International Journal of Research in Biosciences
Vol. 4 Issue 4, pp. (88-97), October 2015
Available online at http://www.ijrbs.in
ISSN 2319-2844

Research Paper

Effect of supplementation of *Entada phaseoloides* seed powder on growth performance, carcass characteristics and haemato- biochemical parameters along with its effect on expression of HSP70 in broiler chicken

***Barua Chandana C.[1], Bora Mondira[1], Purukayastha Arundhati[1], Bodduluru Lakshmi Narendra[1], Hazarika Mineswar[2], Barua Acheenta Gohain[3], Misri Jyoti[4], Dey Sahadeb[5]**

[1]Department of Pharmacology & Toxicology, College of Veterinary Science, Khanapara, Guwahati, INDIA

[2]Department of Livestock Product and Technology, College of Veterinary Science, Khanapara, Guwahati, INDIA

[3]Department of Veterinary Public Health, College of Veterinary Science, Khanapara, Guwahati, INDIA

[4]ICAR, Krishi Anusandhan Bhavan, Pusa, New Delhi, INDIA

[5]IVRI, Izatnagar-243122, UP, INDIA

(Received May 5, 2015, Accepted September 13, 2015)

Abstract

A study was conducted to evaluate *E. phaseoloides* seed powder as a dietary supplementation to study the growth performance, carcass characteristics, haemato-biochemical changes along with its effect on expression of HSP70 in broiler chicks. A total of 90 numbers of one day old chicks were randomly distributed into 3 groups of 3 replicates having 10 birds each. The dietary treatment comprised of a basal diet as control, basal diet supplemented with 0.5% *E. phaseoloides* seed powder level as T_1 and basal diet supplemented with 1% *E. phaseoloides* seed powder level as T_2 group. Body weight, feed consumption were recorded weekly and accordingly FCR (Feed conversion ratio) were calculated. At the end of 5th week, blood was collected from 3 birds from each replicate for haematobiochemical parameters and then sacrificed to determine the carcass characteristics. Supplementation of *E. Phaseoloides* seed powder significantly improved the live weight (P<0.05) and FCR, which was statistically comparable to control and T_1 (0.5%) group. Dietary treatment did not influence the carcass characteristics significantly. Mean values of serum protein, albumin, globulin, A/G ratio, glucose, cholesterol, PCV, ESR, Hemoglobin, SGOT and SGOT were similar among the groups except for significant variation (P<0.001) seen in serum protein and Hb. Gene expression study in spleen and liver tissue showed significant (P<0.05) down regulation of hsp70 gene in T_2 group. Further confirmation by immunoblotting study of HSP70 protein also showed significant decrease in the protein level in liver and spleen of treatment group. Although, expression of the hsp70 mRNA and the HSP70 protein was evident in heart tissue, However it was not significant (P>0.05). From the result of present study it can be inferred that dietary inclusion of 1% *E. phaseoloides* seed powder can promote growth performance in broilers without any side-effects.

Keywords: Carcass characteristics, *E. phaseoloides* seed, Growth performance, Haemato-biochemical, HSP70

Introduction

In the recent years, herbal feed additives of plant origin have been incorporated into diets to improve the productivity of livestock through amelioration of feed properties, promotion of livestock performance and enhancing the quality of livestock products. Use of herbs in pigs and poultry are now gaining momentum as it claimed to have no side effects, safe and eco-friendly. Along with the effect of herb in the growth performance of broiler chickens, optimal environmental conditions also play a crucial role.

Entada phaseoloides (Mimosaceae) is a gigantic climber tree with twisted and tangled stem. Seeds of this plant commonly called as Gilla (Sanskrit), Hathibij (Hindi), Garambi (Marathi) and Gogo are traditionally used worldwide for medicinal purpose[1]. The seeds are hard, circular, chocolate brown with their sides flattened. In India, ground seeds are taken internally for an incredible variety of remedies such as snake bite, constipation and as aphrodisiac. Even the stem, bark and seeds are used as natural shampoo to wash hair and also used as fish poison. The seeds of *Entada phaseoloides* are slightly bitter-acrid in taste and mildly cooling in nature, thus seed pulp paste is used as an herbal medicine to reduce inflammation and pain of joints and lymph nodes, for relieving gastrointestinal disorders and aiding circulation[2]. In addition seeds are reported to have emetic, anthelmintic and antimalignant activities [1,3]. Seeds of Entada were found to be rich in potassium and iron along with rich source of minerals such as magnesium, phosphorus, zinc and manganese. Albumins and globulins constituted the predominant fractions of the seed proteins. The contents of the essential amino acids, isoleucine, leucine, tyrosine and phenylalanine in *E. phaseoloides*, were fairly high. Fatty acids such as oleic and linoleic acids were found to be relatively high [4]. Along with the above beneficial properties, the ethanolic extract stems of *Entada phaseoloides* displayed potent antioxidant activity when assessed by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical-scavenging, reducing power, β-carotene-bleaching and superoxide radical-scavenging assays [2]. Nutritional factors during early development have important effects on growth, body composition and body functions [5].

