PROTEIN PROFILING OF MELANOTIC MEAT IN CROSSES OF KADAKNATH CHICKENS

THESIS

Submitted in partial fulfilment of the requirements for the degree

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POULTRY SCIENCE

By

Dr. Anuj Kumar

Roll No. 4789

To

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DIVISION OF AVIAN GENETICS AND BREEDING
CENTRAL AVIAN RESEARCH INSTITUTE
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Dated : 30/7/2011

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Certified that the research work embodied in this thesis entitled "Protein profiling of melanotic meat in crosses of Kadaknath chickens" submitted by Dr. Anuj Kumar, Roll No. 4789, for the award of Master of Veterinary Science degree in Poultry Science at Indian Veterinary Research Institute, Izatnagar, is the original work carried out by the candidate himself under my supervision and guidance.

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Abbreviations

%	Percentage
µl	Micro liter
ºC	Degree celsius
ANOVA	Analysis of variance
AOAC	Association of official analytical chemists
APS	Ammonium per sulfate
BL	Black-skinned chicken
BMC	Black meat chicken
BW	Body weight
CARI	Central Avian Research Institute
CF	Conversion factor
CP	Crude protein
$CuSO_4$	Copper sulfate
DM	Dry matter
DW	Distilled water
EE	Ether extract
F_1	First filial generation
F_2	Second filial generation
Fig.	Figure
Fm	Fibromelanosis gene
g	Gram
HCl	Hydrochloric acid
H_2SO_4	Sulfuric acid
Hr (s)	Hour (s)
Id	Inhibitor of dermal melanin gene
kDa	Kilodalton
Kg	Kilogram
KN	Kadaknath

mA	Milli ampere
mg	Milligram
mg/dl	Milligram per deciliter
Min	Minute
ml	Mili liter
mM	Mili moler
mm	Mili meter
N	Normal
Na_2CO_3	Sodium bicarbonate
NaOH	Sodium hydroxide
NIN	National institute of nutrition
nm	Nano meter
O.D.	Optical density
PSLW	Pre slaughter live weight
RIR	Rhode Island Red
rpm	Rotations per minute
RTC	Routine table chicken
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
S.E.	Standard error
SL	Slaty-skinned chicken
TEMED	N,N,N^1,N^1-Tetra methyl ethylene diamine
W	White-skinned chicken
WLH	White leghorn
WR	White Plymouth rock
w/v	Weight by volume

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Introduction

In international scenario India posses a unique status for poultry production owing to its contribution to the agricultural economy of the country. Among the various animal species contributing to the national economy, the contribution of poultry is most significant and sizable one. However bulk of the poultry productivity of the country is contributed by the high yielding exotic chicken stocks bred and propagated by the international companies. The native chicken breeds, on account of their low productivity and viability issues, have taken a back seat in their propagation and patronage. These stocks however, are best suited to our Indian climate and backyard-rearing conditions, owing to their adaptability and disease resistance as well as heat tolerance abilities to the tropics. The genes controlling the tropical adaptability conditions ably signify the unique genetic architecture of these breeds of chickens.

Among the various chicken breeds of India, Kadaknath (KN) is an important indigenous breed which has evolved through centuries of natural selection in the tough agro-ecological conditions and is, therefore, well adapted to local environment. This breed has its home tract in Jhabua and Dhar districts of western Madhya Pradesh and adjoining areas of Gujarat and Rajasthan. It is the only Black-Meat Chicken (BMC) breed of India. This breed is locally known as "Kalamasi" meaning fowl having 'black flesh'. It is also known as 'Black gold of Jhabua'. The "Kalamasi" is a sacred bird offered as sacrifice to Goddess, after Diwali (Singh and Singh,1998). The bird is very popular among Adivasis mainly due to its adaptability to local environment, disease resistance, meat quality, texture and flavor. It tolerates extreme climatic conditions of summer heat and cold winter stress and thrives very well under minimal management inputs like poor housing, no health care or supplementary feeding while exhibiting appreciable degree of resistance to diseases compared to other exotic breeds of fowl (Thakur *et al.*, 2006).

Since a long time, Kadaknath breed of chicken was reared by tribals/Adivasies and therefore, through many generations of selection and fixation of genes, some of the important breed characteristics had been established. The flesh of Kadaknath birds is used by these Tribals for treatment of some chronic wasting diseases assigning many medicinal values and aphrodisiac effect to its meat (Singh and Singh, 1998). The KN has special medicinal value in Homeopathy too and is used in treatment of a particular nervous disorder (Anonymous, 2010).

The blackness of KN has been attributed to a character called fibromelanosis (Fm) that is responsible for hyper-pigmentation not only of the external but also in internal organs of the body. Most of the internal organs exhibit intense black coloration, attributed to the deposition of melanin pigment in connective tissues of organs (Rao and Thomas, 1984). Degree wise, it is more pronounced in trachea, thoracic and abdominal air sacs, gonads, elastic arteries at the base of the heart and mesentery. The commonly available varieties of Kadaknath are Jet Black, Penciled and Golden (Thakur *et al.*, 2006). The Jet black adult males and females are black in color. The golden variety adult males and females are basically black in color with golden feathers on neck and head, whereas in penciled variety, adult male and female plumages are black with white feathers on neck. In all the three varieties, skin, beak, shanks, toes and soles of males and females are dark gray-colored, whereas tongue is dark gray or light black in color. Comb, wattles and ear-lobes are light gray to dark gray in color. However, in comb, wattles and earlobes a purplish hue is also observed.

The only other chicken breed having characteristics like Kadaknath, is Silkie (silky), named for its unique fluffy plumage, which is said to be feel like silk. This breed originated in China and have long been used in traditional Chinese medicine. Silkies have black skin, bones and grayish-black meat, often referred as “Crow boned chicken” meaning Black boned chicken.

The Kadaknath breed is poor in egg- production potential, but their black flesh is very delicious and popular among tribal masses. Being well adapted to harsh environment of free range and produces eggs and meat at least possible cost. While Kadaknath is poor in egg production (lays around 105-110 eggs annually) in comparison to exotic layer breeds, it is categorized as a layer among Indian native breed. An egg-quality study (Parmar *et al.*, 2006) showed that Kadaknath hens lay brown-shelled eggs weighing 43 grams, with a yolk proportion of 34 percent and a shell thickness of 0.31 mm. It’s body growth rate is slow as compared to broilers and it attains about 400g weight at 8 weeks of age. The dressing percentage ranges from 60 to 63 percent at the age of 13 to 18 weeks of age (Singh and Singh, 1998).

The meat and eggs of Kadaknath are reckoned to be a rich source of protein (Rao and Thomas, 1984). The eggs of Kadaknath also provide an ideal nutrition, especially for old people and high blood pressure patients, since it has lower cholesterol content and higher free amino acids than white chicken (Arora, 2010). It is believed that due to Melanin deposition, the meat of Kadaknath has 3-4% more crude protein content with less fat content and meat is more tender and juicy as compared to meat from other breeds of chicken

(Mati and Verma, 1983). Kadaknath meat is practically without any fibres, easily digestible and delicious. Its flesh is of higher nutritional value and is being used for the treatment of many diseases in human beings by tribal's/Adivasies living in Jhabua and adjoining districts of Madhya Pradesh. A recent study by the Central Food Testing and Research Institute (CFTRI), Banglore, has confirmed that the bird had medicinal properties as claimed by the tribals. According to the analysis, its meat contained more than 25 per cent protein as compared to an ordinary bird with 18 to 20 percent. The fat content in Kadakanath meat was barely 0.73 to 1.03 percent as compared to higher percentages found in ordinary broilers.

In spite of this nutritional advantage the meat of Kadaknath is not relished in elite markets as an eye appeal plays vital role in the marketing of the products and consumers may not like the Kadaknath meat due to its black color.

