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Esterase variability in different tissues of Naked Neck Fowl (*Gallus gallus domesticus*) of Bangladesh using polyacrylamide gel electrophoresis

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Abstract

Naked neck fowl (*Gallus gallus domesticus*) comprises one of the indigenous chicken populations in Bangladesh. The present investigation was conducted to study the polymorphic pattern of the estrase isozyme in different tissues of the fowl by polyacrylamide gel electrophoresis (PAGE) system. In this experiment, α-napthyle and β-napthyle acetate were used as substrate. Esterase variability of different samples of fore brain, mid brain, hind brain, heart brain, liver, testis, oesophagus, gizzard, bile ducts, proventiculus, small intestine, large intestine pectoral muscle, pelvic muscle, lung, eye, kidney, pancreas, body muscle and blood cells were examined. Altogether four esterase bands were observed and the bands were designated as Esterase Est-1 $^{2.0}$, Est-2$^{1.0}$ Est-3$^{0.75}$ and Est-4$^{o.15}$. Among the four bands Est-1 was the fastest and placed near to anode (+) electrode. Esterase showed tissue specific variation. Est-2 was found in almost all tissues, whereas Est-1 was observed only in liver. Est-3 and Est-4 were expressed exclusively in the nervous system especially in brain. Such findings may provide basic information to analyze the esterase activity in different tissues.

Keywords: Esterase, Isozyme, Naked neck fowl, Tissue

Introduction

Poultry production is a promising sector for poverty reduction in Bangladesh. Approximately 140 million chickens are scattered throughout 68000 villages in the country mostly of indigenous non-descript type (Barua and Howlider, 1990). The production system for indigenous chickens is smallholder backyard scavenging in nature with each family keeping an average of 6-7 chickens to meet family requirements and from which a cash income can also be derived when necessary. Among the indigenous chicken genetic resources non-descript Deshi Aseel and Naked Neck (NN) are noteworthy.

The indigenous chicken population is composed of a number of types such as non-descriptive Deshi, Aseel, NN and Hilly. Some dwarf chickens and Red Jungle Fowls can also be seen in the country. The non-descript Deshi chicken is more acceptable to rural people as an important source of meat and eggs (Barua and Howlider, 1990) due to lower nutritional demand and higher resistance to diseases and heat stress. These rural Deshi chickens provide about 78% of poultry meat and 75% of eggs for domestic consumption. But these potential genetic resources are undergoing genetic erosion due to continuous introduction of exotic stock from developed country and their indiscriminate crossing with the indigenous chickens.

The Deshi and Naked Neck chickens are distributed throughout the country except in some hilly areas. Among the indigenous population egg production is higher in Naked Neck chicken (Hoque *et al*., 2003). Chicken are good models for studying the genetic basis of phenotypic traits, because of the extensive diversity among chickens selected for different purposes. Monogenic traits are well-studied but many interesting traits are complex and determined by an unknown number of genes (Pisenti *et al*., 2001; Dodgson 2004 and Nicholas 2003). Chicken are used as models for studying atherosclerosis, hypertension, cholesterol metabolism, bone development, pathology and surgical studies (Dodgson, 2004).

Isozymes are multiple forms of a single enzyme, which often have different isoelectric points and, therefore, can be separated by electrophoresis. Esterase isozymes are one of the lipid-hydorlizying enzymes, possess high significance in genetics and toxicology (Markert and Moller, 1959 and Callaghan *et al.,* 1994). Electrophoretic studies were done extensively on the different tissues of various animals from which it reveals that the enzyme exists in multi molecular forms (Markert and Moller, 1959). The banding pattern of esterases appears to be genetically controlled and, therefore, it has been used to estimate the genetic distance among different populations or the distance between populations (Turner, 1973). Esterases have also been extensively studied in different insects and vertebrates and for different physiological processes, such as regulational of hormone level, reproduction, functioning of nervous system (Holmes and Whitt, 1970; Champion et al., 1975 and Brestkin *et al*., 1975). Esterases are also used as bioindicators to measure the toxic potency of pesticide residues usually applied in agriculture (Brestkin and Grioria, 1975). The present study was investigated to study the polymorphic pattern of the esterase isozyme in different tissues.