In this context, nutritional factors may account for variable stress response, which may be more or less effective at counteracting the damage. Therefore, “novel” dietary bioactive molecules have to be proven for their stress-associated physiological side effects. Thus the study of the heat shock (HS) response at cellular and molecular levels has provided clues for a better understanding of the mechanism(s) involved in development of thermo tolerance in cultured cells. The phenomenon of HS response has been defined as the expression of a small group of highly conserved polypeptides, known as heat shock proteins (HSP), by prokaryotic and eukaryotic cells in response to high ambient temperatures or other environmental stressor [6-9].The HS response appears to be universal and can be detected in organisms as diverse as bacteria and humans [8].

Thus keeping in mind, the potent antioxidant and beneficial nutritional properties of *E. phaseoloides* seeds the present study was designed to study the effect of the seed of *E. phaseoloides* on growth performance, carcass characteristics and haemato- biochemical parameters along with its effect on expression of *hsp70* in broiler chicken.

Materials and Methods

Experimental Site

The study was conducted at the Department of Pharmacology & Toxicology, College of Veterinary Science, Khanapara, Guwahati-22 for a period of 35 days from 2nd March to 6th April, 2015.The study was carried out as per approval of Animal Ethics Committee guidelines No.770/03/ac/CPCSEA/FVSc, AAU/IAEC/06/20 (21/11/09).

Experimental Birds and Management

A total of 90 numbers of day old chicks were procured from a commercial hatchery of same batch. The chicks were brooded together for first two days of age in order to acclimatize them with the environment. The birds were wing banded and weighed individually and were distributed randomly into three treatment groups, of three replicates with 10 chicks in each group. They were assigned to three different dietary treatment having a commercial feed (crumble form):Diet 1 (Control) only basal diet, Diet 2: Basal diet+0.5% *Entada phaseoloides* seed powder labeled as T_1, Diet 3: Basal diet+1%

Entada phaseoloides seed powder labeled as T_2. Irrespective of treatment, all the chicks were fed with starter diet from 1-21 days and 22-35 days with finisher diet. The chicks were vaccinated (Ranikhet & IBD) and provided with sufficient water throughout the experimental period.

Collection and processing of *Entada phaseoloides* seeds

The *E. phaseoloides* seeds were collected from Sonapur, Assam, shade dried and then dried in hot air oven at a temperature of 40°C for 3-4 h. Dried seeds were powdered in a Grinder and the powder was stored in an air tight container for using in the experimental trial.

Data collection and Analysis

The birds were weighed at the start of the feeding trial and subsequently on weekly basis using a weighing balance. Daily weight gain was calculated from the weekly gain. Feed offered to the birds were weighed weekly and residues were also weighed to determine the feed intake of the birds. At the end of the feeding trial, one bird per replicate i.e. three birds per treatment were randomly selected, weighed, blood was collected (approximately 3ml) for serum biochemical study from the wing vein in a sterilized disposable syringe, transferred to vial and evaluated (Siemen and Synergy Bio). The birds were slaughtered for assessing the carcass characteristics. The statistical analyses of the experimental data (Feed intake, weight gain, and feed conversion ratio, carcass and organ weight) were carried out according to the method described by Snedecor and Cochran [10]. Results are presented as mean ±SD (Standard Deviation).

Detection of hsp70 gene by Reverse Transcriptase PCR

The experiment was designed in such a manner that the form of stress exerted on the birds were mostly the transition and captivity stress. Tissues viz. heart, liver and spleen were carefully excised from the broiler birds of three different groups (control, T_1 and T_2) for the study soon after slaughter. Each tissue was separately homogenized using micro pestle (Tarsons). The total RNA was isolated using TRIzol™ (Ambion). The mRNA was reverse transcribed by RevertAid First Strand cDNA synthesis kit (Thermo Scientific) with random hexamer primers. Initial step included 6.5µl reaction mixture containing 3µl RNA template, 0.5µl Random hexamer and 3µl nuclease free water with PCR condition of 65^0C for 5 minutes. Subsequent step includes 2 µl 5X buffer, 0.5 µl dNTPs, 0.5 µl RNase Inhibitor (Ribolock) and 0.5 µl Reverse Transcriptase making the total volume of reaction to 10 µl. The PCR condition in final step comprised of three phases, initiation at 25^0C for 5 min, elongation 42^0C for 60 min and termination at 70^0C for 15 min and 4^0C hold for infinite time.

