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INFLUENCE OF SOY ISOFLAVONES ON PLASMA ISOFLAVONE AND OESTRADIOL LEVELS IN LAYER CHICKEN

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ABSTRACT

This experiment was conducted to assess the effect of soy isoflavones (daidzein and genistein) on oestradiol concentration in the blood plasma of layer chicken. Group I birds were fed with regular standard maize-soybean meal based diet as per BIS 2007 specification containing 22.5% soybean meal, group II with standard layer diet without soybean meal (BIS, 2007) and group III was supplemented with soy isoflavones enriched diet (0.5 g dried hypocotyl sprout of soybean 100 g^{-1} feed) for a period of 56 days. The levels of free isoflavones in soybean meal and blood plasma derived through *β*-glucuronidase treatment were quantified using liquid chromatography–mass spectrometry (LC-MS) technique. The genistein levels were 2.89 ± 0.02, 1.84 ± 0.13, and 3.25 ± 0.04 µg g^{-1}, while daidzein levels were 1.15 ± 0.26, 0.20 ± 0.03, and 2.40 ± 0.09 µg g^{-1}, respectively, in feeds of groups I, II, and III. The mean genistein and daidzein content in blood plasma was found higher for group III birds compared to other two treatment groups. After feeding trial, plasma concentration of oestradiol 17β was estimated by using radioimmune assay (RIA) technique which was found higher for group II (673.45 ± 7.69 pg mL^{-1}) as compared to the group I (979.21 ± 12.57 pg mL^{-1}) and III (314.23 ± 6.34 pg mL^{-1}). The study showed that the higher amount of isoflavones in feed could lower endogenous production of oestradiol from ovaries.

Keywords: Daidzein, genistein, isoflavones, liquid chromatography-mass spectrometry, phytoestrogens

INTRODUCTION

Isoflavones are a subclass of flavonoids that are the major phytoestrogens found in leguminous plants like soybean. Soybean meal, incorporated up to 25% in animal/poultry feeds, is the main source of protein. Genistein and daidzein are the predominant isoflavones and together make more than 90% of total isoflavones in soy-based food (Liggins *et al*., 2002). Isoflavones have a chemical structure resembling the hormone oestrogen. The structural arrangement of phytoestrogens allows them to potentially bind with oestrogen receptors with high specificity and effectiveness. Therefore, phytoestrogens perform either as oestrogen agonists or antagonists depending on endogenous oestrogen

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oestrogen levels (Gilani and Anderson, 2002) and exert various effects on the physiology of mammals. Soy isoflavones have weak oestrogenic activity but a higher intake of isoflavones would be detrimental to the system (Sirotkin and Harrath, 2014).

Oestradiol 17β (E_2) regulates the integration and coordination of folliculogenesis, accumulation of yolk in the follicles, facilitates luteinising hormone (LH) induced ovulation and development of oviducts. Oestradiol also induces oviductal and uterine glandular development and expression of genes responsible for egg white protein formation (Mishra *et al.,* 2019).

Akiba *et al.* (1982) and Ni *et al*. (2007) using radio-immune assay studied the impact of diet-enriched with isoflavones on serum E_2 levels in laying hens in comparison to the basal control diet. However, they did not determine the correlation between the levels of genistein and daidzein in blood plasma nor studied endogenous oestradiol hormone. Gjorgovska *et al*. (2016) demonstrated the effect of isoflavone supplementation on plasma E_2 concentration and its influence on laying performances in ISA Brown laying hens. However, they did not assess the concentrations of isoflavones in plasma. Saitoh *et al.* (2001) and Saitoh *et al.* (2004) estimated the level of isoflavone transferred to plasma by administering a diet containing a high concentration of soy isoflavones to laying hens. However, they did not assess the endogenous oestradiol hormone levels. Hence, the present study was conducted to determine the genistein and daidzein concentrations in the layer hens' feed and the influence of these dietary soy isoflavones on free isoflavones (daidzein and genistein) as well as on endogenous oestradiol 17β levels in blood plasma of layer chickens.

Phytoestrogens acting as oestrogen mimics may affect the production and breakdown of oestrogen by the body, as well as the circulating levels of oestrogen (Bibu, 2010). Therefore, dietary isoflavones are expected to have a specific impact not only on plasma isoflavone but also on endogenous E_2 levels and can also be used as an alternative to external oestrogen substitutes or in hormone replacement therapy.