During random field survey studies, it was found that the population of Kadaknath (KN) bird is declining rapidly and the breed is under threat of extinction. The breed is presently under ex situ conservation at CARI, Izatnagar (Panda and Praharaj, 2002). Kadaknath has widely been used in crossbreeding with White Leghorn (WLH) and White Rock (WR) breeds to develop KN-WLH and KN-WR crossbreds. The skin (dermis and epidermis) show characteristic blackness along with most of the internal organs. The fibromelanosis in Kadaknath is reportedly caused by an autosomal gene, 'Fm' (Singh and Singh, 1998). The hyperpigmentation of skin is a complex event regulated by an incompletely dominant, sex linked gene: 'inhibitor of dermal melanin (Id)' located on Z chromosome (Mishra *et al.*, 2008a). Studies have also shown distinct association of 'Fm' gene with improved meat quality (significantly lower meat-texture recorded in form of less shearing force) [Arora *et al.*, 2010] and reduction in collagen fibers, which needs elaborate studies.

Very little is known about the pigmentation potential and pattern of melanosis influenced by “Fm” gene in crossbred Kadaknath backgrounds, when the major gene get introgressed by strain or line crosses. Further there is a need to analyze this breed by raising suitable genetic groups via cross-breeding of Kadaknath into diverse recipient backgrounds of chicken (which lack ‘Fm’) like White Leghorn (WLH), broiler breed of White Plymouth Rock (WR) to determine the influence of “Fm” gene on the associated economic characters.

As the literature is largely hollow with respect to protein profiles on Kadaknath meat, it would be appropriate to study the protein-profiling aspects of the melanotic-meat from crosses of Kadaknath chickens (involving White Leghorn and White Rock chickens) displaying variable melanotic intensity both in their skin and meat, to infer the roles of causative genes, in quantity and quality of its protein. Accordingly, the following objectives are proposed.

OBJECTIVES:

1. ***To characterize the melanosis of skin and meat from crossbred-Kadaknath chickens involving non-melanotic chickens, and analyze selected meat-quality parameters in Kadaknath-White Rock crossbred chickens.***
2. ***To subject the melanotic tissue-protein from above crosses to gel-electrophoretic analyses for differentiating their compositional profiles.***

☑☑☑

Review of Literature

KADAKNATH BREED OF CHICKENS

The Kadaknath is an important indigenous breed reared by the tribals since a long time in the Jhabua and Dhar district of the Madhya Pradesh and adjoining areas of the Rajasthan and Gujarat. But, because of indiscriminate crossbreeding with RIR and other breeds, the pure Kadaknath birds are rarely available in Dhar and adjoining areas of Rajasthan and Gujarat. At present, the Kadaknath breed is mainly found in Jhabua district of Madhya Pradesh (Thakur *et al.*, 2006). Most of the internal organs exhibit the intense black coloration, which is due to the deposition of melanin pigment in the connective tissues of the organs and in the dermis (Rao and Thomas, 1984). This blackness has been attributed to a character called Fibromelanosis (Fm) that is responsible for hyper-pigmentation in most of the internal and external organs of the body. The fibromelanosis in Kadaknath is reportedly caused by an autosomal gene, 'Fm' (Singh and Singh, 1998). Recent studies have established that hyperpigmentation of skin is a complex event additionally influenced by an incompletely dominant, sex linked (Z chromosome-linked) gene: 'inhibitor of dermal melanin' 'Id' (Mishra *et al.*, 2008a). Studies have also shown distinct association of 'Fm' gene with improved meat quality (significantly lower meat-texture recorded in form of less shearing force) [Arora *et al.*, 2010] and reduction in collagen fibers (Sachdev *et al.*, 1996), which needs elaborate studies. Recent studies (Kawalkar and Bhambal, 1996) has also hinted that KN's meat is associated with higher content of essential fatty acids (Linoleic acid) and lower in meat-cholesterol, which are highly valued traits from modern-day consumer's point of view.

2.1 MELANIN PIGMENTATION: ITS BIOLOGICAL ROLES, INHERITANCE AND EXPRESSION IN CHICKEN

Melanin, one of the most widespread natural pigment, plays a major role in the pigmentation of feathers, skin, shanks, eye and internal tissues of vertebrates (Arora, 2010). This is produced by highly specialized cells, termed as melanocytes, which arise in embryonic neural crest and migrate during early embryonic development to their ultimate sites of residence. In contrast, ocular melanocytes of the retinal and iridial pigment epithelium and the pectin arise from the outer layer of the embryonic optic cup. Migration of the undifferentiated melanoblasts from the neural crest proceeds rapidly as evidenced by their presence in the limb buds by 80-91 hours of incubation. Shortly after reaching their destination, they differentiate into melanin producing melanocytes, characteristic of the tissue in which they reside. Some like those of the ocular choroid, testes and connective tissue membranes produce their pigment, and thereafter, become melanogenically inactive. In contrast, melanocytes destined to pigment feathers, colonize reservoirs of melanoblasts located in the dermis near the dermal papillae of the developing feathers.

Hirano (1990) studied the distribution of melanin pigment-containing cells in the bones of both young and adult silky fowls. Melanin pigment was detected not only in melanocytes which were mainly distributed in the periosteum, but also in all the other types of cells in the periosteum and bone. The continuity of the number of pigment granules in melanocytes and that in the other pigment-containing cells could not be recognized because the granules in the latter cells were much fewer than those in the former. In young fowls, the pigment-containing cells were distributed in all layers of the periosteum and bone, but their number was low. On the other hand, in aged fowls, most of the cells in the periosteum had pigment granules. In the bone, however, pigment granules were observed only in osteocyte situated near the surface. These findings suggested that the pigment granules which are observed in osteocytes have been transferred from melanocytes to osteogenic cells or osteoblasts before they differentiate to osteocytes, where they are presumed to be digested.

As pointed out by Porto (1981), melanin appeared early in evolutionary history and have largely remained unaltered to the present day. The two main types of melanins are: the eumelanin (black and dark brown) and pheomelanin (red and yellow). The basic eumelanin synthesis pathway involves the oxidation of the amino acid, tyrosine, to Dopa melanin and then Dopa-quinine by the enzyme tyrosinase. This is followed by a series of cyclizations and oxidations leading to the eumelanin polymer. Additional tyrosinase-related proteins (TRP1 and TRP2) are now known to be involved (Hearing, 1993), but their roles are not well understood at this time. Pheomelanin synthesis shares the same Tyrosine Dopa quinone pathway but diverge with the addition of Sulphhydril group to form Cysteinldopa, and ultimately the red and yellow melanins of the feathers. Pheomelanins are found only in the feathers of chickens, with all other melanins of the body being Eumelanins.

The final pigment product involves the attachment of the melanin to a protein matrix to form a pigment granule or melanosome. Thus, melanin exists as cytoplasmic organelles within melanocytes, or in feather keratinocytes following transfer from melanocyte dendrites during feather formation. Bowers (1988) and Smyth (1990) have comprehensively reviewed the available literature on the subject (melanin pigment and melanocyte function) that provides a valuable insight into the events leading to melanin deposition in various body tissues.

Roles played by melanin include display and recognition, as well as protective coloration in females as natural ground nesters. The absence or greatly reduced levels of melanin during embryonic development has also been shown to lead to altered visual pathways connecting the neural retina and the brain in several species. Additionally, melanin appears to serve at the cell level as a scavenger for free radicals, e.g. superoxides. There is also a strong evidence that melanin may be necessary for normal embryonic and early post hatch development in chickens.

2.1.1 Melanosis in the host

Melanin deposition in chicken has been studied in details relating to histopathology parameters and visual examinations of the carcass.

Hutt (1949) described that in the Silky fowl the beak and shank were bluish as in some of the other breeds, but in addition the skin all over the body was black. This usually had a purplish hue, particularly in the wattles, comb and face, from the combined effects of the black pigment in the skin and the red of the blood in capillaries. Hutt’s report recorded that: in some part of the world, this black skinned condition was also found in fowls, but not having the peculiar plumage of the Silkies.

The distribution of melanin in Silkies was studied by Kuklensi (1915), who found it in varying amounts in the dermis of the skin, in the sheaths of muscles and nerves, in tendons, mesenteries, wall of blood vessels and in the duramator and piamator of the brain. The lungs contained only small flecks of black pigment. No melanin was found in bones or in cartilages but periosteal and perichondrial membranes were highly pigmented. Most of the glands had little pigment except for that of their fibrous capsules, but the gonads contained much melanin. There was not at all any pigment, in the liver.