Detection of the *HSP70* gene was performed using Polymerised Chain Reaction (PCR) technique using reported primer having sequence –Forward – AGCGTAACAC CACCA TTCC, Reverse- TGGCTCCCACCCTAT CTC [11] with a concentration of 10pmol and product size of 372bp. The PCR reaction mixture contained a total reaction volume of 25 µl composed of 12.5 µl 2X PCR Master Mix (Fermentus), 0.5 µl of each primer, 1 µl template and 10 µl water. β-actin was used as a positive control for the experiment using reported *hsp70* primers with following sequence Forward- GGAAGTTACTCGCCTCTG, Reverse- AAGACACTTGTTGGGTTAC[12] with a concentration of 10 pmol and product size of 114bp. Amplification was carried out in a thermal cycler (Applied Biosystems- Veriti- Thermal cycler). PCR condition included predenaturation at 95^0C for 5 min, followed by 35 cycles of denaturation at 95^0C for 30sec., annealing at 60^0C, 58^0C for *hsp70* and β-actin respectively for 45 sec. and extension at 72^0C for 45 min followed by final extension at 72^0C for 10min.The PCR product was visualized in 2% agarose gel in 1X TAE. The gel picture was than analyzed by Image Lab software in order to access the relative quantity of the bands.

SDS-PAGE and Immunoblot Analysis of HSP70

Liver, Spleen and Heart samples (30 mg approx.) were homogenized using micro pestle (Tarsons) in 5 mL of chilled lysis Buffer (RIPA Buffer, Amresco, USA) and were centrifuged at 23,000×*g* for 20 min at 4^0C. The protein concentration of the supernatants was quantified by Bradford reagent (Himedia) with BSA as the standard. Fifty micrograms of total protein was loaded and separated in 10% polyacrylamide gels containing SDS[13] using the Hoefer Midi Gel apparatus (Harvard Apparatus, Holliston, MA). Gels were electrophoresed at 150 V until the tracking dye reached the base of the gel. The fractionated proteins were visualized by Coomassie blue staining or transferred to nitrocellulose membrane[14] using semi dry blotting apparatus (Hoefer). The membrane were then blocked using 10ml of cold blocking buffer containing 3% BSA in TBST (Tris Buffer Saline with Tween 20) for 1hr.

The membranes were incubated overnight (4^0C) with 5 mL of 1% BSA in TBST containing antiserum (mouse anti-chicken hsp 70 (Sigma Chemical Co.) against HSP 70 in a 1: 500 dilution. After overnight incubation, the blots were washed 4 times (5 min each) with 10 mL of TBST. The blots were then reacted with goat anti-mouse secondary antibody conjugated to HRP (Santa Cruz Biotechnology) for 1 h. After rinsing with cold TBST, the color reaction on the nitrocellulose membrane were obtained using commercially available Ultra TMB Blotting Buffer (Pierce Biotech, USA).

Statistical Analysis

The statistical package, Graph Pad Prism 5 was used to analyze all results. Values are expressed as mean ± S.E.M. One way ANOVA followed by post hoc analysis (Dunnett's test, Tukey) was used for analysis of data and for comparisons between treated and control groups. $P < 0.05$ was considered significant.

Results and Discussion

It has already been reported from our previous study that the proximate composition of *Entada phaseoloides* seed contains appreciable amount of crude protein (24.06%), crude fat (8.3%), Nitrogen free extract (61.18%), and Total ash (2.5%) calorific value 415Kcal/kg. The minerals, iron, calcium and phosphorous are comparatively high. Similarly the phytochemical screening also reveals the presence of alkaloids, diterpene, triterpene, glycosides, steroids, saponins, phenolics, flavonoids in higher amounts and tannins in trace amount [15]. The crude protein values were slightly higher than the recommended values for starter and finisher ration of broiler.The presence of these essential nutrients and minerals implies that *Entada phaseoloides* seed could be utilized as a feed ingredient in poultry.

Growth Performance of Broiler chicken

The average initial, final body weight, weight gain, feed intake and FCR of the experimental broiler birds are presented in Table 1. Initially, there was no significant difference in body weight (P>0.05). Finally at 35 days of age the body weight differed significantly (P<0.05) between the control and treatment (T_2) group i.e. at 1% *E. phaseoloides* seed powder supplementation group. On an average, the body weight was recorded highest in T_2 (1%) group. Similarly the body weight gain was found to be the highest in T_2 group than control and T_1 group. The FCR in group T_2 was lower as compared to T_1 and control but does not differ significantly.

Similar results were reported by other workers when birds fed with 1 to 2% garlic [16]. Significantly improved live weight and better FCR was recorded @ 2% coriander seed supplemented group [17]. Higher growth and better feed conversion efficiency of the treated group (1%) may be due to beneficial antioxidant activity of *E. phaseoloides* that might have positive effect on nutrient uptake in terms of protection of intestinal villi resulting in more nutrient absorption [18].

