells (SRBC) were used as antigen to quantify the antibody response. Two birds per replicate were injected with SRBC at 60 week of age. After 7 and 14 d of SRBC inoculation, blood samples were obtained from the brachial vein of each hen, and total anti-SRBC, IgG and IgM titers were determined. A significant difference of SB, CSM or their interaction was not observed for total antibody response against SRBC inoculation; however, IgG was significantly increased with 20% CSM at 7 d (7.62 vs. 8.45) and with 2% SB at 14 d (2.79 vs. 3.66) after injection of SRBC. The interaction of SB and CSM for IgG was significantly different among dietary treatments and diet with 2% SB and 20% CSM had the highest titer (5 vs. 2.5) at 14 d after injection of SRBC. In conclusion, SB, CSM or their interactions did not affect blood constituents but significantly changed immune response of the birds.

KEY WORDS blood components, cottonseed meal, humoral immunity, laying hens, sodium bentonite.

INTRODUCTION

Gossypol ($C_{30}H_{30}O_8$) is a polyphenolic pigment that is found in all species of the cotton genus, *Gossypium*. Free gossypol (FG) is the major obstacle in cottonseed meal (CSM) that has limited the utilization of CSM as a protein source for poultry. The absorbed FG from the alimentary tract combines with the proteins and iron in internal organs which inhibits the synthesis of hemoglobin and respiratory enzymes (Ferguson *et al.* 1959; Skutches *et al.* 1973). Danke and Tilman (1966) reported that FG can have a hemolytic effect on the erythrocytes of rats. Likewise, Jalees *et al.* (2011) revealed that hematocrit levels significantly decreased at 42 day of age when 42% CSM was included in Japanese quail diet. Also, Atuaheme *at al.* (1986) reported that hematocrit and number of leukocytes have been reduced in broilers fed 10% CSM. In addition, Mahmood (2006) indicated that feeding broiler breeders with 20 or 30% CSM significantly reduced total numbers of red blood cells (RBC) and white blood cells (WBC). Furthermore, González Garza *et al.* (1986) reported that LDH (lactate dehydrogenase) activity in liver, heart, and testis of male rats fed diets containing CSM was reduced, possibly due to gossypol. Subsequently, impaired nutrition such as feeding of CSM is associated with reduced capacity of bird to form specific antibodies (Mandal *et al.* 2001).

Sodium bentonite (SB) is an aluminum silicate powder that can adsorb different compounds into its three layer structure (Trckova *et al.* 2004). It has been reported that dietary SB may reduce aflatoxicosis (Phillips *et al.* 1988; Kubena *et al.* 1993; Miazzo *et al.* 2005; Kermanshahi *et al.* 2009). Salari *et al.* (2006) demonstrated that 10 to 20 g SB per kg of the diet can be used as a pellet binder with no adverse effect on broiler performance. Hepatic enzymes along with blood components have been evaluated as health indices in various studies (Emadi and Kermanshahi, 2007a; Emadi and Kermanshahi, 2007b; Akbarian *et al.* 2012). For example, Kermanshahi *et al.* (2009) added SB and Aflatoxin B_1 (AFB_1) into broiler's diet. They found that AFB_1 alone into the diets significantly increased the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and LDH in serum. However, 0.5-1.0% SB alone or in combination with AFB_1 had no effect on the above mentioned enzymes. Among the many properties of SB, Hashemipour *et al.* (2010) indicated that SB in diets containing artificial pigments significantly decreased yolk color scale. Thus, it has been hypothesized that incorporation of SB into diets containing CSM might modify the deleterious effects of FG. Therefore, this research was designed to examine the effects of CSM and SB on alteration of blood components and immune response in commercial laying hens.