This type of pigmentation in Silky fowl was designated by Dunn and Jull (1927) as "mesoderma" to differentiate it from the dermal melanin induced by the gene "id".

Since, the melanophores of the silky fowl originated in the neural crest (Eastlick and Wortham, 1946) as do those of the plumage, Hutt (1949) linked to name this condition according to its distribution, rather than by its source in embryo. For that reason, the term "Fibromelanosis" meaning melanotic pigmentation of connective tissues was proposed as more nearly fitting the distribution of melanin found by Kuklensi (1915) in the Silky fowl.

2.1.2 Genetic principles of melanin deposition

The first evidence that the inhibition of dermal melanin was sex linked found by Davenport (1906) in a cross of dark Brahma female (yellow shank) x Tosa male (with willow shank) which yielded males, all with willow shanks. That time, sex linked genes were unknown and the significance of his work was not appreciated. Bateson and Punnett (1911) described that melanotic pigmentation of connective tissues (fibromelanosis) in silky fowl was inherited by a sex linked gene, carried by brown leghorns. This sex linked gene was considered identical to that described by Davenport (1906).

The findings of Bateson and Punnett (1911) were also confirmed by Wreidt (1927), and by Dunn and Jull (1927). They found a similar inhibitor in white leghorn and concluded that it was either identical with the gene "Id" inhibiting dermal melanin or very closely related with it. There was some indication in their birds that the gene for barring, carried by the White leghorn might also reduce somewhat the amount of melanin in birds derived from Silkies.

2.1.3 Molecular genetic studies on Fibromelanosis

Expression analysis of a set of candidate genes for Fibromelanosis was first attempted in the Silkie chicken at the North Carolina University, USA by Dorshorst and Ashwell (2006) to determine their effect on the hyperpigmentation.

Dorshorst *et al.* (2010), through a detailed study, analysed genomic regions associated with dermal hyperpigmentaton, polydactyly and other morphological traits in Silkie chicken. Using Silkie chicken as a classical model, breeding experiments were planned to determine by two interacting genes, the sex-linked inhibitor of dermal melanin (Id) and autosomal Fibromelanosis (Fm) gene. The authors (Dorshorst *et al.*, 2010) suggested the candidate gene *EDN3* (Endothelin 3) to be the putative (candidate) gene for 'Fm' and assigned a supportive role to id^{+}. Regarding the complementary roles of both 'Fm' and 'id' loci, the authors suggested that 'id^{+}' reflected the abnormal migratory properties of melanocyte precursors in the Silkie embryo, which were then maintained by the action of Fm (*EDN3*) throughout the body.

2.2 KADAKNATH CHICKENS AS A UNIQUE MELANOTIC BREED OF INDIA

2.2.1 Breed characters of KN

Thakur *et al.* (2006) studied the growth pattern and gain in body weight of KN breed of poultry available in Jhabua district of Chhattisgarh. The Kadaknath birds attain 1kg body weight between 6 to 7 months of age and the birds reached around 1.5 kg at 1 year of age. The growth trends in both sexes showed linear increase in body weights; however, the rate of increase in body weights was higher in males as compared to females, thus showing clear sex dimorphism.

Chatterjee *et al.* (2007) compared the two Indian native chickens namely, Kadaknath and Aseel, the body weight at all ages except at

2 weeks of age was significantly ($P<0.05$) higher in Aseel than Kadaknath and body weight gain was significantly ($P<0.05$) higher in Aseel than Kadaknath at all periods except between 2–4 weeks of age. All the body conformation traits shank length, keel length and breast angle were significantly ($P<0.05$) higher in Aseel than Kadaknath. The authors recorded live weights for the KN breed as 51.8±0.48, 125±2.27, 275±9.15, 583±18.18 and 861±19.50 g at the 2, 4, 8, 12 and 16 weeks of age respectively.

2.2.2 Carcass quality

A comparative study on Guinea fowl (Pearl) and Desi fowl (Kadaknath) by Singh *et al.* (2006) showed superiority of Pearl over Kadaknath with respect to meat quality traits, cut up yield and by-product yield. Further the changes in moisture and Water holding capacity (WHC) were significant with the increase of the age of birds in both cases. Physico-chemical analysis of raw meat revealed that Kadaknath was significantly ($P<0.05$) higher in ether extract percent and nonsignificantly higher in ash percent at 8, 12 and 16 weeks of age.

Mishra *et al.* (2007) evaluated the effect of genetic background of the native Indian chickens in influencing the meat quality and carcass characteristics at marketable age. The genetic make up of the native chicken breed: Kadaknath (KN) known for harbouring the Fibromelanosis gene (Fm) that rendered the KN chickens to be hypermelanotic across most body tissues, was examined for its modifying effect on meat quality and carcass characteristics. Five distinct genetic backgrounds i.e. breed-crosses sired by Kadaknath chickens with 4 distinct breeds: White Leghorns (WLH), Rhode Island Red (RIR), Aseel-Peela and Aseel-Kagar, along with a pure Kadaknath chicken group, were compared for slaughtered for meat quality (carcass traits). The results showed that genetic make-up of the bird did not influence any of the slaughter quality parameters significantly, except for Preslaughter live weight (PSLW), which was quite expected. The sex of these chickens was, however, significant (P<0.01), in influencing the thigh-meat texture, apart from the PSLW and size of an internal organ: spleen. Numerically, the crosses of KN and WLH exhibited tougher meat-texture as compared to other KN based crosses. The interaction of genetic-group and sex was, however significant (P<0.05) in influencing both the texture of meat at the breast and drumstick, apart from other internal organs weights, i.e. liver, spleen, gizzard, and the back-meat (bony cut-up part). The authors concluded that the KN's genetic background could impart a more desirable meat texture to its crosses, equally, in terms of its breast and leg meat, when bred to the Aseel chickens than to the RIR and WLH based crosses, without affecting other quantitative carcass characteristics.

Mishra *et al.* (2008a) did studies to determine the influence of genetic background of native Indian chickens in affecting meat color (melanosis), texture, crude protein percentage (CP) and carcass quality in crosses of native chicken breed Kadaknath (KN). The impact of Fibromelanosis (Fm) fixed in KN was examined across four genetic backgrounds: White Leghorns (WLH), Rhode Island Red (RIR), Aseel-Peela and Kagar. The crosses of KN with each of these breeds included cross-progeny raised by KN sires and also the ones from KN dams with alternate males. The texture of meat from both breast and drumstick was studied. The analysis showed that genetic make-up of bird did not influence any of the slaughter quality parameters significantly. The sex effect was, however, significant (P<0.01), for thigh-meat texture. The interaction of genetic-group and sex was also significant (P<0.05) for both: texture of meat of breast and drumstick. Analysis of CP of both breast and drumstick meat revealed that CP of breast-meat was significantly ($P<0.05$) affected by sex of chickens (favouring the females) but not by genetic background. When analyzed for groups generated from reverse crosses, no significant influence of sex and genetic background on meat-texture and CP was evident. It was concluded that KN's genetic background did not affect meat quality except for melanotic appearance while, influence of Fm to alter protein concentration and texture of meat in its breed-crosses remains to be researched further.

Mishra *et al.* (2008b) conducted studies to determine the inheritance of the skin-colour pigmentation. Kadakanath (KN) was crossed individually with four other chicken breeds with known background for skin colour genes. Through this study, the authors concluded that fibromelanosis was primarily caused due to a single autosomal gene (Fm) that exhibited incomplete dominance. The study also highlighted the epistatic effect of the dominant sex-linked 'Id' (Inhibitor of dermal melanin) gene in White Leghorns and White Plymouth Rock crosses on 'Fm' induced melanin deposition.