Table 1: Effect of dietary supplementation of *Entada phaseoloides* seed powder on body weight gain (g) and FCR in broiler

Groups	Initial weight (g)	Final weight (g)	Weight gain (g)	Feed intake (g)	FCR
Control	41.53±0.12	963.3±9.23	921.80±21.71	1870.50	2.02±0.11
T_1 (0.5%)	41.59±0.15	1023±24.37^{ac}	981.74±24.25	1947.39	1.98±0.12
T_2 (1%)	41.51±0.50	1177±39.46^{ab}	1135.0±38.93	1964.36	1.73±0.07

Effect on Haemato biochemical parameters

The data pertaining to haematobiochemical profile is presented in Table 2. Blood glucose (mg/dl) level was almost similar in all the groups. Maximum cholesterol level was found in T_2 group (151.2±6.65mg/dl) and lowest in control (92.70±13.75) but the results of glucose and cholesterol did

not differ significantly. Total protein, albumin and globulin levels were found to be the highest in T_2 group (1%) in comparison to T_1 and control group. The protein values differed significantly (P<0.0001) between T_2 and control group. Maximum AST and ALT values were observed in control group (136.86±1.94 and 11.44±1.65 IU/L), while minimum was observed in T_2 group (133.17±4.07 & 6.98±1.74 IU/L) however does not differ significantly (P>0.05). Triglycerides values are almost similar in all the groups. Haemoglobin values in T_2 group was significantly higher (P<0.05) than control and T_1 group. The results are in agreement with another study, who reported significantly higher Hb levels in neem supplemented group @ 2g/kg & 3g/kg than the control in Japanese quails [19]. Similar results were reported on Ginseng administration in animal experiment where they have reported, it greatly enhances the biosynthesis of DNA and protein in bone marrow and increased iron incorporation into RBC [20]. There were no significant differences (P>0.05) in PCV and ESR values between the groups, and the results were found almost similar in all the groups. Therefore, it is evident that the feed supplementation has no adverse effect on any biochemical parameters except elevation or improvement of haemoglobin and protein concentration which may be due to high protein and iron content of *E. phaseoloides* seed itself reported by us [15]. Consequently, biosynthesis of amino acid in hepatic cells might increase the serum protein level.

Table 2: Effect of *E. phaseoloides* seed powder on haemato biochemical profile (mean± S.E.) of broiler birds

Parameters	Control (0%)	T_1 (0.5%)	T_2 (1%)	Level Of Significance
Glucose (mg/dl)	102.6±1.34	106.2±1.84	104.9±1.26	NS
Cholesterol(mg/dl)	92.70±13.75	107.3±23.77	151.2±6.65	NS
Total Protein (g/dl)	3.37±0.06	4.04±0.12	4.45±0.07	***(P<0.0001)
Albumin(g/dl)	1.36±0.05	1.39±0.14	1.61±0.09	NS
Globulin(g/dl)	2.00±0.01	2.65±0.06	2.83±0.16	NS
A/G ratio	0.68±0.02	0.52±0.06	0.57±0.07	NS
AST(IU/L)	136.86±1.94	133.75±1.21	133.17±4.07	NS
ALT(IU/L)	11.44±1.65	7.37±0.19	6.98±1.74	NS
Triglycerides	54.53±3.97	58.85±3.24	56.35±4.00	NS
Hb(g/dl)	6.33±0.29	8.33±0.33	9.06±0.93	*(P<0.05)
PCV (%)	26±2.64	21±6.08	31±1.52	NS
ESR(mm/hr)	0.30±0.10	0.40±0.10	0.46±0.03	NS

LOS- Level of Significance

asterisk* indicate significant difference at 5% level (P<0.05), ***(P<0.0001)indicate significant difference at 1% level, NS=Non Significant, LOS=level of significance

Carcass characteristics

The carcass characteristics of different groups are presented in Table 3. The results revealed no significant difference among the dietary treatment in the carcass traits. The average, live weight of the birds selected randomly on the basis of the treatments showed a maximum dressing percentage (74.88±0.49) in T_2 group (1%). Minimum dressing percentage was found in control group supplied with basal diet. The highest weight of the drumstick was found in T_2 group (122.6±7.88) and the lowest was found in control (117.9±3.31).The results of carcass parameters showed no significant difference between the control and treated group and 0.5% and 1% level of *E. phaseoloides* seed powder did not affect the carcass characteristics in chicks. However at 1% level improved the dressing percent of the chicks, increased mean dressed weight, drumstick weight, thigh, neck, wings, breast, liver, heart, and gizzard weight, probably due to optimum antioxidant activity of *E. phaseoloides* seed at 1% level that stimulated protein synthesis by enzymatic activity of the birds. As *E. phaseoloides* seeds contain a good amount of protein as reported [15]. The present results are in agreement with [21] who reported that supplementation of cinnamon powder @250-2000mg/kg in broiler diets didn't have any influence on carcass parameters. Similarly, it was reported [22] that inclusion of *Moringa oleifera* undecorticated seeds powder (MOUSP) in the broilers diet (0, 0.37, 0.75 and 1.5%) failed to produce significant effect on dressing percentage, liver and heart weights.

Detection of hsp70 gene

The results were evaluated by Image Lab (Bio-Rad) with a relative quantification principle. The results were interpreted as the relative quantity, obtained by keeping the control group as the reference group. Here the gene of concern was *hsp70* and β-actin was taken as an internal control (Figure 1A). Significant variation of expression was observed in spleen (Figure 1B, 2) in both the treatment groups (T_1 and T_2), where the expression was found to be less as compared to the control and similarly significant variation in expression was observed in liver (Figure 1C, 2) where T_2 showed more significant effect ($P<0.001$).Although heart tissue did not show any significant ($P>0.05$) difference in expression of *hsp70*. T_2 group with 1% supplementation showed decrease in expression levels as compared to the control group.

