

## IMPACT OF SODIUM BENTONITE ADDITION TO THE DIETS CONTAINING COTTONSEED MEAL ON PRODUCTIVE TRAITS OF HY-LINE W-36 HENS

A. Gilani, H. Kermanshahi, A. Golian and A. Tahmasbi

Animal Science Department, Faculty of Agriculture, Ferdowsi University of Mashhad, P. O. Box: 91775-1163, Mashhad, Iran  
(Correspondence Author e-mails: gilani.ali@stu-um.ac.ir, gilani@poultry@gmail.com)

### ABSTRACT

This study was conducted to evaluate the effects of cottonseed meal (CSM) treated with sodium bentonite (SB) on the performance of Hy-Line W-36 hens. A 3×3 factorial arrangement with 3 levels of SB (0, 10, and 20 g/kg) and 3 levels of CSM (0, 100, and 200 g/kg) was used with 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. Non-significant effect of SB, CSM, or their interaction were noted on egg specific gravity, shell weight percentage, shell thickness, or the percentage of soft-shelled, cracked, or broken eggs. Feeding a diet with a high level of CSM (200 g/kg) resulted in significant ( $P<0.05$ ) reduction in hen-day egg production, egg weight, and daily egg mass. Hens fed 200 g CSM /kg had the most feed consumption, the worst feed conversion ratio, and the least hen-day egg production and daily egg mass. Hen-day egg production and daily egg mass were improved ( $P<0.05$ ) in hens fed diet containing 10 g/kg SB. In conclusion, feeding the hens with 10 g SB and 100 g CSM per kg of diet resulted in the best productive characteristics with no detrimental implication.

**Key words:** cottonseed meal, Hy-Line W-36 hens, performance, sodium bentonite.

### INTRODUCTION

Gossypol ( $C_{30}H_{30}O_8$ ), a polyphenolic pigment, is present in all species of the cotton genus, *Gossypium*. Free gossypol (FG) is the main antinutritional factor in cottonseed meal (CSM) that caused limited utilization of CSM as a plant protein source in poultry diets. Feeding FG to hens may lead to drop in egg production (Panigrahi *et al.*, 1989) or eggs with olive or brown yolk discoloration after cold storage (Davis *et al.*, 2002; Lordelo *et al.*, 2007). The FG in diets of chicken also resulted in poor growth rate (El Boushy and Raterink, 1989; Lordelo *et al.*, 2005).

Supplementation of diet with iron slats to counteract FG and to eliminate its adverse effects on bird's performance proved beneficial (Panigrahi *et al.*, 1989; Panigrahi and Morris, 1991). However, Panigrahi and Plumb (1996) indicated that treatment of CSM with crystalline ferrous sulphate heptahydrate (FSH) reduced the phosphorus bioavailability and hen performance. Excess iron can also interfere with the absorption of phosphorous (Mc Donald *et al.*, 1981), manganese (Matrone *at al.*, 1959), and copper (Hill and Matrone, 1961). In addition, ferrous sulphate may cause feed to turn black (Zhang *et al.*, 2007). Thereby, other kinds of treatments have been evaluated to identify simple and inexpensive procedures for gossypol detoxification in poultry diets.

Sodium bentonite (SB) is an aluminum silicate powder that can adsorb different compounds into its three layer structure (Trckova *et al.*, 2004). This cost effective additive has a variety of applications in poultry industry.

It has been reported that SB in the diet may ameliorate aflatoxicosis (Phillips *et al.*, 1988; Kubena *et al.*, 1993; Miazzo *et al.*, 2005; Kermanshahi *et al.* 2009). Furthermore, 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. Among the many properties, the SB has strong affinity for pure carotene (Erwin *et al.*, 1957). It has been suggested that SB is not specific for carotene and apparently binds other non-carotenoid pigments as well. Hashemipour *et al.* (2010) indicated that SB in diets containing artificial pigments significantly decreased yolk colour scale. Therefore, it was postulated that incorporation of SB into diets containing CSM might relieve the deleterious effects of FG as a pigment. Thus, this research was designed to explore the interactive effects of SB and FG of CSM on the performance of laying hens.