MATERIALS AND METHODS

Experimental layout

The study was conducted at department of Veterinary Physiology for a period of 56 days (from 01/08/2022 to 26/09/2022) on thirty-six Gramasree layer birds of 28 weeks age procured from the University Poultry and Duck Farm (UPDF), Mannuthy, KVASU. The experiment was approved by the Institutional Animal Ethics Committee (IAEC) of College of Veterinary and Animal Sciences, Mannuthy (no. IAEC/22/16 dated 06.04.2022). The birds were randomly distributed in a completely randomised experimental design and placed into three treatment groups, each with 12 birds. Group I birds were provided standard layer diet containing soybean meal (BIS, 2007) while group II were maintained on standard layer diet without soybean meal (BIS, 2007) and group III were provided soy isoflavones enhanced diet (0.5 g dried hypocotyl sprout of soybean 100 g^{-1} feed) in addition to standard layer diet with soybean meal (BIS, 2007).

Experimental birds, housing and management

On completion of 28 week-age, the birds were housed in well-ventilated cages of animal house. They were maintained at 26.5±0.5˚C temperature and 70-80% relative humidity. A photoperiod of 16 h day^{-1} was ensured throughout the study period. The total ration was provided as a single lot in the morning for all the groups and water was provided *ad libitum* throughout the experimental period of 56 days. Three isocaloric and isonitrogenous experimental feeds, with variable isoflavone levels were provided to laying hens. The composition of diets formulated for treatment groups is given in Table 1. The diet for group III was formulated according to BIS 2007 standards as done for group I and additionally enriched with dried hypocotyl sprout of soybean at the level of 0.5 g 100 g^{-1} feed (Vargas Galdos, 2009). Soybean seeds were sown in soil after soaking them for overnight and the hypocotyl sprout

Table 1: Composition of diets formulated for treatment groups of layer chicken

S. No.	Feed ingredients	Composition (%)		
		Group I[#]	Group II[#]	Group III[#]
1.	Yellow maize	53.16	53.65	53.16
2.	De-oiled rice bran	8.85	-	8.85
3.	Soybean meal	26.86	-	26.86
4.	Groundnut cake	-	20.58	-
5.	Wheat bran	-	7.07	-
6.	Gingelly oil cake	-	5.00	-
7.	Dried fish	-	3.78	-
8.	Dicalcium phosphate	1.29	1.04	1.29
9.	Grit	5.00	4.23	5.00
10.	[1]Calcite powder	3.98	4.00	3.98
11.	[2]DL – Methionine	0.21	0.19	0.21
12.	[3]L – Lysine	0.20	0.20	0.20
13.	Soda bicarb	0.05	0.05	0.05
14.	Salt	0.40	0.21	0.40

[#]Group I: birds fed with standard layer diet containing soybean meal
Group II: birds fed with standard layer soybean meal free diet
Group III: Birds fed with soy isoflavones enhanced diet (0.5 g dried hypocotyl sprout of soybean 100 g^{-1} feed) + standard layer diet containing soybean meal

harvested on 5th day of germination. The sprouts were dried and ground to use it as feed ingredient for group III birds.

Standards and mobile phase for LC-MS

Liquid chromatography-mass spectrometry (LC-MS) electron spray ionisation (ESI) detection using reverse-phase C18 stationary matrices is the method of choice for determining daidzein and genistein in soy-based feed formulations, blood and tissues (Vargas Galdos, 2009). The chemicals and solvents of HPLC grade were procured from Thermo Fisher Scientific, USA while genistein and daidzein standards were obtained from Sigma Aldrich, USA. To create stock solutions of free isoflavones (daidzein and genistein) 2 mg of standard substance was dissolved in 2 mL of (HPLC-MS) grade methanol (99.9%). Composite working standard solutions ranging from 1 to 500 µg $mL^{-1}$was generated for quantification and recovery experiments. These working solutions were prepared by diluting the stock solution with appropriate amounts of methanol, as per Hu *et al.* (2019). Two different gradient elution system were used as mobile phases to separate genistein and daidzein from samples (Saitoh *et al*., 2004). These gradient systems consisted of two components: an aqueous solvent A (containing 0.1% acetic acid and 5% acetonitrile in water) and an organic solvent B (containing 0.1% acetic acid in acetonitrile).