Table 3: Carcass Characteristics of Broiler fed with different proportion of *Entada phaseoloides* seed powder

Parameters	Control (0%)	T_1 (0.5%)	T_2 (1%)
Live weight (Kg)	1.05±0.05	1.04±0.06	1.06±0.01
Dressed weight (Kg)	0.757±0.034	0.763±0.05	0.798±0.014
Dressing percentage (%)	72.06±2.63	73.30±0.91	74.88±0.49
Drumstick (%)	117.9±3.31	120.2±2.06122	122.6±7.88
Thigh (%)	111.50±7.9	117.40±9.20	118.61±4.37
Breast (%)	232.03±14.90	244.60±7.64	246.56±18.01
Neck (%)	60.86±5.07	61.3±5.35	64.35±0.54
Back (%)	134.93±4.55	135.08±12.82	145.27±5.23
Wing (%)	94.12±4.35	89.56±2.38	96.65±1.52
Liver (%)	18.68±1.19	18.88±1.04	22.56±1.79
Gizzard (%)	20.60±0.83	22.5±2.10	23.1±3.46
Heart (%)	5.73±0.27	6.42±0.20	6.54±0.311

Values represent mean ± standard deviation (SD) of triplicate results,
Group C=represent broiler chicken fed Basal diet, T_1=represents broilers fed basal diet +0.5% *Entada phaseoloide*s powder, T_2=represents broilers fed with basal diet+ 1% *Entada phaseoloide*s powder.

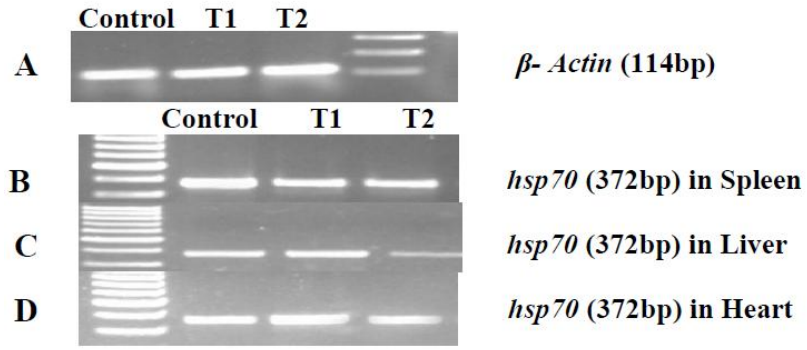

Figure 1 : Effect of *Entada phaseoloides* seed powder on protein expression of *hsp70* mRNA in the B- liver, C- spleen and D- heart of control and treatment groups of broiler. Lane 1 corresponds to tissue lysate of control group and Lane1 and 2 corresponds to tissue lysates of treatment groups. A- Represents β- Actin mRNA expression in corresponding groups in tissue lysates

The *hsp70* is synthesized constitutively under normal growth conditions but its expression increase following thermal challeng orstimulation from a variety of other environmental stressors (transportation and captivity stress in our experiment) and its stress-induced response varies between different tissues [11]. Our finding reveled expression of the *hsp70* gene but decreasing trend was observed in spleen and liver tissue (Figure 1, 2) of treatment group which was in partial agreement with Akbarian *et al* [23]. The antioxidant property of [15],[24] *Entada phaseoloides* seed powder might have protective impact on cellular stress by neutralizing free radicals and other harmful molecules generated in the

body during stress as compared to the control group. It has been documented that production of free radicals and expression of antioxidant enzymes during oxidative stress increases, which can be considered as protective response of cells against oxidative stress [25]. High nutritive properties of the *E. phaseoloides* seed powder can also be taken in to account in having the ability to combat the stress by having least toxic effect on the vital tissues. It has been documented that stressors other than thermal stressors, for example, exposure to heavy metals, toxins, oxidants, bacterial and viral infections [26], may also elicit hsp response.