Materials and Methods

Sample collection

The whole carcass of one Naked neck fowl was collected from Gazipur district in Bangladesh. The collected carcass was dissected to collect the required tissues viz. forebrain, midbrain, hindbrain, heart, liver, testis, oesophagus, gizzard, crop, proventiculous, small intestine, large intestine, pectoral muscle, pelvic muscle, lung, eye, kidney, pancreas, body muscle and blood. Separate special sterile bags were used for carrying the samples to avoid further contamination. The samples were than transported to the laboratory in an insulated box with ice to maintain the temperature around 4-6°C. In the laboratory, each sample was taken into eppendorf tube and kept in deep freeze at -80ºC until further processing.

Preparation of the sample

About 0.016gm different tissues of the Naked Neck fowl were taken in to an eppendrof tubes and squashed in 1 × Tris-Borate EDTA buffer adding about 40 ml. After squashing 2 × Bromophenol blue solution was added to each sample as the amount of 10 ml. The samples were then centrifuged at 12600 rpm for 15 min at 25°C. Ten microlitre of supernatant was used in each slot of the gel.

Preparation of Gel

Seven and half percent (7.5%) gel was prepared using 7.5 ml of Acrylamide-bisacrylamide (30:0.8; Bio Basic Inc); 6.0 ml of 5 × of TBE buffer; 0.150 ml of 10% ammonium persulphate (AMPS; Fulka Biochemica); 0.140 ml of TEMED and 16 ml of sterilized distilled water.

Electrophoresis of gel

The gel was run at 120V constant voltage for at least I hr and 20 min. until the tracking dye (bromophenol blue) reached to the bottom of gel. The gel was recovered by dismantling the glass plates sinking in water very cautiously.

Staining and scoring of bands

The gel was taken in a staining tray and 30 ml of substrate mixture poured on to it for 15 min. at room temp. After 15 min, the substrate mixture was out poured and 30 ml of fast blue RR solution added to the gel and incubated at 37ºC for 25 min. Photograph of the gel was taken by a digital zoom lens camera laying the gel on a white back ground (Samsung Kenox-SHD lens, 3.2 mega pixels). Bands were scored on the stained gel from the highest to the lowest mobility and numbered accordingly (Webb, 1964). Relative mobilities were estimated towards the commonly found band Est-2 as the band for unity.

Statistical analysis

The data generated from this experiment were entered in Microsoft Excel worksheet, organized and processed for further analysis. Data were analyzed using the general linear models procedure with the help of Statistical Package for Social Sciences (SPSS) 10/11.5.

Result and Discussion

The esterase isozyme bands were analyzed by 7.5% polyacrylamide gel electrophoresis (PAGE). Elevation of esterase banding patterns was done in the presence of the two substrates, α and β napthyle acetate in different tissues of native naked neck fowl. Altogether four esterase bands (Est-1, Est-2, Est-3, Est-4) were found in the fowl with different mobilities (Fig. 1). The relative mobility of these esterase bands were 2 ± .02, 1 ± .02, .75± .02, 0.15 ± .02 cm, respectively (Fig. 2). All bands stained dark red color in α staining and purple in β staining. Based on intensity of staining, esterase isozyme bands could be arbitrarily described in the 3 categories such as deep stained, medium stained and faint stained (Table 1). All mentioned esterase bands had tissue and substrate specific expression. Ahuja and Schawb (1977) also found that tissue specific expression of esterases in sword tail fish (Xiphophorus helleri). The bands also showed an intensity variation among different tissues. Based on staining intensity, esterase isozyme bands could be arbitrarily described in the three categories such as- (a) deep stained, (b) medium stained and (c) faint stained.