Feed sample preparation to estimate daidzein and genistein content

The levels of isoflavones in three experimental diets was measured as per Lin *et al.* (2004) with slight modification. One gram feed was weighed and extracted using 10 mL of 80% methanol. The samples were placed on an incubator orbital shaker (M/s Thermo Fischer Scientific, USA) for 1 h. The mixture was centrifuged in high speed refrigerated microcentrifuge (M/s Thermo Electron LED, Germany) for 20 min and supernatants were collected. The precipitate was re-dissolved in 10 mL of 80% methanol and re-centrifuged. The collected supernatants were pooled and the total extracted volume was reduced to < 2 mL using an incubator set at 40ºC. Subsequently, these extracts were mixed with 10 mL of 0.1*M* sodium acetate buffer (pH 5) and 75 µL of *β*-glucuronidase (*Helix pomatia*, 1000 Fishman units). The solution was incubated overnight at 37ºC and finally passed through C-18 purification kit (Bond Elut 500 mg 3mL^{-1}). Finally, the isoflavones of interest were recovered from the cartridge using 80% methanol, followed by a mixture of ethyl acetate and methanol in 80:20ratio, with a total volume of 2 mL.

Following the C-18 clean-up process, the samples were concentrated through evaporation. The resulting extracts were brought back to a volume of 2 mL by reconstitution with 20% methanol. These reconstituted solutions were then passed through a 0.45 µm syringe polypropylene filter and injected into the LC-MS system (M/s Thermo Fischer Scientific, USA). Each sample was run in triplicate.

Plasma sample preparation to estimate daidzein and genistein content

Blood samples were collected at the end of experiment in EDTA vials at room temperature. Plasma was

separated by centrifuging at 3000 rpm for 15 min and then 1 mL 0.1 *M* sodium acetate buffer (pH 5.0) and 1000 Fishman units of *β*-glucuronidase were added to 1 mL plasma. The mixture was incubated at 37°C overnight (Saitoh *et al*., 2001). Isoflavone was collected from the hydrolysate by extracting it twice with 4.7 mL absolute ethanol and 1 ml 70% ethanol, respectively. The pooled extract was evaporated to dryness and dissolved in 1 mL 70% aq. ethanol before LC-MS analysis (Saitoh *et al*., 2004).

Chromatographic and mass spectrometric conditions

Standard chromatogram and mass spectra were generated for each injected standard and test sample using DAD of chromatograph with a time window of 30 min for daidzein and 28 min for genistein. The temperature of column was maintained at 35°C throughout the experiment. An autosampler was used to inject 20 µL of blank (mobile phase), standards, test samples and samples from control birds into C18 column (3 µm, 2.1X 150 mm). The samples were separated by reverse phase column elution using the mobile phase at a flow rate of 1 mL min^{-1}). A gradient program implemented for mobile phases of both daidzein and genistein elution as recommended by Saitoh *et al.* (2001) was as follows:

Time (min)		% mobile
Daidzein	Genistein	phase B
0	0	0
1	1	15
5	5	27.5
29	25	32.5
30	28	0

The UV-VIS detection was carried out at 260 and 262 nm wavelength specific for daidzein and genistein, respectively. The MS detector was set with vapouriser temperature of 35°C and a pressure of 152-155 bar. The ions were monitored through the single ion monitoring (SIM) method at mass/charge (m/z) ratio of 256.14 and 270.23 for daidzein and genistein, respectively.

Linearity of daidzein and genistein concentration versus area curve

Daidzein and genistein standards were analysed using liquid chromatography-mass spectrometry (LC-MS) to create calibration plots. Peak areas for each dilution were determined from the chromatogram and the values plotted against the corresponding concentration. The reproducibility of results was verified at least three times with each concentration of standards. A regression equation was calculated using MS-Excel® based on the data obtained from the analysis.

Limit of detection and Limit of quantification

The limit of detection (LOD) is the lowest concentration in a sample that can be detected, but not quantified, from background noise. The LOD for phytoestrogen standards was determined by average concentration that gave the signal three times the background noise. The limit of quantification for phytoestrogen standards was found by determining the average concentration that gives the signal ten times the background noise.

Estimation of plasma oestradiol 17β concentration by radio immune assay

Radio immune assay was carried out as per the manufacturer's protocol (Beckman Coulter, California, USA) for *in vitro* assay of oestradiol 17β in plasma. Blood samples collected at the end of experiment and plasma was separated by centrifugation. Then 100 µL of calibrator, control and test samples were added to the anti-oestradiol antibody coated labelled tubes. Tracer (500 µL) was added to each tube and thoroughly mixed by vortexing. Exactly 500 µL tracer was added to two new tubes and used to obtain total counts min^{-1} (CPM). The tubes were incubated for 3 h at 18-25°C with continuous shaking in an orbital shaker at 350 rpm. After incubation, the contents in tubes were aspirated (except the tubes for CPM). The tubes were placed in a gamma counter set for Iodine-125 (^{125}I) and radioactivity was estimated in CPM (1 min $tube^{-1}$). The results obtained from standard curve interpolation were expressed in pg mL^{-1}.