MATERIALS AND METHODS

Nine mash diets in a 3 × 3 factorial arrangement and in a completely randomized design with 4 replicates of 8 birds each were fed to 288 Hy-Line W-36 laying hens with uniform body weight from 51-63 wk of age. Nine isocaloric and isonitrogenous diets comprised 3 levels of SB (0, 10 and 20 g/kg of diet) and 3 levels of CSM (0, 100 and 200 g/kg of diet). The diets were formulated to meet or exceed the nutrient requirements of laying hens as recommended. The hens were acclimatized to the experimental diets one week prior to the beginning of the experiment. Feed and water were provided *ad libitum* throughout the experiment. The CSM had a FG content of 0.071% which provided the amount of 0, 71 and 142 mg FG per kg of diet when CSM was used at the level of 0, 100 and 200 g/kg of diet, respectively (FG was determined based on ISIRI, (1997). Dry matter, ash, crude protein, and ether extract of all diets were analyzed according to the standard procedures of AOAC (1996). The composition of experimental diets is shown in Table 1. Water was provided to hens by automatic drinker with one nipple per cage with 4 hens each and the diets were provided via feed trough. Cage dimensions were 45 × 45 cm, so that each hen had 506.25 cm^2 floor space. Hens were exposed to a daily lighting schedule of 16L:8D. Blood samples were collected with heparinized syringe from the brachial vein of one bird per replicate (4 replicates per treatment) to measure blood cells at the end of the experiment. Also, another blood sample for every replicate was achieved to measure the enzyme activity of ALT, AST, and LDH in serum.

Blood chemicals were analyzed using standard kits with chemistry analyzer (Bayer RA1000, Germany). Sheep red blood cells (SRBC) were used as antigen to quantify the antibody response. Eight birds per treatment were injected with SRBC (one mL of 3% suspension in phosphate-buffered saline in the breast muscle of each hen) at 60 weeks of age. At 7 and 14 days after injection, blood samples were obtained from the brachial vein of each hen, and total anti-SRBC, IgG and IgM titers were determined as previously described by Qureshi and Havenstein (1994) and Cheema *et al.* (2003). The data were analyzed using the GLM procedure of SAS 9.1 (SAS, 2004). Tukey's Studentized Range (HSD) test was used to compare the means (P<0.05). The model for a factorial experiment with two factors A (SB) and B (CSM) is:

$$Y_{ijk}= \mu + A_i + B_j + (AB)_{ij} + e_{ijk}$$

Where:

i: 1,…,a; j = 1,…,b; k = 1,…,n.

y_{ijk}: observation k in level i of A and level j of B.

μ: the overall mean.

A_i: the effect of level i of A.

B_j: the effect of level j of B.

$(AB)_{ij}$: the interaction effect of level i of factor A with level j of factor B.

e_{ijk}: random error.

A: number of levels of factor A.

b: number of levels of factor B.

n: number of observations for each A × B combination.

RESULTS AND DISCUSSION

The effects of SB, CSM, and their interaction on blood constituents are shown in Tables 2 and 3. There was no significant effect of SB, CSM, or their interaction on blood constituents and enzyme activity of AST, ALT, and LDH. In this trial, CSM did not affect hematocrit value of laying hens. Contrary, Atuaheme *at al.* (1986) demonstrated that hematocrit and number of leukocytes have been declined in broilers fed 10% CSM.

Table 1 Composition of nine diets containing three levels of sodium bentonite (SB, g/kg of diet) and three levels of cottonseed meal ((CSM, g/kg of diet)) unless otherwise stated)