Another study was conducted by Mishra *et al.* (2010) to determine the positive influence of hyperpigmentation due to Fibromelanosis gene on meat texture and leanness in exotic and indigenous breed crosses of Kadaknath (KN). Their experiment involved study of the texture and leanness of dressed meat from crosses of KN with different chicken-breeds, viz., White Rock (WR) and White Leghorn (WLH) and Aseel which included different proportion of KN blood (Pure, F1 and Back-crosses). The muscle-tenderness (shearing force) and abdominal fat (AF) at 12 weeks of age were significantly ($P<0.05$) affected by varying KN genomic-proportion. Among cut-up parts, the texture of thigh meat was more desirably improved than that of breast meat due to introgression of KN-genome into recipients. Particularly, interaction of genetic-group and sex was significant for texture of thigh-meat. It was concluded that increased proportion of KN genome in introgressed broiler-background resulted a positive impact on meat tenderness and reduction in abdominal fat (AF) than crosses of White Leghorn and Aseel and therefore, increased proportion of KN blood in broiler-crosses irrespective of 'Fm' was advantageous for meat-tenderness and leanness.

Arora *et al.* (2010) evaluated the effect of Kadaknath genomic proportion on various economic traits viz., bi-weekly body-weight, carcass quality and leanness parameters in crossbreds which included F_1 and back-crosses of Kadaknath with respective breeds i.e. White Leghorns, White Plymouth Rock and Aseel-Peela. Analysis of quantitative traits indicated a significantly favourable impact of Kadaknath genomic proportion when raised to 50% or beyond, on meat-texture and carcass leanness. However, desirable improvement in leanness was noticeable in the White Rock crosses but not in White-Leghorn and Aseel-Peela crosses. Thigh-meat texture was more influenced by enhanced Kadaknath genomic proportions than the breast-meat. It was concluded that introgression of Kadaknath genomic proportion beyond 50% in a cross with meat-type chickens, irrespective of Fm's impact, brought distinct improvement in meat quality whereas no such advantage was realized for growth traits.

2.3 PROTEIN PROFILE OF MEAT SAMPLES OF KADAKNATH VERSUS OTHER CHICKENS

Sachdev *et al.* (1996) found that multifold increase in poultry meat production was associated with huge yields of gizzard and other less valued chicken organs. In order to explore the possibility of using gizzard proteins, their qualitative characters in fibromelanotic Kadaknath as well as broiler birds were determined through poly-acryl amide gel electrophoresis. Protein matrix in raw (3 hour postmortem) and deep fried (130±10°C) breast muscles of these birds were studied under scanning electron microscope to identify the ultra structural changes caused by thermal treatment during the process of cooking. The electrophoretic profile of total proteins from gizzard of Kadaknath evidenced the presence of three additional proteins in the molecular weight range of 55.4 and 99.7 kilodaltons which illustrated the protein enriched character of Kadaknath gizzards. Electron micrographs of breast muscle from Kadaknath revealed meagre quantities of interfibrillar connective tissue and melanin pigment grains of 30 µm diameter whereas, the samples of broiler showed dense network of such tissues. Their study also established the better tenderness of the Kadaknath meat (muscles) than broilers, through shear press measurements. The authors concluded that in spite of the poor sensory scores, the protein profile of Kadaknath meat suggested its potential for commercial utilization in processing convenient poultry products.

2.3.1 Protein composition analysis of Chicken Meat using SDS-PAGE methods

Protein composition analysis of chicken meat other than Kadaknath meat has been attempted by many workers, using standard electrophoretic methods like Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis procedures, to make one dimensional analysis of protein fractions.

Gras *et al.* (2001) conducted studies for the quantitative determination of titin and nebulin in chicken meat by SDS-PAGE electrophoresis technique which was developed by application of β-galactosidase as the internal standard. The method was tested first on marker protein samples of known concentrations (myosin, transferrin, glutamic dehydrogenase) and then the method was used in the determination of titin and nebulin content in chicken meat.

The method demonstrated high accuracy, confirmed by correlation coefficient 0.91±0.99. Two gel analysis techniques, i.e. scanning and densitometry were also compared. By the use of marker proteins as well as titin and nebulin, higher accuracy and precision were achieved in scanning than in the densitometric technique.

Wang *et al.* (1979) conducted studies on the electrophoretic analyses of protein components of striated muscle myofibril purified from various vertebrate and invertebrate species, revealed that proteins much larger than myosin heavy chain are present in significant amounts. The study concluded that titin is a structurally conserved myofibrillar component of vertebrate and invertebrate striated muscles.

Fritz *et al.* (1989) analyzed the factors affecting PAGE and blotting of high-molecular-weight proteins. Electrophoresis of the high-molecular-mass proteins (>500 kDa) of muscle myofibrils is difficult using conventional procedures. The mobility of these proteins was influenced by the heating time in sample buffer, the use of 2-mercaptoethanol in the upper reservoir buffer, and the pH of the resolving gel in a stacking sodium dodecyl sulfate gel system. Heating samples for 4 min (versus shorter times), addition of 2-mercaptoethanol to the upper reservoir buffer, and reducing the pH of the resolving gel to 8.6 all enhanced the mobility and resolution of the high-molecular-weight proteins on polyacrylamide gels. The sulfhydryl reducing agents commonly used in protein sample buffers (2-mercaptoethanol and dithiothreitol) were found to migrate at the electrophoresic dye front. Inclusion of 10 mm 2-mercaptoethanol in the upper reservoir buffer or blocking free sulfhydryl groups with N-ethylmaleimide prevented intermolecular disulfide bond formation during electrophoresis. The addition of 10 mm 2-mercaptoethanol to the buffer used for electro-blotting also improved efficiency of protein transfer to nitrocellulose.

2.3.2 Improved resolution and efficiency of electrophoresis of meat proteins by SDS PAGE

A study was conducted by Porzio and Pearson (1976) on standard experimental procedures for continuous polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate, which were modified to give more effective separation and improved resolution of myofibrillar proteins. The system utilized a running gel consisting of 10% acrylamide with 0.1% bisacrylamide cross-linker (100:1) incorporating 400 mM Tris/glycine (pH 8.80), 0.1 mM ethylenediaminetetraacetate, 5% glycerol and 0.1% sodium dodecyl sulfate. Electrophoresis was performed at 1 mA per gel with corresponding running times of 4-6 hrs. The authors concluded that myosin heavy chain migrated as a narrow symmetrical band while the smaller regulatory proteins of the myofibril got resolved.

Kumar *et al.* (1993) conducted studies to decipher the multiple bands realized on the SDS-PAGE analysis of proteins due to Intermolecular Disulfide cross-linking. Gel electrophoresis has been used extensively as an analytical technique to check the purity and to determine the molecular weight of proteins. Improved levels of detection of proteins by silver staining of the gels have made the technique more sensitive in detecting heterogeneity. They reported some interesting observations and the reasons there of about the anomalous behavior of some proteins like appearance of multiple bands, after silver staining, on the SDS-PAGE gels of proteins which are shown to be homogenous in high performance liquid chromatography (HPLC).

Poduslo (1980) compared different tris-based buffer systems used in SDS-PAGE procedures. The accuracy of molecular-weight estimates for glycoproteins was evaluated by sodium dodecyl sulfate-pore gradient electrophoresis. The study suggested that both the gradient gel system as well as the formation of borate complexes with the individual carbohydrate moieties of the glycoprotein contribute to the precision of the molecular-weight estimate. Deviations in the molecular-weight estimates in the reduced and non-reduced state, however, imply that the physical properties of the individual glycoprotein ultimately govern the accuracy of the molecular-weight estimate. Based on these observations, a strategy has been presented for estimating the molecular weights of glycoproteins by sodium dodecyl sulfate-pore gradient electrophoresis, particularly as related to complex membrane systems.