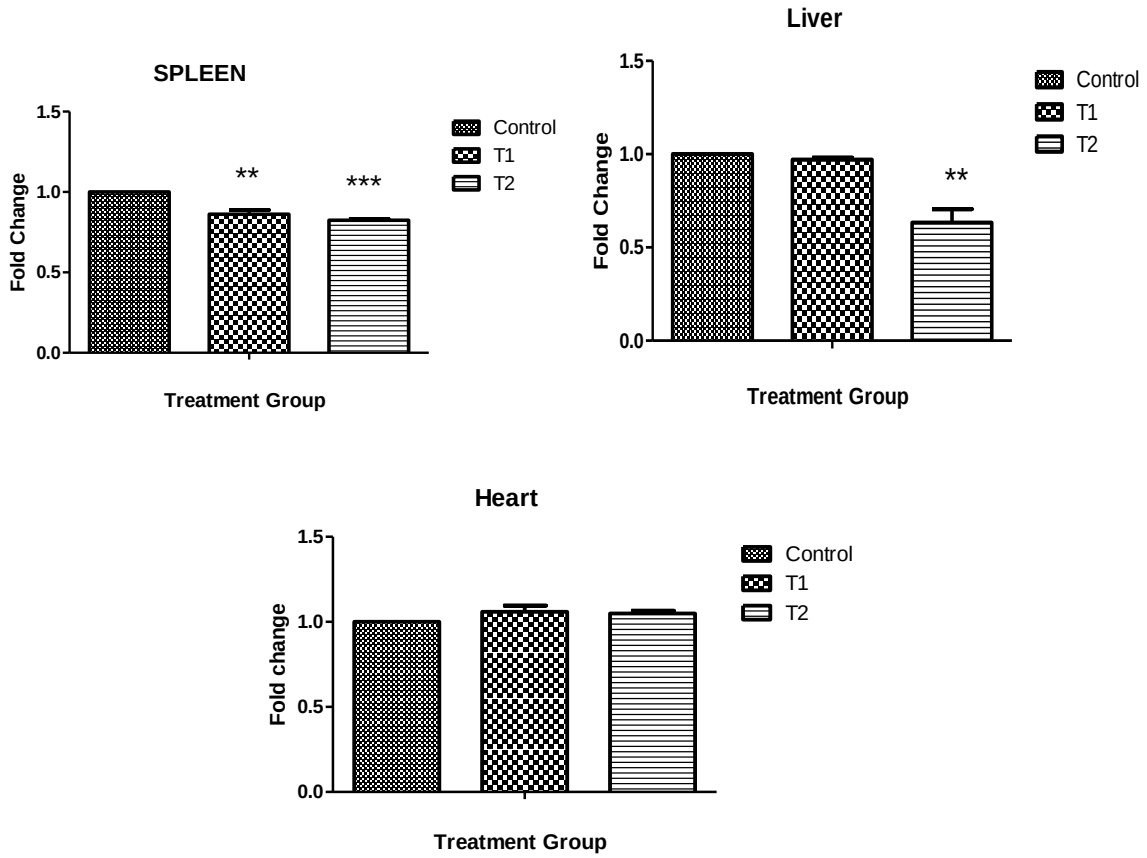

Figure 2: Quantitative Expression of hsp70 in corresponding tissues in Control, T_1 and T_2 treatment group. Values expressed as percentage fold change of expression of hsp70 in corresponding tissues. Values are expressed as Mean ± SEM (n=3)
***P<0.05 as compared to the control group**

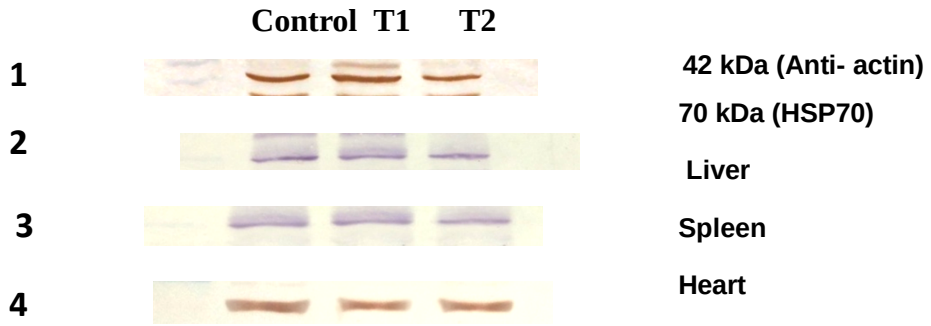

Figure 3: Effect of Entada phaseoloides seed powder on protein expression of HSP70 protein in the 2- liver, 3- spleen and 4- heart of control and treatment groups of broiler. Lane 1 corresponds to tissue lysate of control group and Lane1 and 2 corresponds to tissue lysates of treatment groups

Detection of HSP70 by Immunoblotting

Further confirmation of the RT-PCR results was done by immunoblotting technique. The results were obtained by analyzing the membrane picture by Image J (NIH, Bethesda MD, USA) as relative quantification. The analysis revealed similar trend to that of RT-PCR with significant (P< 0.05) decrease in HSP70 protein concentration in liver and spleen tissue (Figure 3,4) of treatment group when compared to control group. Similarly, heart tissue have not shown any significant level of expression of HSP70 protein (P>0.05). Here Anti- actin was taken as internal control and the expression of HSP70 protein was compared with Anti- actin expression.