SB	0			10			20		
CSM	0	100	200	0	100	200	0	100	200
Ingredients (g/kg of diet, as-fed basis)									
Corn grain	584.3	548.5	513	585.5	550	514.5	587	551.5	516.1
Soybean meal (44%)	229.6	161.1	92.7	229.3	160.8	92.4	229	160.8	92.1
Cottonseed meal (33%)	0	100	200	0	100	200	0	100	200
Bone meal	22.2	21	19.9	22.2	21	19.9	22.2	21	19.9
Limestone	91.1	92.2	93.2	90.8	91.9	93	90.6	91.6	92.7
Vitamin and mineral premix[1]	5	5	5	5	5	5	5	5	5
Common salt	3.8	3.7	3.7	3.4	3.3	3.3	3	2.9	2.8
Tallow	42.7	46.6	50.5	42.1	46	50	41.5	45.5	49.4
DL-Methionine	1.7	1.5	1.4	1.7	1.5	1.4	1.7	1.5	1.4
L-lysine HCl	0.1	0.3	0.5	0.1	0.3	0.5	0.1	0.3	0.6
Sodium bentonite	0	0	0	10	10	10	20	20	20
Fine sand	20	20	20	10	10	10	0	0	0
Calculated composition									
ME (kcal/kg)	2816	2816	2816	2816	2816	2816	2816	2816	2816
Crude protein	152.5	152.5	152.5	152.5	152.5	152.5	152.5	152.5	152.5
Crude fiber	29.5	40.9	52.3	29.5	40.9	52.3	29.4	40.9	52.3
Calcium	42.5	42.5	42.5	42.5	42.5	42.5	42.5	42.5	42.5
Available Phosphorus	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2
Linoleic acid	26.6	29.2	31.8	26.5	29.1	31.7	26.4	29	31.6
Sodium	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
Lysine	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8
Methionine + Cystine	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7
Free Gossypol (mg/kg of diet)	0	71	142	0	71	142	0	71	142
Analysed composition									
Dry matter	936.8	935.5	940.9	937.4	938.1	941.6	935.1	934.7	936.8
Ash	190.4	188.1	211.4	172.9	192.9	165.4	178.9	185.8	161.4
Crude protein	149.4	149.8	146.3	155.8	150.4	148.9	149.6	142.8	150.3
Ether extract	61.8	67.8	75.6	60.5	71	82.8	56.8	77.5	81.7

[1] Vitamin and mineral premix supplied per kilogram of diet: vitamin A: 8800 IU; vitamin D3: 2500 IU; vitamin E: 11 IU; vitamin K3: 2.2 mg; Thiamin: 1.5 mg; Riboflavin: 4 mg; Pantothenic acid: 8 mg; Niacin: 35 mg; vitamin B6: 2.5 mg; Folic acid: 0.5 mg; vitamin B12: 0.01 mg; Biotin: 0.15 mg; Betain: 190 mg; Choline chloride: 50 mg; Mn: 75 mg; Zn: 65 mg; Fe: 75 mg; Cu: 6 mg; I: 0.9 mg and Se: 0.2 mg.

Also, Mahmood (2006) showed that feeding broiler breeders with 20 or 30% CSM significantly decreased total numbers of leukocytes and erythrocytes. The maximum FG concentration in the diets of this study was 142 mg/kg of diet and remarkably lower than those already studied. For instance, Lordelo *et al.* (2005) reported that broilers fed 400 mg gossypol per kg of diet had lower hematocrit than birds fed the control diet.

Nevertheless, Lordelo *et al.* (2007) indicated that the hematocrit value of laying and broiler breeder hens was not influenced by the same amount of gossypol. These results might indicate that young chickens are more susceptible to gossypol.

These findings coincide with the observations of Henry *et al.* (2001) which demonstrated no difference in the erythrocyte and leukocyte count in broiler chickens fed 20% CSM and control group. Interestingly, Jalees *et al.* (2011) reported that CSM at the levels of 13 and 27% did not affect on hematocrit, erythrocyte as well as leukocyte counts of Japanese quail.

However, they found that hematocrit levels significantly diminished at 42 d of age when 42% CSM was incorporated in the quail's diet. They hypothesized that hypoproteinemia and hypoalbuminemia at the high level CSM fed quail might be due to reduced synthesis of albumin in the degenerated hepatocytes. As compared with these results about SB, Safaeikatouli *et al.* (2010) indicated that broilers given diet containing 1.5% SB had significantly decreased AST and glucose as compared with control. Levels of globulin, urea, creatinine, cholesterol, triglyceride, high density lipoprotein, low density lipoprotein, very low density lipoprotein, alkaline phosphatase, creatine kinase, glutamyltransferase, and LDH did not significantly affect by SB. The effects of SB, CSM, and their interaction on total anti-SRBC, IgG and IgM titers are shown in Table 4. There was no significant impact of SB, CSM, or their interaction for total antibody response to injection of SRBC; however, IgG was increased in birds fed diets with 200 g CSM/kg of dietand with 20 g SB/kg of diet, at 7 and 14 d post injection of SRBC, respectively.