2.4 PROXIMATE ANALYSIS OF CHICKEN MEAT INCLUDING KN-MEAT

Kawalkar and Bhambal (1996) investigated the clinical and nutritional significance of black meat chicken (BMC) kadaknath (KN) over routine table chicken (RTC) white leghorn employing the AOAC (1975) and the NIN-India (1971) methods. In this study, linoleic acid, in BMC was observed as 24% as against the 21% of RTC, claiming its nutritional superiority clinically. BMC protein content 91.94% on DM basis is of interest comprising of essential amino acids (EAA). It is enigmatic that the concentration of cholesterol 184.75mg/100g in BMC meat was significantly lower ($P<0.01$) than that of RTC meat 218.12 mg/100g, in spite of significantly ($P<0.01$) higher blood cholesterol 352.37mg/dL in BMC than RTC 253.12 mg/dL. Thus, it was inferred that Kadaknath meat was richer in certain proteins, and was low in cholesterol [while its fat possessed higher degree of unsaturation in terms of linoleic acid] than the routine table chicken, which needed to be studied in details from healthy-nutrition point of view.

Mati and Verma (1983) compared the proximate composition of the light and dark meat of the Kadaknath, the study clearly suggested that the Kadaknath meat contained more crude protein and significantly less fat percentage as compared to the other exotic breeds of chicken. The following table contains the proximate composition of light and dark meat of Kadaknath :

Table 2.1 : Proximate composition of light and Dark meat (Mati and Verma, 1983)

Proximates	Light Meat	Dark Meat
Protein	25.16%	24.47%
Moisture	72.69%	70.35%
Ash	1.08%	1.60%
Muscle fat	0.73%	1.03%

☑☑☑

Materials & Methods

In order to achieve the objectives of the present investigation as detailed in earlier sections, efforts had been made to evaluate the effect of fibromelanosis on skin coloration, proximate composition, sensory evaluation scores, plasma protein estimation and electrophoretic characterization of the proteins in crosses involving an F_2 cross of Kadaknath and White Plymouth Rock along with an advanced intercross of Kadaknath and White Leghorn. All the experimental procedures and statistical techniques employed during this study at the Central Avian Research Institute (CARI), Izatnagar are briefly discussed here under.

3.1 EXPERIMENTAL DESIGN

3.1.1 Genetic groups and cross setting

Germplasm : Three major chicken germplasm belonging to following breeds were used.

1. Kadaknath (KN)
2. White Leghorn (WLH)
3. White Rock (WR)

The study utilized both purebred and crossbred chickens, raised from above, in the manner described below.

3.1.2 Generation of Crossbreds

Keeping the fully-melanotic Kadaknath purebred chickens in view, customized KN-crosses were studied against the latter two breeds of chickens (non-melanotic phenotypes). It was proposed to use two existing sources of crossbred-KN chickens (available at Experimental Native fowl farms, CARI) for these purposes. These crossbred resources were : (a) KN-WLH advanced intercrosses which segregate for alternative alleles of Fibromelanosis (Fm vs. fm) locus (which are already fixed for the 'Id' allele at the sex linked 'Id' locus); (b) KN-WR F_1 progeny which contain alternative alleles for both 'Fm' & 'Id' locus for supporting further crosses.

Using the above two germplasm resources, two customized crosses were raised. The 1st cross: (A) was generated by using the moderately-pigmented (slaty) looking males and females of the KN-WLH intercross as parents to generate all the possible genotypes for Fm alleles, i.e. Fm/Fm, Fm/fm^{+}, fm^{+}/fm^{+} following procedures of Pratap *et al.* (2010). As per the expectations, these cross-progeny primarily included three types of skin-pigmentation, i.e. completely-pigmented, moderately pigmented (slatey), non-pigmented chickens, which were used in qualitative analysis of skin proteins as well as in skin phenotyping (carcass melanosis assessment) studies.

The 2nd customized cross, i.e. (B) was generated by an F_2 cross (second filial generation) resulted from the matings of non-pigmented KN-WR F_1 males (having few spots) with moderately-pigmented (slaty) looking KN-WR F_1 females, to generate all possible combination of genotypes from the 'Fm' and 'Id' locus, as has been cited by Arora, (2010). As per the expectation of this cross, the progeny included three types of skin-pigmentation similar to the above, i.e completely-pigmented (black), moderately-pigmented (slatey), non-pigmented (white) chickens which constituted the research materials for meat protein profile analysis, proximate analysis (Moisture, Crude protein and Ether extract only), biochemical (total plasma protein content in blood) and sensory evaluation studies.

Therefore, together these germplasm included: (A) resources which segregate for alternative alleles of 'Fm' but fixed for 'Id' allele; and (B) Resources which segregate for alternative alleles from 'Fm' & 'Id' locus. Besides these, purebred KN, WLH and WR were included in the study as controls to aid in differential quantitative and qualitative protein-profiling studies.

3.1.3 Egg Collection and Incubation

All the matings were conducted by artificial insemination of above adult parents which were housed in individual cages. Fertile eggs were collected consecutively for a period of 14 days, hen wise, and then set in automatic forced-draft incubators for raising the chicks of various genetic groups. The egg were transferred to the pedigree boxes and then placed onto hatching trays on 18^{th} day of incubation. The chicks were taken out from the hatchers on the 22^{nd} day of incubation. On hatch, chicks were wing-banded and vaccinated as per standard healthcare schedule of CARI, Izatnagar.

3.1.4 Brooding and Management practices

Chicks of same genetic group were brooded and reared together, in battery brooders upto 8^{th} weeks of age, beyond which they were subjected to growing in floor pens. Standard management practices were followed throughout the experimental period and these were kept uniform for all genetic groups. Uniform numbers of chick were maintained in each genetic group, as far as possible. The feeding and watering were provided *ad-libitum*, throughout the rearing period.

Hundred straight-run chicks from above crossbred groups of chickens (A and B each) were raised along with thirty each of WLH, WR and KN chickens, which were reared in the CARI, Izatnagar farms under uniform managemental and healthcare measures. The biweekly body weights were recorded w.e.f. 4^{th} till to 12^{th} week of age. The purebred Kadaknath, White Leghorn and White Plymoth Rock chickens served as control groups in respective experiments.

3.2 GROWTH PERFORMANCE

The body weights were recorded for the purebred chickens of WLH, WR and KN along with their progeny of advanced intercrosses (Cross-A and Cross-B) at 4, 6, 8, 10 and 12 weeks of age, using electronic balances to minimize the error in data recording. The sampling of the chickens used for each group was made randomly which excluded the sex- bias by including equal number of males and females within each phenotype. A uniform number of 30 chickens each were retained in each genetic group for recording of performance.

3.3 SKIN-PHENOTYPING STUDIES AND CARCASS MELANOSIS ASSESMENT

At the age of 12 week, 10 birds falling from the three major skin-color phenotypes [completely pigmented (black), moderately pigmented (slaty) and non-pigmented (white)] from Crosses: A and B were slaughtered to study the carcass appearance and melanosis assessment across skin, body, shank region and internal organs by visual observation.

3.3.1 Analysis of Fibromelanosis character

The impact of Fibromelanosis on the other economically-important traits like; body weight and performance traits of cross bred chickens was analyzed to evaluate the impact of Fibromelanosis in the various genomic back ground. The phenotypic effect of 'Fm' was investigated by analyzing the KN crossbreds (F_2 and advanced intercrosses) populations versus purebred KN to ascertain its influence on majority of economic traits of relevance.

All the KN crossbreds (KN-WLH & KN-WR) chickens were phenotyped for the 'Fm' gene induced characters, which included skin phenotype as well as performance traits. Accordingly, the skin pigmentation was categorized as-

(a) Completely-pigmented (Black-skinned)

(b) Moderately-pigmented (Slaty-skinned)

(c) Non-pigmented skin (White-skinned)

3.3.2 Determination of Pigmentation in the Skin in Cross-A and Cross-B Chickens

For delineating the skin pigmentation due to presence of ‘Fm’ alleles [‘Fm’ versus ‘fm’ as hypothesized by Hutt, (1949)] in the above resources were analyzed from evaluation of both live birds and carcasses (post-slaughter). All the individuals were photographed for their live-skin and their respective carcass-skin appearance in presence of two independent observers to minimize the risk of mismatching in the recording of pigmentation intensity in the skin.

