EFFECT OF EXTRUDED AND NO EXTRUDED SOYBEANS SUPPLEMENTS IN FODDER ON ANTIOXIDANT LIVER ACTIVITY IN BROILERS

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Abstract: The aim of the trial was to evaluate the effect of extruded and no extruded soybeans substitute of soybean meal and soybean oil in fodder on the broiler liver antioxidant activity. Metallothionein was used to measure oxidative stress in liver. There were used the substitute of 15% soybean meal and soybean oil by extruded soybeans, the substitute of 10% soybean meal and soybean oil by extruded soybeans and the substitute of 10% soybean meal and soybean oil by no extruded soybeans of a feed ration from 10 to 35 day of age. The trial was carried out on the 156 female chickens Ross 308. The chickens were kept in the double-floor cage technology. All of them were fed by the same complete feed mixture BR1 (Broiler No. 1) for the first 10 days. After 10 days of age, the birds were randomly allocated to 12 cages in both tiers corresponding to 4 dietary treatments with three replicates of each treatment. Each group comprised 3 cages containing 13 broilers each. The dietary treatments included a control diet and the diets containing 15% extruded soybean substitute, 10% extruded soybean substitute and 10% no extruded soybean substitute of soybean meal and soybean oil with the other components remaining the same as in the control diet. The level of the metallothionein content was determined in samples obtained from 35 days old animals by adsorptive transfer stripping differential pulse voltammetry. The fodder substitute of the extruded and no extruded soybeans had significant ($P<0.05$) effect on the level of the metallothionein level in the liver of the broiler chickens.
The highest level of the metallothionein was measured in the 10% substitute of extruded soybeans. The lowest level of metallothionein level was measured in the control group. The difference found was significant ($P<0.05$).

Key Words: oxidative stress, metallothionein, Ross 308

INTRODUCTION

Soybean derivatives as soybean oil, soybean flour and so on are considered as sources of a large variety of antioxidant compounds. Those compounds belong to the family of isoflavone glycosides and their derivatives, phospholipids, tocopherols, amino acids and peptides (Gyorgy et al. 1964 cit. in Shahidi, Naczk 2003). For instance, soybean protein hydrolysates possess antioxidant activity which is associated with free amino acids and lower molecular weight peptides. Soybean flour possesses antioxidant compounds as isoflavone glycosides and their derivatives, phospholipids, tocopherols, amino acids and peptides (Hayes et al. 2006). An obvious connection between the tocopherol content of soya oil and its resistance to oxidative deterioration has been discovered since 1930s. Soybeans further contains lecithin which also was shown to have antioxidant properties (Buck 1981).

Metallothionein (MT) is a protein which occurs in heavy metal homeostasis and detoxification (Haidara et al. 1999). Generally, MTs as a group, constitute of low-molecular-weight, cysteine-rich, metal binding and nonenzymatic proteins appearing in the animal kingdom. Despite the fact that MT was detected many years ago, there are still some uncertainties in its physiological functions. The structure of amino acids forming MT seems to be uncommon. There are no aromatic amino acids.

Furthermore, one third of its residues are cysteines (Klaassen et al. 1999). MT occurrence is linked with oxidative stress. It participates in an array of protective stress responses (Andrews 1999).

The objective of the trial is to evaluate the effect of extruded and no extruded soybeans substitute of soybean meal and soybean oil in fodder on the broiler liver antioxidant activity by MT expression.

MATERIAL AND METHODS

The experiment was carried out on 156 female chickens during 35 days. One day old hybrids Ross 308 were used. The average weight of chickens was 43.8 g. Chickens were kept in double-deck cage technology and in the top part of double-deck cage technology during the first 10 days. All chickens were fed by the complete feed mixture Broiler No 1 (BR1) for the first 10 days. Thereafter, female chickens were divided into 4 groups (Table 1). Each group had 3 repetitions with 13 members. Chickens were fed by the complete feed mixture Broiler No 2 (BR2) after the first 10 days. Chickens were fed ad libitum. The diet was given twice daily. In the last week of the experiment, the diet was supplied three times weekly. The compositions of complete feed mixtures are shown in Tables 2 and 3. Main components of complete feed mixture BR2 were the same for all chickens in all groups. The experimental group was fed by the complete feed mixture BR2 with extruded or no extruded soybean substitute. The substitutes were E15 (15% soybean meal and soybean oil substituted by extruded soybeans), E10 (10% soybean meal and soybean oil substituted by extruded soybeans) and B10 (10% soybean meal and soybean oil substituted by no extruded soybeans). The CO (control group) was fed by the complete feed mixture BR2 with no extruded or no extruded soybeans (Table 3).