### MATERIALS AND METHODS

**Diets and birds' housing:** Nine mash diets in 3×3 factorial arrangement with 4 replicates of 8 birds each were fed to 288 Hy-Line W-36 hens of uniform body weight from 51 week of age for 12 weeks. These isocaloric and isonitrogenous diets comprised 3 levels of SB (0, 10, and 20 g/kg) and 3 levels of CSM (0, 100, and 200 g/kg). These diets were formulated to meet or exceed the nutrient requirements of laying hens as recommended by Hy-Line W-36 management guide (2007). 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. Dry

matter, ash, crude protein, and ether extract of all diets were analysed (AOAC, 1996). The composition of experimental diets is shown in Table 1.

Water was provided to the hens by one nipple drinker per cage with 4 hens each. The diets were offered through feed trough. Cage dimension was 45×45 cm, to provide 506.25 cm<sup>2</sup> floor space per hen. Daily lighting schedule of 16L:8D was provided. The experimental protocols were approved by the Animal Care Committee of Ferdowsi University of Mashhad, Iran.

#### **Determination of laying hen performance and fresh egg quality:**

Egg production, soft-shelled eggs, cracked or broken eggs, and mortality were recorded daily. Feed consumption was measured during each period of 28 days. Body weight of hens in each replicate was determined at the beginning and at the end of the trial. Four eggs were randomly collected from each replicate and weighed. The egg specific gravity (SG) was determined by the floatation method (Holder and Bradford 1979) every other week. The volume of eggs was calculated by using the formula described by Etches (1996) as; egg volume (cm<sup>3</sup>) = 0.913 egg weight (g). After that, each egg was broken and yolk colour was scored based on Roche Yolk Colour Fan (Vuilleumier, 1969). Eggshells were washed, air-dried, and weighed. Eggshell thickness was measured at the equator of the egg with attached shell membranes using a digital micrometer (model DSWQO-100, Made in China).

**Statistical analyses:** The data were analysed using the GLM procedure of SAS 9.1 (SAS Institute, Inc., 2004). Tukey's Studentized Range (HSD) test was used to compare means (P<0.05).

## **RESULTS AND DISCUSSION**

The effects of SB, CSM, and their interaction on hen-day egg production, daily egg mass, daily feed intake, FCR (g feed per g egg), percentage of soft-shelled, cracked or broken eggs, body weight change, and mortality are given in Table 2. Hen-day egg production and daily egg mass were significantly (P<0.05) decreased while daily feed intake and FCR were significantly (P<0.01) increased when 200 g CSM/kg diet was used. The SB at the level of 20 g/kg significantly (P<0.01) decreased hen-day egg production, but it had no adverse effect on daily egg mass. There was non-significant effect of SB, CSM, or their interactions on percentage of soft-shelled eggs, and cracked or broken eggs. Hens that were nourished with a diet containing 200 g/kg CSM lost their weight during the trial.

The effects of SB, CSM, and their interaction on weight, volume, specific gravity (SG), shell thickness, shell weight percentage, and the Roche Yolk Colour Fan scale of the eggs are shown in Table 3. The non-

significant effects of SB, CSM, or their interaction were noted on SG, shell weight percentage, and shell thickness. Weight and volume of egg were significantly (P<0.05) diminished and the Roche Yolk Colour Fan scale was significantly (P<0.01) increased as the inclusion level of CSM in the diet raised. Conversely, SB significantly (P<0.01) decreased the yolk colour scale.

High inclusion rate of CSM in layer ration for a long time resulted in poor performance. These results are in agreement with Panigrahi *et al.* (1989) who reported CSM could be included in layer diets up to 75 g/kg, but at 150 g / kg diet (130 mg FG per kg CSM), the egg production was depressed towards the end of 10 week feeding period.

Increase in feed intake due to high inclusion rate of CSM is inconsistent with the results of Watkins and Waldroup (1995) who indicated that CSM inclusion up to 200 g/kg diet did not affect feed consumption. However, feed intake significantly decreased at CSM inclusion up to 300 g. This difference can be related to distinct genotypes of the birds. Likewise, Panigrahi and Morris (1991) observed the pronounced effects of genotype on feed intake and egg production of hens fed diets containing CSM and iron treated CSM. Davis *et al.* (2002) also indicated that some Hy-Line W-36 hens were resistant to gossypol and did not show yolk discoloration. Augmentation of feed consumption in hens fed rations containing 200 g CSM/kg might be due to higher crude fiber (CF) of the CSM (170 g CF/kg CSM in comparison with 70 g CF/kg soybean meal in this study). Higher amounts of cell wall constituents cause faster passage rate of digesta (Nagalakshmi *et al.*, 2007) and subsequently can result in more feed intake.