Statistical analysis

The data were expressed as mean ±S.E. The statistical significance of difference or relation between the treatments were analysed by one-way ANOVA using Statistical Product and Service Solutions (SPSS) software version 24.0 (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Preparation of calibration curve for daidzein and genistein

Standard solutions were injected three times into the LC-MS column and the height and area of peaks were found to be increased with increasing concentration of standards (Table 2). The retention time RT) of standards were 23.51 to 24.44 min for daidzein and 12.78 to 12.95 min for genistein. The mass spectrum of the sample peak fraction coinciding with this retention time were corresponding to that of daidzein and genistein, respectively. Calibration curve with the standards 1.95, 3.90, 7.81, 15.625, 31.25, 62.5, 125, 250 and 500 µg mL^{-1} were constructed for daidzein and genistein. The chromatogram of daidzein and genistein standards (500 µg mL^{-1}) are shown in Fig. 1 and Fig. 2. Representative spectrum of standards are shown in Fig. 3 and Fig. 4 since the detection was carried out in MS detector with SIM method.

Table 2: LC-MS retention time (RT), area, expected amount and calculated amount of daidzein and genistein standards

Daidzein				Genistein			
Concentration (µg mL^{-1})	RT (min.)	Area (min x mAu)	Calculated amount (µg mL^{-1})	Concentration (µg mL^{-1})	RT (min.)	Area (min x mAu)	Calculated amount (µg mL^{-1})
1.9531	24.44	1.86	2.272	1.9531	12.92	3.37	1.961
3.9063	24.42	3.84	4.689	3.9063	12.92	6.62	3.858
7.8125	23.42	7.47	9.126	7.8125	12.78	12.91	7.521
15.625	24.14	13.90	16.980	15.625	12.78	24.77	14.431
31.250	24.12	28.09	34.316	31.250	12.28	51.06	29.751
62.500	24.12	58.44	71.383	62.500	12.76	101.98	59.418
125.000	23.74	112.14	136.979	125.000	12.76	209.71	122.185
250.000	23.63	203.47	248.550	250.000	12.95	418.05	243.572
500.000	23.51	428.93	523.966	500.000	12.92	865.79	504.439

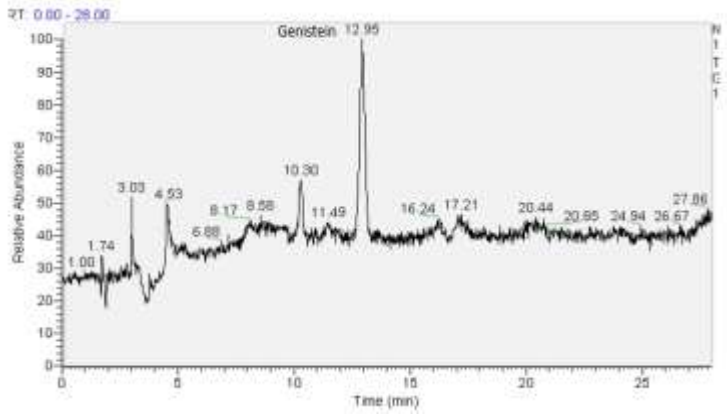

Fig. 1: Chromatogram of eluted genistein standard 500 ug mL^{-1}

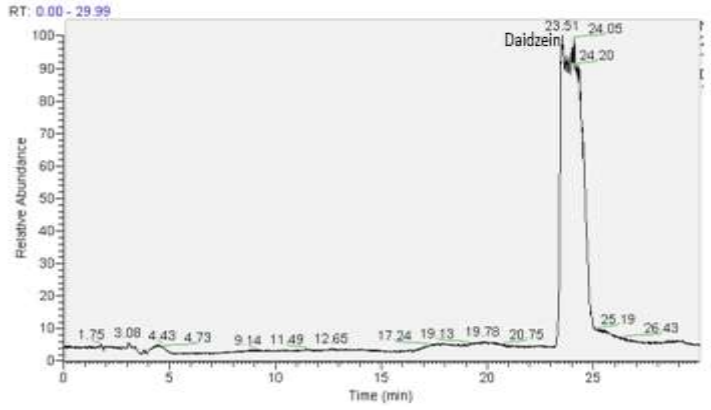

Fig. 2: Chromatogram of eluted daidzein standard 500 ug mL^{-1}

Free isoflavone (genistein and daidzein) content of feed samples

In current study daidzein and genistein content of feed samples were significantly different ($p \leq 0.01$) among the treatment groups. Higher mean values of genistein and daidzein were detected in group III feed samples (Fig. 5 and