Table 1. Electrophoretic banding pattern showing the intensity variation of esterase isozymes in different tissues of naked neck fowl (scored from α, β stained gel)

Sl. No.	Tissues	Est-1	Est-2	Est-3	Est-4
1	Fore Brain	-	DS	MS	DS
2	Mid Brain	-	DS	FS	MS
3	Hind Brain	-	DS	MS	DS
4	Heart	-	FS	-	-
5	Liver	MS	DS	-	-
6	Testis	-	DS	-	-
7	Oesophagus	-	DS	-	-
8	Gizzard	-	FS	-	-
9	Crop	-	DS	-	-
10	Proventiculous	-	DS	-	-
11	Small Intestine	-	DS	-	-
12	Large Intestine	-	MS	-	-
13	Pectoral Muscle	-	FS	-	-
14	Pelvic Muscle	-	FS	-	-
15	Lung	-	DS	-	-
16	Eye	-	FS	-	-
17	Kidney	-	FS	-	-
18	Pancreas	-	FS	-	-
19	Body Muscle	-	FS	-	-
20	Blood	-	DS	DS	-

DS=Deep stained
MS=Medium stained
FS=Faint stained

These esterase bands showed tissue specific variation. Among them Est-2 is common in almost all tissues. This finding indicates that Est-2 has a physiological activity in different tissues of the body of Naked Neck fowl. The staining intensity in esterase zymogram shows different levels of esterase band activity, denoted by different thickness and degree of staining, were another kind of variation that has been observed among the different tissues.

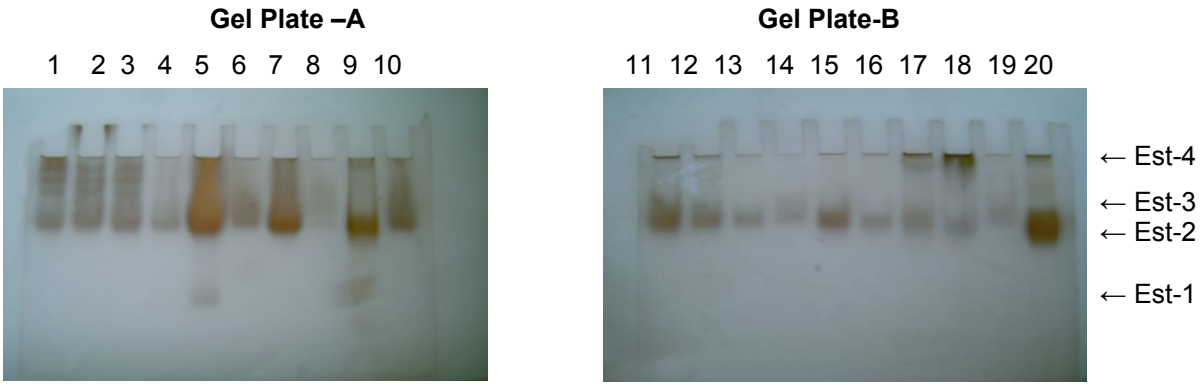

Fig. 1. Polyacrylamide gel showing esterase bands in different tissues of the naked neck fowl (Scored from α,β Stained Gel). 1-Fore brain, 2-Mid brain, 3-Hind brain, 4-Heart, 5-Liver, 6- Testis, 7-Oesophagus, 8-Gizard, 9-Bile, 10-Proventiculus ,11-Small intestine, 12- Large intestine, 13-Pectoral muscle, 14-Pelvic muscle, 15-Lung, 16- Eye , 17-Kidney, 18-Pancreas, 19- Body muscle, 20- Blood.