Table 2 The effects of dietary sodium bentonite (SB) and cottonseed meal (CSM) on hematocrit (HT), hemoglobin (HG), white blood cells (WBC), lymphocyte, heterophil, monocyte and eosinophil of laying hens at the end of the experiment

Item	HT (%)	HG (g/dL)	WBC (n/μL)[1]	Lymphocyte (WBC %)	Heterophil (WBC %)	Heterophil to lymphocyte ratio	Monocyte (WBC %)	Eosinophil (WBC %)
SB (g/kg of diet)								
0	27.75	8.65	18583	58.50	35.75	0.61	5.50	0.25
10	27.83	8.81	18458	58.41	36.66	0.61	5.58	0.33
20	27.58	8.70	18000	58.16	35.58	0.61	5.25	0.41
SEM	0.299	0.117	309.7	0.373	0.340	0.008	0.291	0.146
CSM (g/kg of diet)								
0	27.91	8.93	18250	58.75	35.33	0.60	5.33	0.33
100	27.63	8.58	18750	58.00	35.75	0.61	5.83	0.41
200	27.58	8.65	18041	58.33	35.91	0.61	5.16	0.25
SEM	0.299	0.117	309.7	0.373	0.340	0.008	0.291	0.146
Interactions (g/kg of diet)								
0 SB × 0 CSM	28.25	9.02	19000	58.75	35.50	0.60	5.50	0.25
0 SB × 100 CSM	27.50	8.32	19250	58.50	35.25	0.60	5.75	0.50
0 SB × 200 CSM	27.50	8.60	17500	58.25	36.50	0.62	5.25	0.23
10 SB × 0 CSM	27.50	8.78	18000	59.00	35.00	0.59	5.75	0.25
10 SB × 100 CSM	28.25	9.00	19000	57.50	36.50	0.63	5.75	0.25
10 SB × 200 CSM	27.75	8.70	18375	58.75	35.50	0.60	5.25	0.50
20 SB × 0 CSM	28.00	9.02	17750	58.50	35.50	0.60	5.75	0.50
20 SB × 100 CSM	27.25	8.42	18000	58.00	35.50	0.61	6.00	0.50
20 SB × 200 CSM	27.50	8.65	18250	58.00	35.75	0.61	5.00	0.25
SEM	0.581	0.302	536.5	0.627	0.589	0.015	0.504	0.184
Source of variation				P-values				
SB	0.83	0.59	0.39	0.80	0.94	0.99	0.70	0.72
CSM	0.71	0.10	0.29	0.37	0.46	0.38	0.25	0.72
SB × CSM	0.58	0.18	0.25	0.77	0.37	0.42	0.78	0.63

[1] Number of white blood cells per microliter.
SEM: standard error of the means.

Table 3 The effects of dietary sodium bentonite (SB) and cottonseed meal (CSM) on alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) activity of laying hens at the end of the experiment

Item	AST (IU/L)	ALT (IU/L)	LDH (IU/L)
SB (g/kg of diet)			
0	198.46	24.93	2210.00
10	163.08	28.08	2304.50
20	173.62	23.66	2276.08
±SEM	15.440	4.981	82.860
CSM (g/kg of diet)			
0	190.29	28.70	2241.00
100	179.58	28.97	2341.75
200	165.29	19.00	2207.83
±SEM	13.690	4.360	73.184
Interactions (g/kg of diet)			
0 SB × 0 CSM	189.00	33.25	2195.75
0 SB × 100 CSM	216.25	32.12	2345.63
0 SB × 200 CSM	190.13	21.00	2088.63
10 SB × 0 CSM	183.38	37.50	2215.00
10 SB × 100 CSM	161.25	36.70	2344.88
10 SB × 200 CSM	144.62	28.25	2353.63
20 SB × 0 CSM	198.50	38.25	2312.25
20 SB × 100 CSM	161.25	30.37	2334.75
20 SB × 200 CSM	161.12	33.37	2181.25
±SEM	23.597	9.501	126.073
Source of variation		P-values	
SB	0.26	0.79	0.70
CSM	0.39	0.15	0.33
SB × CSM	0.62	0.48	0.75

SEM: standard error of the means.