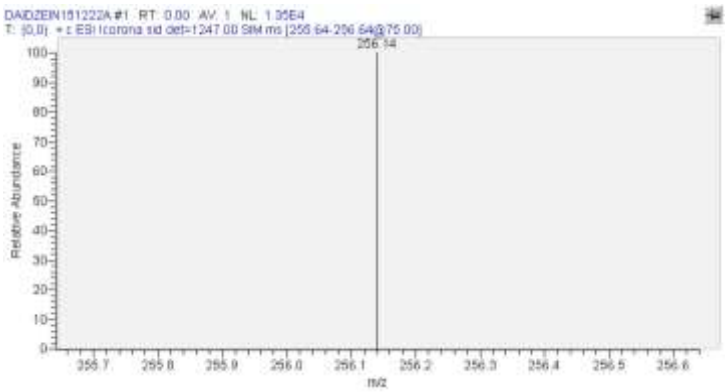

Fig. 3: MS spectrum of daidzein standard 500 ug mL^{-1}

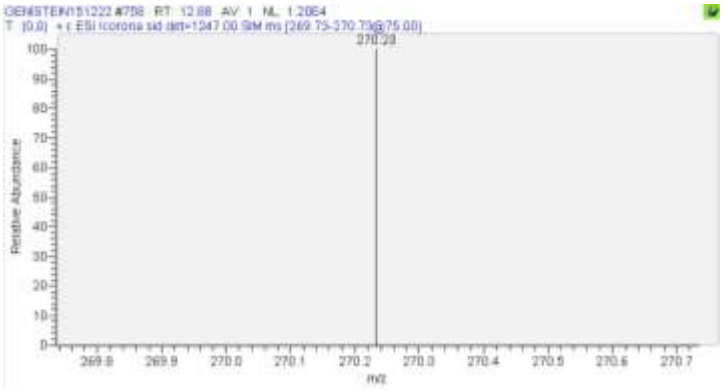

Fig. 4: MS spectrum of genistein standard 500 ug mL^{-1}

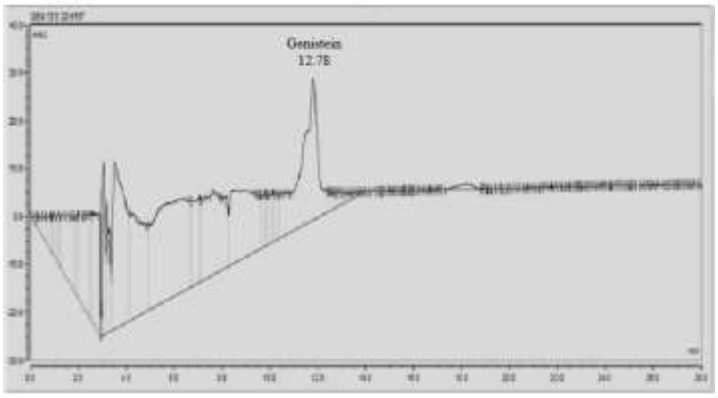

Fig. 5: Chromatogram of eluted genistein in group III feed

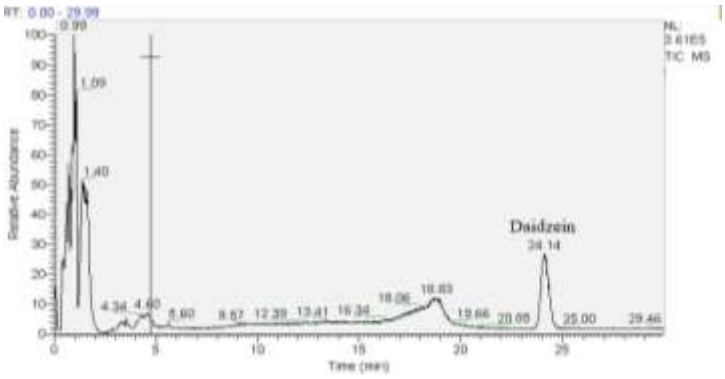

Fig. 6: Chromatogram of eluted daidzein in group III feed

Fig. 6) followed by group I feed. On the other hand, group II feed samples showed lower mean value for both daidzein and genistein as compared to the other two groups (Table 3). Boettger-Tong *et al*. (1998) also found soy supplements as richest source of isoflavones with concentrations ranging from 118 to 306 mg 100 g^{-1} and these are the common ingredients of laboratory animal feed. Gilani and Anderson (2002) reported that soy products prepared from hypocotyledon have >20 mg isoflavones g^{-1}. Grgic *et al*. (2021) also detected the presence of soy isoflavones in 88% of the poultry feed samples (n = 263). Aglycones were detected in lower concentrations as compared to the respective glycosides. The maximum concentration was 102 mg kg^{-1} for genistein and 465 mg kg^{-1} for its glucosidic conjugate. Hashem and Soltan (2016) have shown that isoflavones could affect animal fertility and growth, quality of animal products and human health. Soybean meal is a source of unsaturated fatty acids for energy utilisation in livestock, especially in poultry feed industry. Soy products are commonly used as animal feed due to their high protein content of about 38% (Grgic *et al*., 2021), healthful fat and high fibre. This was the major driving force that stimulated the production and consumption of soybean all over the world (Boonnoon *et al*., 2016).