The SB at the level of 10 g/kg of diet raised the hen-day egg production and daily egg mass. This effect might be related to the reduction of feed transit time through the alimentary tract (Kurnick and Reid, 1960). Nevertheless, reduced egg production and daily egg mass with 20 g SB/kg of the diet maybe due to non-specific selective characteristics of aluminosilicates (zeolite and bentonite) as previously described for adsorption of nutrients such as vitamins (Briggs and Spivey, 1956) and pigments (Hashemipour *et al.*, 2010; Kermanshahi *et al.*, 2011). Fethiere *et al.* (1990) indicated that egg weight decreased when 0.75% synthetic sodium aluminosilicate was supplemented to the diet of Leghorn-type laying hens. Nevertheless, Miles and Henry (2007) demonstrated that supplementing the diet with 1 or 2% IMTX (Improved Milbond-TX as a hydrated sodium calcium aluminosilicate) for Hy-Line W-36 laying hens had no detrimental effects on egg weight, eggshell weight, albumen quality, feed consumption, or FCR. Also, Hashemipour *et al.* (2010) reported that hen-day egg production, egg weight, shell thickness, and shell weight percentage were not influenced by the dietary SB.

**Table1. Ingredient and nutrient composition of experimental diets containing three levels of sodium bentonite (SB) and three levels of cottonseed meal (CSM) (g/kg unless otherwise stated)**

| SB<br>CSM                          | 0     |       |       | 10    |       |       | 20    |       |       |
|------------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|                                    | 0     | 100   | 200   | 0     | 100   | 200   | 0     | 100   | 200   |
| <b>Ingredients (as-fed basis)</b>  |       |       |       |       |       |       |       |       |       |
| Corn grain                         | 584.3 | 548.5 | 513   | 585.5 | 550   | 514.5 | 587   | 551.5 | 516.1 |
| Soybean meal (CP 44%)              | 229.6 | 161.1 | 92.7  | 229.3 | 160.8 | 92.4  | 229   | 160.8 | 92.1  |
| Cottonseed meal (CP 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  |
| Vit. and Min. premix <sup>1</sup>  | 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     |
| <b>Calculated composition</b>      |       |       |       |       |       |       |       |       |       |
| 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) <sup>2</sup> | 0     | 71    | 142   | 0     | 71    | 142   | 0     | 71    | 142   |
| <b>Analysed composition</b>        |       |       |       |       |       |       |       |       |       |
| 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  |

<sup>1</sup> Vitamin and mineral premix supplied per kilogram of diet: vitamin A, 8800 IU; vitamin D<sub>3</sub>, 2500 IU; vitamin E, 11 IU; vitamin K<sub>3</sub>, 2.2 mg; thiamin, 1.5 mg; riboflavin, 4 mg; pantothenic acid, 8 mg; niacin, 35 mg; vitamin B<sub>6</sub>, 2.5 mg; folic acid, 0.5 mg; vitamin B<sub>12</sub>, 0.01 mg; biotin, 0.15 mg; betaine, 190 mg; choline chloride, 50 mg; Mn, 75 mg; Zn, 65 mg; Fe, 75 mg; Cu, 6 mg; I, 0.9 mg; Se, 0.2 mg.

<sup>2</sup> The cottonseed meal (CSM) had a free gossypol (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, respectively (FG of CSM was determined based on ISIRI, 1997).