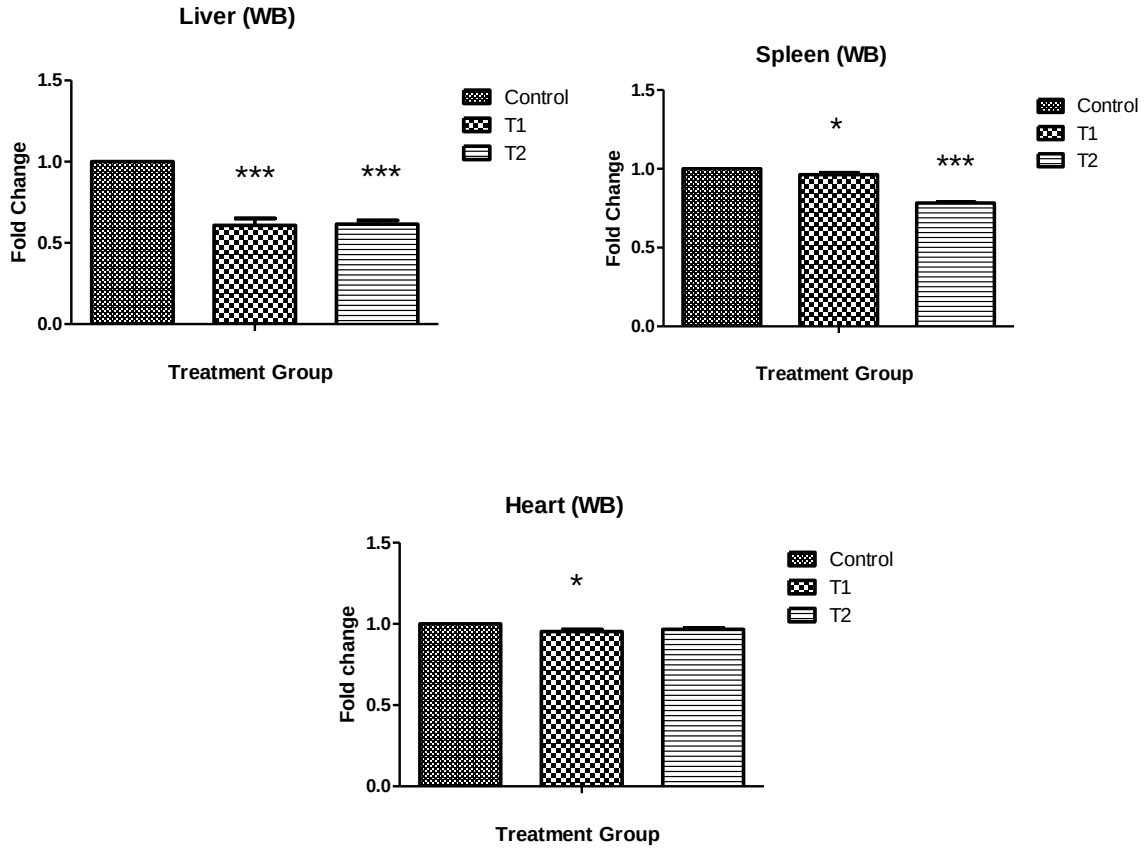

Figure 4: Quantitative data expressing the HSP70 protein levels was assessed using NIH Image J software and is expressed as percentage fold change compared with loading control (β-actin). All the values are represented as mean±SD (n=3). Statistical significance was determined by one-way ANOVA followed by Dunnet post hoc test, *P<0.05 as compared to the control group

Conclusion

From this study it may possibly be concluded that following supplementation of 1% *E. phaseoloides* seed powder, growth performance, FCR, serum protein, Hb of broilers were improved. The expression of *hsp70* showed significant down-regulation in T_2 group specially in spleen and liver. Spleen, being the recycling unit of blood cells has very important role in combating stress and liver plays an important role in metabolism of xenobiotics, there is positive effect on both the organs post supplementation with *E. phaseoloides*. On the basis of the results obtained through *hsp70* expression pattern it can be concluded that *E. Phaseoloides* might have the ability to combat stress. No reports of *E. phaseoloides* seed powder has been found to be used as an additive in broilers. Hence the present study unveils the effect of *Entada phaseoloides* as feed supplementation in broiler chick advantageously for the first time.

Acknowledgement

The authors are grateful to Indian Council of Agricultural Research, Govt. of India, New Delhi for providing financial assistance. Facilities provided by Biotech Hub FVSC, Khanapara and Gauhati University and The Director of Research (Vety), CVSc, Khanapara are also gratefully acknowledged.

References

1. Kirtikar K.R., Basu B.D. and Blatter E., Indian Medicinal Plants, Lalit Mohan Basu Publishers, Allahabad, 906–907 **(1935)**

2. Barua C.C., Hazorika M. and Misri J., An Overview of *Entada phaseoloides*: Current Research and Future Prospects, Journal of Pharmacy and Pharmacology, 2(1), 1-18 **(2014)**

3. D-E. The wealth of India, A Dictionary of on Indian Raw Materials and Products, Industrial National Institute of Science Communication and Information Resources. New Delhi, 3, 174-175 **(1948)**

4. Mohan, V.R. and Janardhana K., Chemical and nutritional evaluation of raw seeds of the tribal pulses *Parkia roxburghii* G. Don. and *Entada phaseoloides* (L.) Merr., International Journal of Food Sciences and Nutrition*,* 44(1), 47-53, **(1993)**