Free isoflavone (daidzein and genistein) content in plasma

In present study the plasma levels of daidzein and genistein concentrations were significantly different ($p \leq 0.01$) for the treatment groups (Table 4). Higher plasma concentrations of daidzein

Table 3: Concentration of daidzein and genistein feed samples (mean ± SE)

Treatment groups[#]	Mean conc. (µg g^{-1}) in feed samples	
	Genistein	Daidzein
Group I	$2.89^{a} \pm 0.02$	$1.15^{a} \pm 0.26$
Group II	$1.84^{b} \pm 0.13$	$0.20^{b} \pm 0.03$
Group III	$3.25^{c} \pm 0.04$	$2.40^{c} \pm 0.09$
p-value (99% level)	≤ 0.01**	≤ 0.01**

Means with different superscripts in the same column differ significantly; ** Highly significant ($p \leq 0.01$)

[#]Group I: birds fed with standard layer diet containing soybean meal
Group II: birds fed with standard layer soybean meal free diet
Group III: birds fed with soy isoflavones enhanced diet (0.5 g dried hypocotyl sprout of soybean 100 g^{-1} feed) + standard layer diet with soybean meal

Table 4: Concentration of daidzein and genistein in plasma samples (mean ± SE)

Treatment groups[#]	Mean conc. (µg mL^{-1}) in plasma	
	Genistein	Daidzein
Group I	$1.84^{a} \pm 0.02$	$0.28^{a} \pm 0.08$
Group II	$0.02^{b} \pm 0.02$	$0.13^{a} \pm 0.09$
Group III	$2.45^{c} \pm 0.09$	$10.35^{b} \pm 2.08$
p- value (99% level)	≤ 0.01**	≤ 0.01**

Means with different superscripts in the same column differ significantly; ** Highly significant ($p \leq 0.01$)