**Table 2. The effects of dietary sodium bentonite (SB) and cottonseed meal (CSM) on overall percentage of hen-day egg production, daily egg mass, daily feed intake, feed conversion ratio (FCR), percentage of soft-shelled and cracked or broken eggs, and body weight change**

| Item                       | Hen day egg production (%) | Daily egg mass (g /hen) | Daily feed intake (g/hen) | FCR (g feed per g egg) | Soft-shelled eggs (%) | Cracked or broken eggs (%) | Body weight change (g) |
|----------------------------|----------------------------|-------------------------|---------------------------|------------------------|-----------------------|----------------------------|------------------------|
| <b>SB (g/kg)</b>           |                            |                         |                           |                        |                       |                            |                        |
| 0                          | 80.52 <sup>ab</sup>        | 51.82 <sup>ab</sup>     | 115.47                    | 2.24                   | 0.73                  | 1.77                       | 11.93                  |
| 10                         | 82.99 <sup>a</sup>         | 53.24 <sup>a</sup>      | 115.68                    | 2.19                   | 0.69                  | 1.61                       | 45.98                  |
| 20                         | 77.80 <sup>b</sup>         | 50.39 <sup>b</sup>      | 115.62                    | 2.31                   | 1.56                  | 1.30                       | 26.95                  |
| ±SEM                       | 1.467                      | 0.590                   | 1.421                     | 0.040                  | 0.463                 | 0.470                      | 13.896                 |
| <b>CSM (g/kg)</b>          |                            |                         |                           |                        |                       |                            |                        |
| 0                          | 81.72 <sup>a</sup>         | 53.14 <sup>a</sup>      | 112.78 <sup>b</sup>       | 2.13 <sup>b</sup>      | 1.18                  | 1.32                       | 62.67 <sup>a</sup>     |
| 100                        | 82.27 <sup>a</sup>         | 53.31 <sup>a</sup>      | 114.23 <sup>b</sup>       | 2.15 <sup>b</sup>      | 0.26                  | 1.75                       | 57.27 <sup>a</sup>     |
| 200                        | 77.33 <sup>b</sup>         | 48.99 <sup>b</sup>      | 119.77 <sup>a</sup>       | 2.46 <sup>a</sup>      | 1.56                  | 1.62                       | -35.07 <sup>b</sup>    |
| ±SEM                       | 1.467                      | 0.579                   | 1.421                     | 0.040                  | 0.463                 | 0.470                      | 13.896                 |
| <b>Interactions (g/kg)</b> |                            |                         |                           |                        |                       |                            |                        |

|                     |                    |         |        |         |       |       |         |
|---------------------|--------------------|---------|--------|---------|-------|-------|---------|
| 0 SB × 0 CSM        | 79.45              | 54.84   | 116.61 | 2.15    | 0.56  | 1.10  | 36.26   |
| 0 SB × 100 CSM      | 81.91              | 53.88   | 117.27 | 2.17    | 0.00  | 1.05  | 47.81   |
| 0 SB × 200 CSM      | 80.20              | 51.17   | 122.09 | 2.40    | 0.00  | 2.38  | -48.28  |
| 10 SB × 0 CSM       | 84.84              | 54.74   | 115.31 | 2.08    | 0.00  | 1.99  | 56.60   |
| 10 SB × 100 CSM     | 85.67              | 55.54   | 117.23 | 2.05    | 0.52  | 1.46  | 75.00   |
| 10 SB × 200 CSM     | 78.45              | 49.15   | 120.75 | 2.45    | 1.06  | 1.21  | 6.36    |
| 20 SB × 0 CSM       | 80.87              | 52.40   | 115.84 | 2.17    | 0.62  | 1.73  | 59.15   |
| 20 SB × 100 CSM     | 79.21              | 50.43   | 115.22 | 2.23    | 0.00  | 2.38  | 49.00   |
| 20 SB × 200 CSM     | 73.33              | 48.62   | 118.82 | 2.53    | 1.05  | 1.40  | -63.30  |
| ±SEM                | 2.541              | 2.165   | 3.902  | 0.069   | 0.571 | 1.342 | 24.069  |
| Source of variation | -----P-values----- |         |        |         |       |       |         |
| SB                  | 0.048              | 0.010   | 0.994  | 0.118   | 0.338 | 0.778 | 0.239   |
| CSM                 | 0.047              | <0.0001 | 0.004  | <0.0001 | 0.145 | 0.803 | <0.0001 |
| SB×CSM              | 0.051              | 0.171   | 0.913  | 0.765   | 0.153 | 0.509 | 0.266   |

<sup>a, b</sup> Means with different superscript for every effect in each column are significantly different (P<0.05).