All the chickens from each group (consisting of both the sexes) were subjected to feed withdrawal overnight (12 hours) and slaughtered followed by dressing for the proper visualization of skin color. The individual carcasses with intact skin were photographed and analyzed. The hyperpigmentation of the skin of purebred KN carcasses was categorized as intensely-pigmented and a similar melanosis found in both crossbred groups (A and B) were categorized as completely-pigmented. The skin and other body parts examined for melanin deposition included comb, wattle, shank, beak and skin over the legs and breast. Visceral organs were visually evaluated for pigmentation, mainly heart, spleen, gizzard, trachea, liver, gonads besides the meaty cuts (muscles and bones from legs and breast). The carcasses of these genetic groups were compared with each other for differential skin colouration. All the slaughtered individuals were categorized as per skin-colour phenotypes namely, completely-pigmented (black), moderately-pigmented (slaty) and non-pigmented (white) respectively, as ascertained thoroughly, by two observers. The fully non-pigmented carcasses of both crossbred groups, scored at par with the WR.

3.4 GEL ELECTROPHORETIC ANALYSIS OF MELANOTIC TISSUE PROTEINS

This experiment included the analysis of the melanotic tissue proteins to study their compositional profiles by SDS-PAGE method.

3.4.1 Gel electrophoretic Analysis of skin proteins

For gel electrophoretic characterization of skin proteins, skin samples were taken from chickens of Cross A. Three (3) chickens were selected randomly from each genetic group i.e. completely-pigmented; moderately-pigmented and non-pigmented of Cross-A. From each chicken, skin samples were collected; followed by homogenization of skin-samples, they were blended together (pooled) within each genetic group and subsequently subjected to polyacrylamide gel electrophoresis (Laemmli, 1970).

3.4.2 Gel electrophoretic Analysis of meat proteins

Cross-B chickens were utilized for the gel electrophoretic characterization of meat proteins. From Cross-B, 3 chickens were selected randomly from each genetic group i.e. completely-pigmented; moderately-pigmented (slaty) and non-pigmented to collect the meat tissue samples (from breast and thigh muscles), followed by sample homogenization and these samples were further subjected to Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (Laemmli, 1970). Brief description of the procedure used is given hereunder.

3.4.3 Casting of gel

A linear resolving gel of 10% was prepared in the assembled glass plate sandwich of the electrophoresis apparatus. The top of the gel surface was covered by gently layering with water- saturated Isobutanol against the edges of the spacers. The gel was allowed to polymerize at room temperature. After polymerization of the resolving gel, Isobutanol was drained off and the upper surface of the gel was thoroughly washed with distilled water, followed by rinsing the surface with distilled water. The 5 % stacking gel was gently poured into the center of the sandwich over the 10% resolving gel. Slot forming Teflon comb was carefully inserted into the top of the stacking gel and left at room temperature for polymerization. Completion of polymerization was assessed by the appearance of sharp optical discontinuity around the wells. The comb was removed carefully and the wells were thoroughly washed with electrode buffer to remove the Isobutanol and also the unpolymerized gel material, if any, present inside the wells.

3.4.4 Preparation of the samples

The skin and meat tissue proteins were extracted by homogenizing the tissue samples with an extraction solution. The homogenized tissue samples were then centrifuged in order to obtain the protein supernatant, which served as the actual tissue protein sample.

The skin and meat tissue protein samples were mixed in an equal proportion with tracking dye (5 µl protein sample + 5 µl dye). The samples were then loaded into the wells as per the method described by Franceshetti and Brantwein, (1993).

3.4.5 Electrophoresis

Samples were loaded in wells in such a way that the protein concentration was about 5 µl in each well. Electrophoresis was carried out at room temperature at a constant mode of 18 mA/slab until the tracking dye reached the resolving gel. Then the current was increased to 25 mA/slab and electrophoresis was continued till the tracking dye reached the bottom of the resolving gel. The standard molecular weight marker was also run parallel along with the samples (PageRuler™ unstained broad range protein ladder SM# 1881 by Fermentas with molecular weight range of 5 kDa-250 kDa).

3.4.6 Fixing, staining and destaining of the gel

After completion of electrophoresis, the gel was carefully removed from the glass plate sandwich. One corner of the gel was cut as identification for particular well number. The gel was stained for overnight in staining solution in a staining tray, kept on gel rocker. The gel was then destained with several changes of destaining solution. When the background was thoroughly destained, the gel was photographed and stored in 7% Glacial acetic acid.

3.5 PROXIMATE ANALYSIS OF MELANOTIC TISSUES FOR MOISTURE, CRUDE PROTEIN AND ETHER EXTRACT

Chickens of Cross-B were utilized for this purpose, 3 chickens from each genetic groups i.e. completely pigmented; moderately pigmented (slaty) and non-pigmented were selected randomly, followed by skin and meat tissue sample (from breast and thigh muscles) collection from each chicken, which were further subjected for the estimation of moisture content, Crude protein and Ether extract as per AOAC (2000) procedures.

3.5.1 Moisture content

Moisture content was determined according to the method described in AOAC (2000). The minced meat sample [5 gm] was transferred into pre-weighed flat bottom Aluminum dish, which was then transferred to hot air oven at 101±1°C for 16-18 hr. Meat samples were weighed until their dried weight became constant. Dried sample were then placed in desiccators having silica gel as desiccant. After 1 hr, the Aluminum dishes were weighed. Moisture content of the meat samples were calculated by applying the following formula:

$$\text{Moisture (\%)} = \frac{W2-W3}{W2-W1} \times 100$$

Where,

W1 = weight of empty dish

W2 = weight of dish + sample

W3 = weight of dish + dried sample

3.5.2 Crude Protein Content

Crude protein content was determined following methods described in AOAC (2000). Small quantity of meat and skin tissues (2-2.2 gm) were digested using Micro-Kjeldhal digester in presence of catalyst (95 part Sodium sulfate/Potassium sulfate + 5 part Copper sulfate) and 15 ml Sulfuric acid. The Flask was placed in inclined position and heated gently until frothing ceased and then boiled rapidly until the solution became clear. The digested sample was then cooled and distilled water was added to make the volume upto 250 ml. The diluted sample (25 ml) was distilled with 10 ml of 40% NaOH solution using Micro- Kjeldhal distillation unit. The resultant steam was distilled over 3% boric acid (10 ml) containing mixed indicator (1 part 0.2% methyl red + 2 part 0.2% bromocresol green dye) for 10 minutes. The Ammonia trapped in Boric acid was estimated by titrating with 0.1 N Sulfuric acid. The Nitrogen percentage was finally calculated using the following formula.

$$\text{Nitrogen (\%)} = \frac{(A - B) \times 0.0014 \times \text{Total volume made}}{\text{Weight of sample taken} \times \text{Volume distilled}} \times 100$$

Where,

A = Titrated value for sample

B = Titrated value for blank

Protein percentage was thereafter determined indirectly from the Nitrogen percentage by multiplying it with a conversion factor (CF) of 6.25, with the assumption that all the Nitrogen in meat was presented as protein i.e. Crude Protein percentage = N% x CF (6.25).

3.5.3 Ether extract content

Ether extract (E.E.) content was determined with Soxhlet Extraction Unit as described by AOAC (2000). Soxhlet Extractor was set with reflux condenser and distillation flask which was previously dried and weighed. The meat sample [0.5g] was taken in to fat free extraction thimble, dried in oven for 6 hrs at 100-102°C and placed in extraction apparatus (Soxhlet). Then 150 ml of petroleum ether (BP: 60-80°C) was poured into extraction flask and condenser was joined and placed on electric heater in order to boil the solvent gently. Extraction was carried out for 6-8 hrs. The solvent was removed. The E.E. content was calculated by using the following formula :

$$\text{Ether extract (\%)} = \frac{W2-W1}{W3} \times 100$$

Where,

W1 = weight of empty distillation flask

W2 = weight of distillation flask + Fat

W3 = weight of sample taken

3.6 BIOCHEMICAL ANALYSIS

Chickens of Cross-B were utilized for this experiment. Blood plasma samples from the selected chickens (10 samples from each melanotic shade i.e. [completely-pigmented (black), moderately-pigmented (slaty) and non-pigmented (white) of Cross-B were estimated for total protein content using Folin-phenol method (Lowry *et al.*, 1951).