The temperature, humidity, light intensity and air convection were monitored during the experiment. The temperature was 30°C in the shed on the first day. Thereafter, the temperature reduced gradually to 20°C. It was difficult to maintain required indoor temperature because of high outside temperature. The relative humidity was around 60%. The light intensity and light mode were regulated (Table 4). The light intensity was 40 lux for the first 15 days of the experiment and 20 lux from 15 to 35 days.

Six chickens from every group were killed by decapitation the 35th day of the experiment. The average weight of live chickens was 1.770 g. Samples of liver were collected immediately after the decapitation. Livers were stored in polystyrene box with the ice. Liver samples were processed during the day of decapitation.

MT as an oxidative stress value was measured in the liver. The antioxidant activity was expressed by a trolox equivalent (TE).

The measurement of antioxidant activity was carried out by using an adsorptive transfer stripping differential pulse voltammetry.

Table 1 The scheme of the experiment

Group	Substitution portion (%)	Number of repetitions	Number of chickens
CO	0.0	3	39
B10	10.0 (no extruded soybeans)	3	39
E10	10.0 (extruded soybeans)	3	39
E15	15.0 (extruded soybeans)	3	39

Table 2 The composition of complete feed mixture BR1

Components	BR1 (%)
Wheat	27.5
Corn	25.0
Soybean Meal/Soybean-oil	30.0/2.3
Fish Meal	1.0
Substitute*	0.0
Premix	4.2

Table 3 The composition of complete feed mixture BR2

Components of BR2 (%)	CO	B10	E10	E15
Wheat	39.1	39.1	39.1	39.1
Corn	25.0	25.0	25.0	25.0
Soybean Meal/Soybean-oil	27.3/4.5	19.1/2.7	19.1/2.7	15.0/1.8
Extruded soybeans	0.0	0.0	10.0	15.0
No extruded soybeans	0.0	10.0	0.0	0.0
Premix	4.1	4.1	4.1	4.1

Table 4 The light schedule in the chicken shed

Days of the experiment	The length of lighting (h)
1–7	23
8–33	18
34–36	23

The statistical analysis was performed using program Unistat 5.1 (Unistat Ltd., England). The liver characteristics were expressed as the mean. Data variability was quantified by the coefficient of variation. Differences between groups were analyzed by Kruskal-Wallis one-way analysis of variance.

RESULTS AND DISCUSSION

The attention was focused on the oxidative stress in liver. Protein MT has been taken as an oxidative stress value.

For the control group (CO), it was predicted that the MT level would be greater than for the experimental groups. Furthermore that the MTlevel would be the lowest for the group with the 15% extruded soybeans substitution (E15).

The expression and the induction of MT is related with oxidative stress and cells apoptosis (Yang et al. 2006).The results showed some differences among the fodder substitutes in antioxidant activity in the chicken liver. The effect of the fodder substitutes in the diet on the antioxidant activity (expressed by MT content) is shown in Table 5.

The MT concentration (Table 5) was higher (P<0.05) for E10 (24.1 μM TE) than for B10 (9.6 μM TE) and CO (8.2 μM TE) in the liver. These results are not consistent with experiment of Lee et al. (2005) who confirmed that some soybean´s substances as isoflavones and their glykosides possess antioxidant activity. Also Huang and Chen (2004) has demonstrated the antioxidant activity of soybean. Furthermore Wiseman et al. (2000) reported that consumption of soy decreased lipid peroxidation in vivo and elevated the resistance of low-density lipoproteins to oxidation due to naturally occurring amounts of isoflavone phytoestrogens. In spite of, it has been proved that soybean isoflavones and their glycosides possess antioxidant activity they seem to be like ineffective antioxidants in comparison with tea epicatechins and alpha-tocopherol (Lee et al. 2005).

No others statistically significant differences were found between the groups.

The results did not confirm the positive effect of extruded and no extruded soybeans substitute of soybean meal and soybean oil in the chicken diet on antioxidant activity as was expected.

Table 5 Mean values of MT in the liver of broilers fed with fodder contains soybean meal and soybean oil, extruded or no extruded soybeans

Parameter (μM TE)	CO	B10	E10	E15
MT	8.2^{a}	9.6^{a}	24.1^{b}	12.6

a, b, c—different letters mean differences at P<0.05.

CONCLUSION

The objective of the trial was to examine the effect of extruded and no extruded soybeans substitute of soybean meal and soybean oil in the chicken diet. The oxidative stress was measured by MT content in the chicken liver.

The highest antioxidant activity expressed by MT liver content and caused by the diet substitutes was found for the E10 group (24.1 μM TE). On the contrary, the lowest MT content in liver was found for CO group (8.2 μM TE). The differences among group E10 (24.1 μM TE) and groups B10 (9.6 μM TE) and CO (8.2 μM TE) were statistically significant ($P<0.05$). The positive effect of extruded and no extruded soybeans substitute of soybean meal and soybean oil in the chicken diet on antioxidant activity was not confirmed in this trial.

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