[#] Group I treatment details same as mentioned in the footnote of Table 3

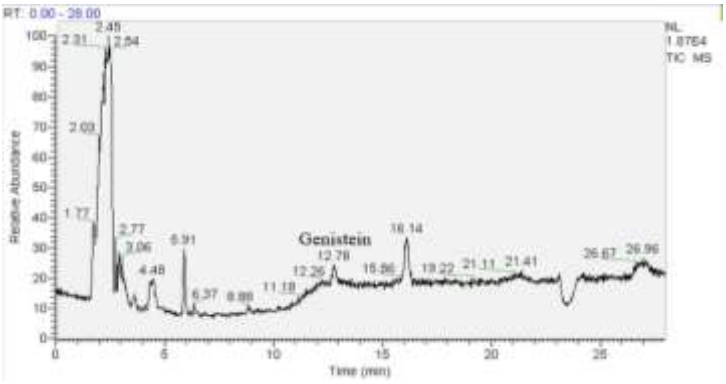

Fig. 7: Chromatogram of eluted genistein in group III plasma

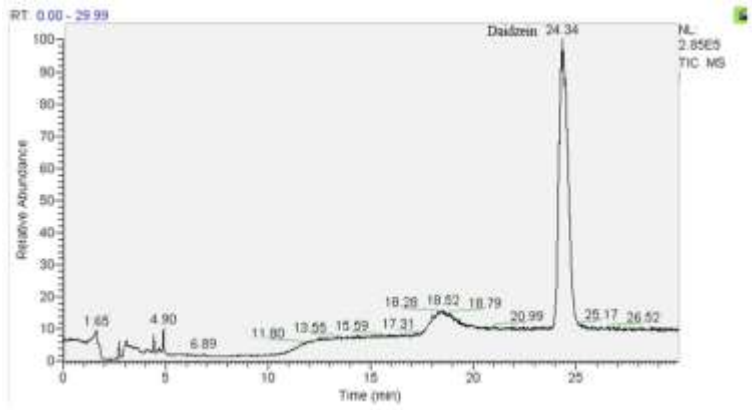

Fig. 8: Chromatogram of eluted daidzein in group III plasma

and genistein were detected in treatment group III (Fig. 7 and Fig. 8). Genistein plasma concentration was lower for treatment group II as compared to the group I. Plasma daidzein concentrations were statistically at par in group I and II.

Several studies conducted worldwide had proved that isoflavones can be transferred from feed into the tissues, eggs and milk of livestock species via blood (Grgic *et al*., 2021). It would also be of great interest to determine the ratio of this transfer and the transfer into other chicken tissues, such as heart and liver, which are consumed in several different countries. Isoflavones were maternally transferred to the egg through circulatory system. Vitellogenin was synthesised to act as an egg yolk precursor in bloodstream of egg birds. Both dietary isoflavones and vitellogenin were co-transported as complexes via bloodstream into the oocyte before ovulation (Saitoh *et al*., 2004).

It was elucidated that every process within the body which was controlled and maintained by oestrogens could be influenced by phytoestrogens also (Wang, 2002). Hence the level of isoflavones in blood would also affect the affinity of oestradiol to its receptors. Hashem and Soltan (2016) studied the affinity of different phytoestrogens to the oestrogen receptors (ER) using the receptor binding affinity test. The ranking of the oestrogenic potency of phytoestrogens for ER was found like oestradiol > genistein > daidzein. The differential potency between genistein and daidzein could be ascribed to the presence of the 5-hydroxyl group of genistein which provides more identical

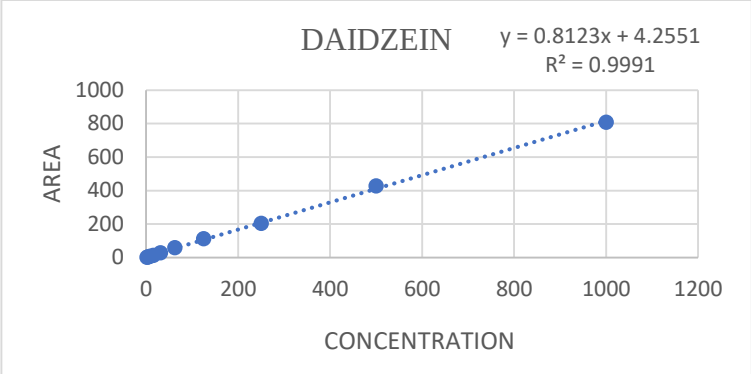

Fig. 9: Linearity of daidzein standards and corresponding area

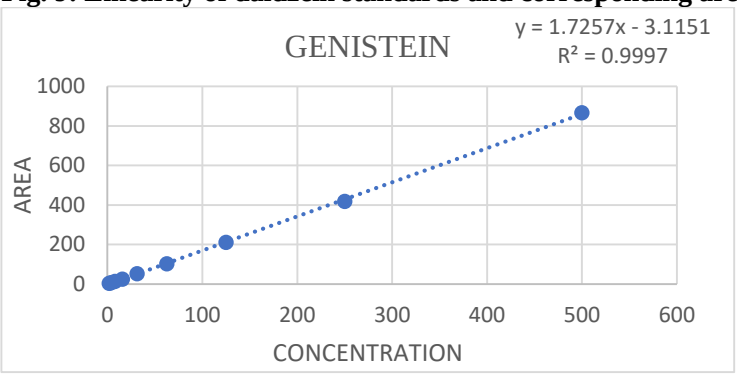

Fig. 10: Linearity of genistein standards and corresponding area

chemical similarity to mammalian oestradiol.

Linearity of standard concentration of daidzein and genistein v/s area curve

A good linearity of the method was observed with a correlation coefficient of 0.999 between the concentration range of 1 to 500 µg mL^{-1} for daidzein and genistein(Fig. 9 and Fig. 10).

Linear regression analysis was done using MS-Excel®. The concentration of free isoflavones (genistein and daidzein) in feed and blood samples were quantified using the regression equation.