3.6.1 Materials

(i) Solution A : 2 % Sodium carbonate in 0.1 N Sodium hydroxide

Na_2CO_3	-	2 g
NaOH	-	0.4 g
D.W.	-	100 ml

(ii) Solution B : 0.5 % Copper sulfate in 1% Sodium potassium tartarate

$CuSO_4.5H_2O$	-	0.5 g
Sodium potassium tartarate	-	1 g
D.W.	-	100 ml

(iii) Solution C :

Solution A	-	10 ml
Solution B	-	0.2 ml

These solutions were prepared fresh before use.

(iv) Bovine Serum Albumin (BSA) standard : 1mg/ml

3.6.2 Procedure

Three test tubes were taken and marked as test, standard and blank. This experiment was performed in the manner described below.

	Test	Standard	Blank
	0.1 ml	0.1 ml	-
Distilled water	0.9 ml	0.9 ml	1.0 ml
Solution C	5 ml	5 ml	5 ml

(a) After shaking the content of test tubes, there was an incubation time for 10 minutes.

(b) Folin reagent was mixed with distilled water in 1:1 ratio.

(c) In each tube, 0.5 ml of Folin reagent was added.

(d) The test tubes were kept for 30 minutes at room temperature in dark.

(e) O.D. of the test and standard were taken at 750 nm against blank.

(f) Total plasma protein content was calculated by using following formula :

$$\text{Total plasma protein (g/dL)} = \frac{\text{O.D. of test sample}}{\text{O.D. of standard}} \text{ X Conc. of standard X 100}$$

3.7 SENSORY EVALUATION USING AN EMULSION BASED MEAT PRODUCT

This evaluation was aimed to analyze the sensory attributes of melanotic meat of chickens from Cross-B to delineate effect of varying shades of malanosis on sensory scores.

Crossbred KN-WR chickens were sacrificed at 12 week of age, and the carcasses were dressed and eviscerated as per procedures of Jones *et al.* (1984). The carcasses were graded into different melanotic classes i.e. completely pigmented (black), moderately pigmented (slaty) and non-pigmented (White) and then subjected to deboning as per the standard procedures. Subsequently, an emulsion based meat product, i.e. chicken nuggets, was prepared using standard procedures.

Sensory evaluation of emulsion based meat product i.e. chicken nuggets was conducted using an 8 point descriptive scale (Keeton, 1983) with minor modifications, where 8 was extremely desirable and 1 was extremely poor. The sensory panel consisted of scientists and postgraduate students of the CARI, Izatnagar. The panelists were briefed with the nature of the experiments without disclosing the identity of the samples and requested to rate them on an 8 point descriptive scale on the sensory evaluation proforma for different attributes. Chicken nuggets were warmed (45ºC) in an oven for 1 min and served to the panelists. Water was provided to rinse the mouth between tasting of each sample. The panelists evaluated the samples for attributes such as general appearance, flavor, juiciness, texture, binding and overall acceptability and inferences on the melanotic shades of the product, if any, on the final sensory scores of the product were drawn, as per suitable statistical methods.

3.8 DATA COMPILATION AND ANALYSIS

All the experimental data collected, following above procedures were tabulated, compiled, and subjected to standard statistical analysis. The means and standard error (S.E.) for various genetic groups were calculated and Analysis of Variance (ANOVA) was carried out using SPSS™ (version, 16).

☑☑☑

Results

All the investigations were undertaken at the laboratories of the division of Avian Genetics and Breeding, CARI, Izatnagar. The data obtained on these parameters were analyzed statistically, for their treatment (genetic) effects and the results have been presented in this chapter.

4.1 GROWTH PERFORMANCE

The data pertaining to growth performance as influenced by different genetic backgrounds (pure-breds) as well the crossbreds being categorized into three major phenotypes i.e. completely pigmented (black-skinned), moderately pigmented (slaty-skinned) and non-pigmented (white-skinned) chickens of both Cross-A (KN-WLH) and Cross-B (KN-WR), are elucidated here under.

4.1.1 Body weight

Data on biweekly BW (4-12 weeks of age) for the experimental chickens (including all three purebred groups as well as the crossbred chickens belonging to three melanotic phenotypes i.e. black, slaty and white of both Cross-A and Cross-B) have been presented in tables 4.1-4.3.

4.1.1.1 Body weight of purebreds

The mean body weights of purebred WLH chickens were recorded significantly higher ($P<0.05$) than the purebred KN chickens at 4th, 6th, 8th and 10th weeks of age. Though at 12th week of age purebred WLH chickens had numerically higher body weight than their KN counterparts, it remained non-significant.

However, purebred WR chickens maintained their superiority in mean body weight over the remaining two purebreds (WLH and KN) at 4th, 6th, 8th and 10th and 12th week of age and these differences were found to be significant ($P<0.05$) at all ages. Purebred WR chickens were even better than the crossbreds at all ages of the study. The purebred KN chickens had the lowest body weights across these ages of measurement. The mean body weights with standard errors for the purebred chickens have been presented in the table 4.1.

4.1.1.2 Body weight of Cross-A (KN-WLH intercross) chickens

The mean Body Weights (BW) at 6, 8, 10 and 12 weeks were recorded significantly higher for the chickens of white-skinned group as compared to the chickens of slaty and black. The numerical superiority of the White group chickens over the other two phenotype at the 4th week of age was however not significant. Again, between the two melanotic shades, the BW in slaty group was recorded higher than those of black group except at 4 weeks of age. The body weight of all the crossbreds (KN-WLH), however, remained in between those of WLH and KN, where the WLH maintained its superiority over all the crossbreds and the KN-purebreds, across all ages. The mean body weights with standard errors of these crossbred phenotypes along with those of purebred KN and WLH groups have been presented in the table 4.2.

4.1.1.3 Body weight of Cross-B (KN-WR intercross) chickens

The chickens of completely pigmented (black) genetic group had significantly higher body weights than the chickens of slaty (moderately-pigmented) phenotypes, at all ages. However, at 10th week of age the difference in the bodyweight was numerically higher but not significant. Simultaneously, when the bodyweights of chickens

Table 4.1 : Body weight in grams (Mean±SE) of the purebred chicken groups

Genetic groups	N	Mean±S.E.				
		BW4	BW6	BW8	BW10	BW12
WR Purebred	30	343.7 ± 8.73^{a}	706.06 ± 20.83^{a}	1436.8 ± 49.11^{a}	1674.4 ± 59.57^{a}	2087.1 ± 88.62^{a}
WLH purebred	30	197.3 ± 4.88^{b}	333.6 ± 10.71^{b}	510.8 ± 16.63^{b}	621.7 ± 23.43^{b}	726.0 ± 32.51^{b}
KN purebred	30	101.7 ± 3.83^{c}	203.43 ± 8.79^{c}	349.13 ± 13.48^{c}	515.5 ± 18.96^{c}	667.6 ± 21.41^{b}

Means bearing different superscripts column wise are significantly different ($P<0.05$)

Table 4.2 : Body weight in grams (Mean±SE) of the KN-WLH crossbreds, purebred KN and WLH chickens

Genetic groups	N	Mean±S.E.				
		BW4	BW6	BW8	BW10	BW12
KN-WLH BL	30	169.47 ± 4.17^{b}	252.73 ± 7.46^{b}	321.23 ± 7.69^{a}	390.1 ± 8.04^{a}	459.2 ± 8.49^{a}
KN-WLH SL	30	159.03 ± 4.92^{b}	293.06 ± 10.21^{c}	408.6 ± 17.61^{b}	515.2 ± 24.31^{b}	692.9 ± 35.70^{b}
KN-WLH W	30	169.3 ± 5.09^{b}	307.0 ± 10.51^{c}	449.9 ± 15.46^{c}	579.1 ± 19.71^{c}	826.1 ± 25.30^{c}
WLH purebred	30	197.3 ± 4.88^{c}	333.6 ± 10.71^{d}	510.8 ± 16.63^{d}	621.7 ± 23.43^{c}	726.0 ± 32.51^{b}
KN purebred	30	101.7 ± 3.83^{a}	203.43 ± 8.79^{a}	349.13 ± 13.48^{a}	515.5 ± 18.96^{b}	667.6 ± 21.41^{b}

Means bearing different superscripts column wise are significantly different ($P<0.05$)

from completely pigmented group were compared to those of the non-pigmented (white-skinned) phenotypes, the weights remained higher at all ages, though the differences were significant only at 6th and 8th week and 12th week.