5. Koletzko B., Aggett P.J., Bindels J.G., Bung P., Ferré P., Gil A., Lentze M.J., Roberfroid M. and Strobel S., Growth, development and differentiation: A functional food science approach. British Journal of Nutrition, 80, S5-S45 **(1998)**

6. Ashburner M. and Bonner J.J., The induction of gene activity in Drosophila by heat shock, Cell.,17, 241-254 **(1979)**

7. Craig E. A., The heat shock response, Critical Reviews in Biochemistry, 18, 239-280 **(1985)**

8. Lindquist S., The heat shock responses, Annual Review in Biochemistry, 55, 1151-1191**(1986)**

9. Lindquist S. and Craig E.A., The heat shock proteins, Annual Review in Genetics, 22, 631-637 **(1988)**

10. Snedecor G.H. and Cochran W.G., Statistical Methods*.* 8th Edition, The Iowa State University press, Ames, I.A. **(1994)**

11. Yu J. and Bao E., Effect of Acute Heat Stress on Heat Shock Protein 70 and Its Corresponding mRNA Expression in the Heart, Liver, and Kidney of Broilers, Asian-Australian Journal of Animal Science, 21(8), 1116 -1126 **(2008)**

12. Amrutkar S.A., Saxena V.K., Tomar S., mRNA profiling of *HSP-70* under different tropical stress conditions in various broilers, Veterinary World, 7, 100-108 **(2014)**

13. Sambrook J., Fritsch E.F., Maniatis T., Molecular cloning: a laboratory manual, 2nd edition, Cold Spring Harbor: Cold Spring Harbor Laboratory Press, **(1989)**

14. Towbin H., Staehelin T., Gordon J., Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications, Proceedings of National Academy of Sciences, USA, 76, 4350-4354 **(1979)**

15. Barua C.C., Hazorika M., Misri J., Sen S., Bora M., Saikia B.N. and Hazarika M., Phytochemicals, In vitro Antioxidant activity and proximate composition of Seeds of *Entada Phaseoloides Linn.* Merrill. International Journal of Pharma and Bio Science, 6(3), 366 - 376 **(2015)**

16. Bampidis V.A., Christodoulou V., Florou-Paneri P., Christaki E., Chatzopoulou P.S., Tsiligianni T. and Spais A.B., Effect of dietary dried oregano leaves on growth performance, carcass

characteristics and serum cholesterol of female early maturing Turkeys, British Poultry Science.46: 595-601 **(2005)**

17. Hamodi S.J., Al-Mashhadani E.H., Al-Jaff F.K. and Al-Mashhadani H.E., Effects of coriander seeds (*Corriandrum sativum L.)* as diet ingredient on broilers performance under high ambient temperature, International Journal of Poultry Science, 9(10), 968-971 **(2010)**
18. Akhtar S., Mohammad K.R., Akhtar S., Muhammad S. and Ali A., Nutritional value and glycaemic index of KFC and Local burgers in normal and diabetic human subjects, Journal of Applied Pharmacology, 2(3), 191-196 **(2011)**
19. Kaushal S., Kumar A., Palod J., Patoo R.A., Soni R. and Singh M.K., Haematobiochemical studies in Japanese quails on diet supplemented with Neem (*A. Indica*) leaf powder, Indian Veterinary Journal, 91(6), 90- 93 **(2014)**
20. Huang C.K., The Pharmacology of Chinese Herbs, 2nd edition, CRC press, LLC, 37**(1999)**
21. Koochaksaraie R.R., Irani M.and Gharavysi S., The effect of Cinnamon powder feeding on some blood metabolites in broiler chicks, Brazilian Journal of poultry Science, 13,197-201 **(2011)**
22. Abbas T.E. and Ahmed M.E., Use of *Moringa oleifera* seeds in broilers diet and its effects on the performance and carcass characteristics, International Journal of Applied Poultry Research, 12(1), 1-4 **(2012)**
23. Akbarian A., Michiels J., Golian A., Buyse J., Wang J. and Smet S. De, Effects of plant-derived oils on HSP70 and antioxidant enzyme gene expression in Broilers under high ambient temperatures, Conference Proceedings. 19th European symposium on Poultry Nutrition, Germany, http://hdl.handle.net/1854/LU-4127264, **(2013)**
24. Aktar F., Kuddus Md. R., Faruque S. O., Rumi F., Quadir M.A. and Rashid, Md. A. Antioxidant, Cytotoxic, Membrane Stabilizing & Antimicrobial Activities of Bark & Seed of *Entada phaseoloides* (L.) Merr., A Medicinal Plant from Chittagong Hill Tracts, Journal of Pharmacy and Nutrition Science,1, 171-176 **(2011)**
25. Mcardle A. and Jackson M.J., Exercise, oxidative stress and ageing, Journal of Anatomy, 197, 539–541 **(2000)**
26. Morimoto R.I., Cells in stress: Transcriptional activation of heat shock genes, Science, 259, 1409–1410 **(1993)**