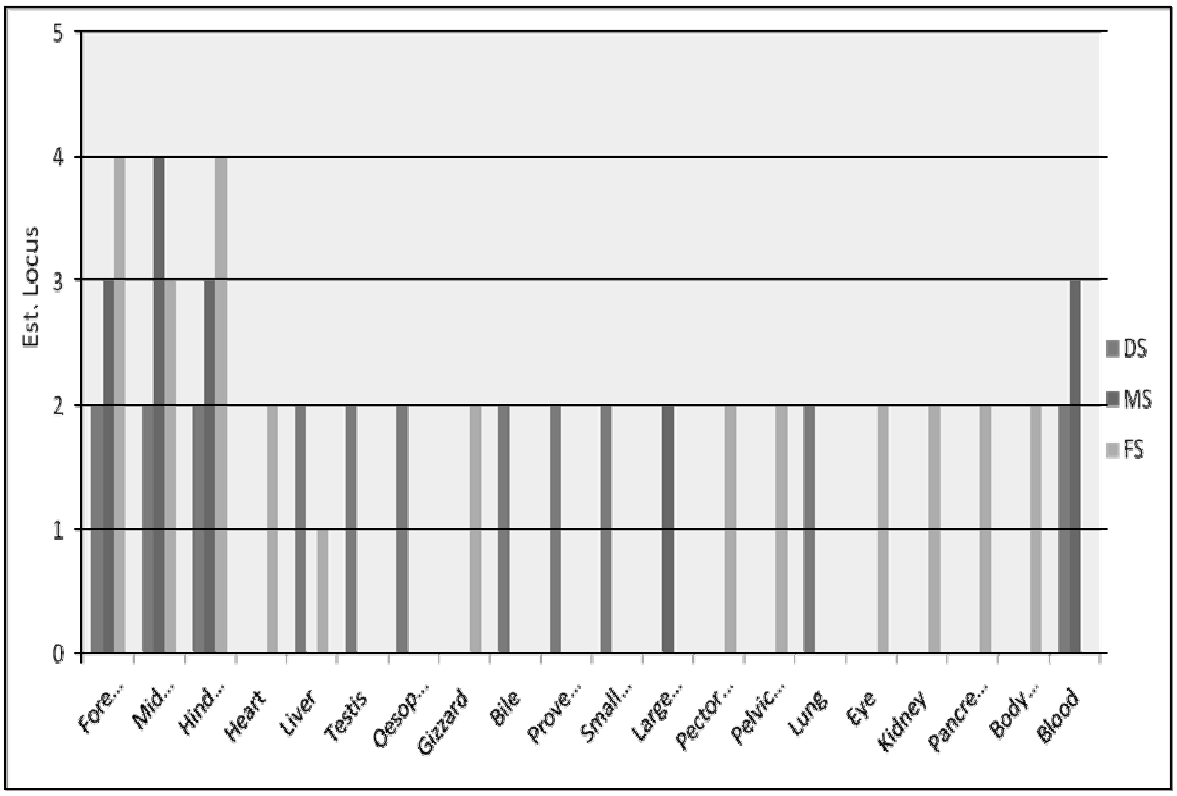

Fig. 2. Distribution of esterase bands in different tissues of naked neck fowl and their staining intensity

In the present study the maximum number of bands (Est-2, 3 and 4) were found in all the three parts of the brain tissues (fore, mid and hind) where Est-2 and Est-4 were deep stained and Est-3 was medium stained. This result indicates that Est-genes are active in the brain tissues. Similar result was observed in the brain of different species (Brestkin *et al*., 1975). In the liver, Est-2 was found more intensively. This indicates, Esterase showed the strong enzymatic activity in the liver tissue. Hirji and Courtney (1983) also found strong enzymatic activity in the liver tissues of perch fish (*Perca fluvitalis).* Liver esterases could be

associated with digestion and metabolism of different esters e.g. fat, cholesterol etc (Jones and Brancoft, 1986 and Sastry, 1974).The present study revealed that Est-1 was unique for liver. In the muscles (pelvic, pectoral and body), large intestine, heart, pancreas, gizzard, and eye esterase bands were expressed as faint. This finding indicates that the esterase action is poorly performed in those tissues.

Conclusion

The results suggested tissue wise variation in the banding pattern of esterase and may be used as a good genetic marker in terms of species identification.

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