Table 4 The effects of dietary sodium bentonite (SB) and cottonseed meal (CSM) on immune response of laying hens, 7 and 14 days after injection of sheep red blood cell (SRBC) at 60 weeks of age

	7 days after injection of SRBC[1]			14 days after injection of SRBC[1]		
Item	Total anti-SRBC antibodies	IgG	IgM	Total anti-SRBC antibodies	IgG	IgM
SB (g/kg of diet)						
0	8.37	8.16	0.20	4.25	2.79[b]	1.45
10	7.96	7.58	0.37	3.70	2.91[b]	0.79
20	7.87	7.54	0.33	4.04	3.66[a]	0.37
SEM	0.395	0.333	0.201	0.408	0.242	0.361
CSM (g/kg of diet)						
0	8.08	7.62[b]	0.45	3.53	2.82	0.70
100	7.54	7.20[b]	0.33	3.89	3.13	0.82
200	8.58	8.45[a]	0.12	4.27	3.41	0.85
SEM	0.395	0.333	0.201	0.408	0.242	0.36
Interactions (g/kg of diet)						
0 SB × 0 CSM	7.75	7.37	0.37	3.37	2.5[b]	0.87
0 SB × 100 CSM	8.87	8.75	0.12	4.87	3.25[b]	1.62
0 SB × 200 CSM	8.5	8.37	0.12	4.5	2.62[b]	1.87
10 SB × 0 CSM	8.37	7.87	0.50	4.37	3.5[ab]	0.87
10 SB × 100 CSM	7.12	6.62	0.50	3.12	2.62[b]	0.76
10 SB × 200 CSM	8.37	8.25	0.12	3.63	2.62[b]	1.01
20 SB × 0 CSM	8.12	7.62	0.50	3.75	3.37[ab]	0.37
20 SB × 100 CSM	6.62	6.25	0.37	3.38	2.63[ab]	0.75
20 SB × 200 CSM	8.87	8.75	0.12	5.00	5.00[a]	0.74
SEM	0.685	0.577	0.348	0.708	0.419	0.626
Source of variation	P-values					
SB	0.92	0.83	0.05	0.32	< 0.001	0.41
CSM	0.08	0.006	0.87	0.30	0.57	0.55
SB × CSM	0.20	0.07	0.99	0.18	0.01	0.70

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

In previous study, Tang *et al.* (2012) demonstrated that broilers fed 4% and 8% fermented cottonseed meal had a significant increase in IgM at 21 d of age compared with the birds fed soybean meal. A significant higher IgM and IgG was also observed in birds fed 8% fermented cottonseed meal compared with the control from 22 to 42 d. The ameliorative effect of graded levels of dietary SB (0.2, 0.4 and 0.6 percent of feed) on *in vitro* impaired phagocytosis and suppressed immune response to Newcastle disease vaccine during aflatoxicosis in broiler chicks was investigated by Ibrahim *et al.* (2000).

The presence of SB in the diet depressed the immune response of chicks as measured by haemagglutination inhibition test. Sodium bentonite was also effective in ameliorating the suppressive effect on the titre in chicks vaccinated against Newcastle disease. The best results obtained when SB was added at the rate of 0.4 percent of feed to the AF-containing diets. Also, in the study of Hasan *et al.* (2010) the addition of SB was effective in ameliorating the negative effect of AF on antibody production against Newcastle disease. This effect could be attributed to the role of SB as a sequestering agent against AF present in the diet through reducing its bioavailability in the gastrointestinal tract (Araba and Wyatt, 1991).

The toxic symptoms of gossypol vary depending on strain and age of bird and differences in protein quality and quantity in ration and various mineral contents in the diet (Nagalakshmi *et al.* 2007).
Moreover, Saqib *et al.* (2012) concluded that alteration in leukocytes varies from one experiment to another one indicating that change is absolute, but varies from one group to another.

From the view point of performance in this trial, diet containing 2% SB and 20% CSM as compared with the control group significantly decreased hen-day egg production (73 vs. 79%) and daily egg mass (47 vs. 52 g). These findings concur with the results of Koenen *et al.* (2002) who found that there is a negative correlation between body weight gain and antibody response (total antibody response and specific anti-SRBC) in broilers.

CONCLUSION

Under the conditions of this experiment, high stimulation of humoral immune response and the reduction of performance by 200 g CSM/kg of diet and 20 g SB/kg of diet may indicate a negative correlation between immune response and performance criteria, especially egg production.

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