**Table 3.**The effects of dietary sodium bentonite (SB) and cottonseed meal (CSM) on overall egg weight, egg volume, egg specific gravity (SG), shell thickness, shell weight percentage and Roche yolk colour scale of fresh egg

| Item                | Egg weight (g)      | Egg volume (cm <sup>3</sup> ) | Egg SG | Shell thickness (mm) | Shell weight % | Yolk colour scale   |
|---------------------|---------------------|-------------------------------|--------|----------------------|----------------|---------------------|
| SB (g/kg)           |                     |                               |        |                      |                |                     |
| 0                   | 64.38               | 58.48                         | 1.078  | 0.339                | 8.88           | 7.239 <sup>a</sup>  |
| 10                  | 64.09               | 58.51                         | 1.077  | 0.334                | 8.74           | 7.050 <sup>b</sup>  |
| 20                  | 64.82               | 59.18                         | 1.077  | 0.339                | 8.86           | 7.099 <sup>b</sup>  |
| ±SEM                | 0.431               | 0.394                         | 0.0005 | 0.0030               | 0.074          | 0.0344              |
| CSM (g/kg)          |                     |                               |        |                      |                |                     |
| 0                   | 65.07 <sup>a</sup>  | 59.16 <sup>a</sup>            | 1.077  | 0.338                | 8.76           | 6.939 <sup>b</sup>  |
| 100                 | 64.80 <sup>ab</sup> | 59.16 <sup>ab</sup>           | 1.077  | 0.336                | 8.74           | 7.167 <sup>a</sup>  |
| 200                 | 63.41 <sup>b</sup>  | 57.89 <sup>b</sup>            | 1.078  | 0.339                | 8.97           | 7.284 <sup>a</sup>  |
| ±SEM                | 0.431               | 0.394                         | 0.0005 | 0.0030               | 0.074          | 0.0344              |
| Interactions (g/kg) |                     |                               |        |                      |                |                     |
| 0 SB × 0 CSM        | 65.56               | 59.86                         | 1.077  | 0.340                | 8.79           | 7.156 <sup>a</sup>  |
| 0 SB × 100 CSM      | 64.64               | 59.01                         | 1.078  | 0.337                | 8.89           | 7.170 <sup>a</sup>  |
| 0 SB × 200 CSM      | 62.93               | 57.46                         | 1.078  | 0.340                | 8.97           | 7.392 <sup>a</sup>  |
| 10 SB × 0 CSM       | 64.64               | 59.02                         | 1.077  | 0.336                | 8.72           | 6.739 <sup>b</sup>  |
| 10 SB × 100 CSM     | 64.87               | 59.23                         | 1.076  | 0.334                | 8.62           | 7.166 <sup>a</sup>  |
| 10 SB × 200 CSM     | 62.75               | 57.29                         | 1.077  | 0.332                | 8.87           | 7.246 <sup>a</sup>  |
| 20 SB × 0 CSM       | 65.00               | 59.35                         | 1.077  | 0.337                | 8.97           | 6.923 <sup>ab</sup> |
| 20 SB × 100 CSM     | 64.90               | 59.25                         | 1.077  | 0.346                | 8.70           | 7.166 <sup>a</sup>  |
| 20 SB × 200 CSM     | 64.55               | 58.94                         | 1.078  | 0.345                | 9.08           | 7.208 <sup>a</sup>  |
| ±SEM                | 0.747               | 0.682                         | 0.0009 | 0.0050               | 0.129          | 0.0597              |
| Source of variation | -----P-values-----  |                               |        |                      |                |                     |
| SB                  | 0.495               | 0.494                         | 0.336  | 0.414                | 0.360          | 0.0018              |
| CSM                 | 0.025               | 0.024                         | 0.449  | 0.724                | 0.070          | <0.001              |
| SB×CSM              | 0.580               | 0.578                         | 0.926  | 0.813                | 0.800          | 0.0225              |

<sup>a-c</sup> Means with different superscript for every effect in each column are significantly different (P<0.05).

Whereas, the addition of SB decreased the specific gravity and yolk color index compared to the control diet.

In conclusion, 200 g CSM/kg diet had negative effects on body weight, egg production, egg weight, daily egg mass, and yolk colour of laying hens. Under the situation of this trial, feeding the hens with 10 g SB accompanied by 100 g CSM per kg of diet had the best productive characteristics with no adverse impact on performance.

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