Limit of detection (LOD) and Limit of quantification (LOQ)

The LOD of this method was 4.81 and 3.06 µg 100 g^{-1} for daidzein and genistein, respectively, with acceptable accuracy and precision. The method demonstrated the LOQ of 14.59 µg 100 g^{-1} for daidzein and 9.58 µg 100 g^{-1} for genistein, which is determined consistent with guidelines.

Circulating plasma oestradiol 17β concentration

Phytoestrogen can act like endogenous oestrogen at low doses but block oestrogen at high doses which agrees with our study results. In present study the calibration curve of oestradiol 17β was prepared by using counts min^{-1} (CPM) values and calculated concentrations of oestradiol standards (Fig. 11). A significant difference ($p \leq 0.01$) was observed in oestradiol concentrations at the end of experiment. Total mean plasma oestradiol level was lower for group III as compared to the group I and II (Table 5). In present study, the feeding of isoflavones for 56 days @ 500 mg kg^{-1} decreased the plasma oestradiol concentration. However, Lu *et al.* (2017) found that a nominal isoflavone concentration of 200 mg kg^{-1} is not expected to cause adverse effects following daily administration to laying hens for

Table 5: Mean ± SE plasma oestradiol 17β hormone level

Treatment groups[#]	Oestradiol 17β (pg mL^{-1})	p value (99 % level)
Group I	$673.45^{a} \pm 7.69$	
Group II	$979.21^{b} \pm 12.57$	≤ 0.01**
Group III	$314.23^{c} \pm 6.34$	

Means with different superscripts in the same column differ significantly; ** Highly significant ($p \leq 0.01$)
[#]Group I treatment details same as mentioned in the footnote of Table 3

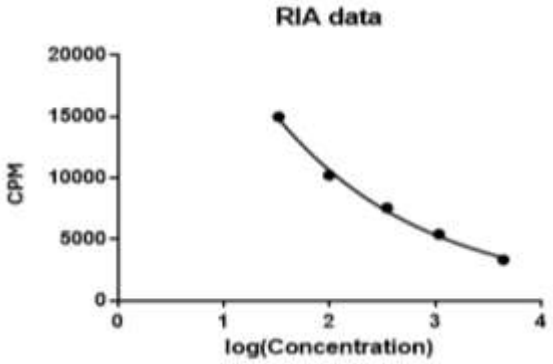

Fig. 11: Calibration curve of oestradiol 17β with different standards [CPM, counts min^{-1}]

84 days. Ni *et al*. (2007) selected ISA laying hens during the post peak period of egg laying and fed with basal diet and the daidzein supplemented diet at the level of 10 mg kg^{-1} diet, respectively, for 9 weeks. The serum E_2 was measured with radioimmunoassay using commercial kits.

Serum concentrations of E_2 was 160 pg mL^{-1} for daidzein supplemented groups compared with their control counterparts (175 pg mL^{-1}) which in agreement with our study. Isoflavones can competitively bind oestrogen receptors but generally have less potential than endogenous oestrogens (Boonnoon *et al*., 2016). Madnurkar *et al*. (2014) presumed that the success of the breeder and table egg industries relied significantly on endocrinological changes involving E_2. The involvement of E_2 in activating thereproductive axis, promoting the production of yolk precursor vitellogenin in the liver, synthesis of egg white proteins in the oviduct, and storage of calcium in medullary bone demonstrated that this hormone played a role in coordinating and contributing to all aspects of egg formation and laying performance. Hence in our study supplementation of isoflavones in excess @ 0.5mg g^{-1} feed may impart negative effects on reproduction traits by decreasing the plasma oestradiol 17β concentration.

Conclusion: Isoflavones are non-steroidal oestrogen-like compounds which can alter growth, development, and function of oestrogen-dependent target tissues. The study revealed that daidzein and genistein concentrations in blood plasma of laying hens can increase significantly by supplementing the diet with an isoflavone-rich source like dried soy hypocotyl sprout. The mean plasma oestradiol concentration estimated by radioimmuno assay was significantly higher ($p \leq 0.01$) for treatment group without soybean meal supplementation (979.21 ± 12.57 pg mL^{-1}). A dose of 0.5 g 100 g^{-1} soybean sprout in addition to the BIS standard diet reduced the plasma oestradiol level to 314.23 ± 6.34 pg mL^{-1}. Hence, higher amount of phytoestrogens in feed could lower the endogenous production of oestradiol from ovaries. Phytoestrogens exert a natural balancing action in body. If oestrogen is low, phytoestrogens will increase oestrogen activity in body, However, it will compete for oestrogen receptor sites and decrease the effects of oestrogen in presence of high phytoestrogen levels.

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