Purebred WR chickens maintained their superiority (significant at $P<0.05$) in bodyweights over all the three KN-WR crossbreds as well as the purebred KN chickens, at all ages. The mean body weights with standard errors of these crossbred phenotypes along with those of purebred KN and WR groups have been presented in the table 4.3.

4.2 CARCASS MELANOSIS ASSESSMENT

Data pertaining to melanosis in the skin as influenced by the genetic make-up of the chickens in three major phenotypes i.e. completely pigmented (black), moderately pigmented (slaty) and non-pigmented (white) of both Cross-A and Cross-B chickens have been presented pictorially in figures.

The chicken carcasses were also photographed post-slaughter to determine the extent of melanosis across following tissues, i.e. shank, meaty cuts and selected visceral organs. The melanosis in the skin, meaty cuts and visceral organs of both Cross-A and Cross-B crossbred chickens has been shown in figures 4.1-4.6.

4.2.1 Melanosis assessment in Cross-A (KN-WLH) chickens

4.2.1.1 Melanosis assessment of chickens with skin

When the carcass melanosis of KN-WLH [Cross-A] chickens was assessed visually, all the experimental chickens separated out into three distinct skin phenotypes. i.e. chickens with completely pigmented or black skinned bearing completely black beak and shanks (matching to those of purebred KN); the moderately-pigmented (slaty) skin which had intermediate melanosis across their body; and a totally non-

Table 4.3 : Body weight in grams (Mean±SE) of the KN-WR crossbreds, purebred KN and WR chickens

Genetic groups	N	Mean±S.E.				
		BW4	BW6	BW8	BW10	BW12
KN-WR BL	30	276.3±7.69^{c}	546.9±9.26^{c}	883.5±19.27^{c}	1080.6±19.38^{b}	1409±19.26^{c}
KN-WR SL	30	249.6±9.33^{b}	446.9±20.02^{b}	729.9±29.94^{b}	1002.1±33.02^{b}	1252.16±37.37^{b}
KN-WR W	30	271.93±5.54^{c}	486.8±11.20^{b}	796.06±19.78^{b}	1020.9±31.81^{b}	1310.6±41.89^{b}
Purebred WR	30	343.7±8.73^{d}	706.06±20.83^{d}	1436.8±49.11^{d}	1674.4±59.57^{c}	2087.06±88.62^{d}
Purebred KN	30	101.7±3.83^{a}	203.4±8.79^{a}	349.13±13.48^{a}	515.5±18.96^{a}	667.6±21.41^{a}

Means bearing different superscripts column wise are significantly different ($P<0.05$)

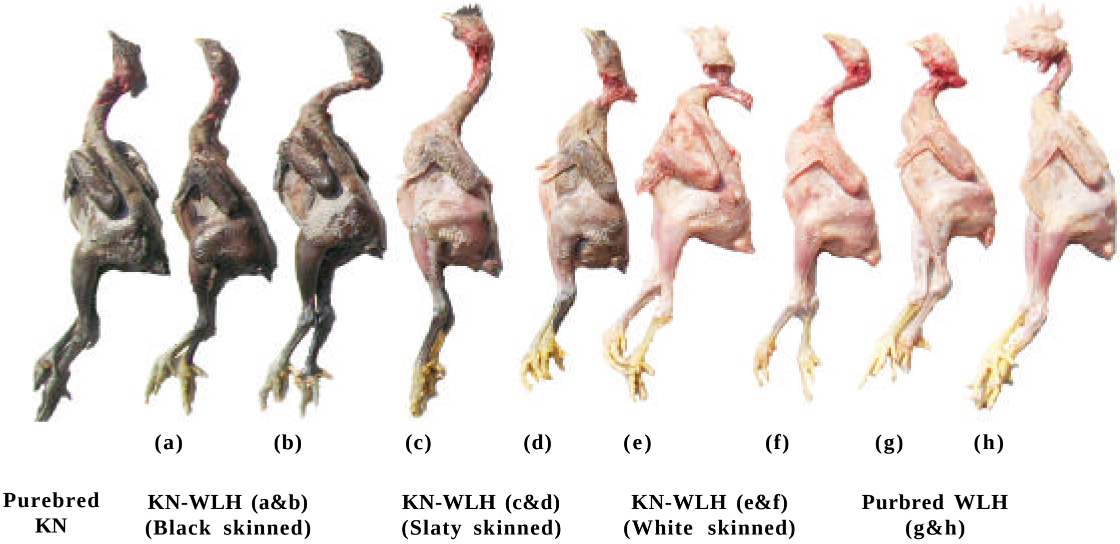


Fig. 4.1 **Shows the variable shades of melenosis in the 3 phenotypes visible on slaughter of Cross-A chickens alongwith the skin melanosis of purbred KN and WLH chickens**

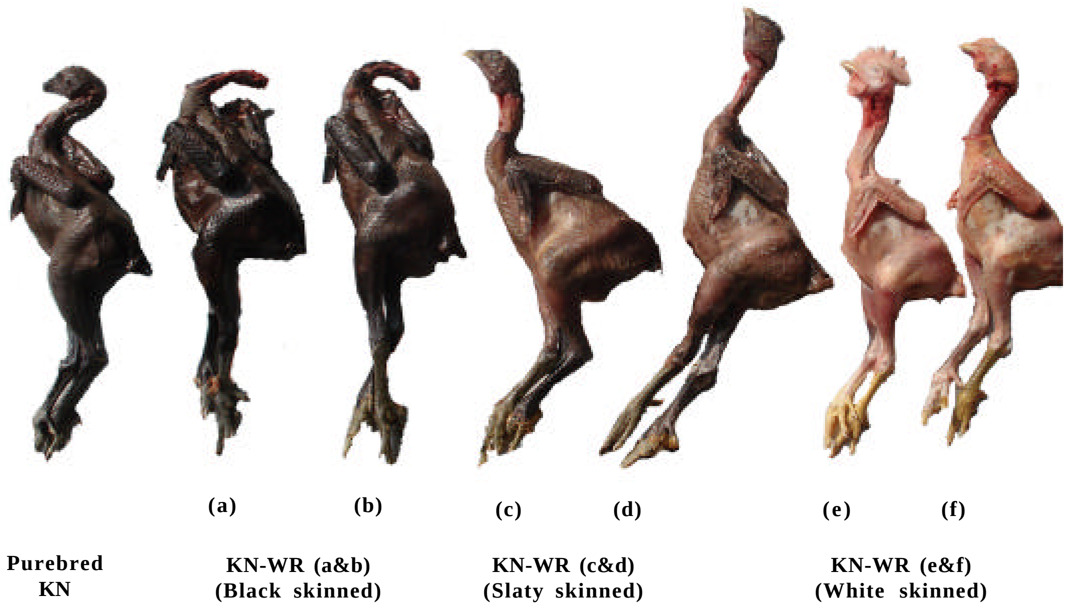


Fig. 4.2 **The intensity scale for skin melanosis visible in various phenotypes of Cross-B alongwith that of purebred KN is highlighted**

pigmented skin (chickens possessing no melanosis across their body, beaks, combs and wattles). However the shanks of the non-pigmented group had slightly green patches, which varied between willow-green to light green. Some of the slaty group chickens had patchy melanosis in beaks while their shanks were of greenish-black color. The figure 4.1 depicts the melanosis in skin of chickens of Cross-A along with that of purebred Kadaknath and White leghorn chickens.

4.2.1.2 Melanosis assessment of meaty cuts and selected visceral organs

Chickens of completely pigmented groups had intense melanosis across the muscle tissues of thigh, breast, back, wings and neck. However, the melanosis was much more pronounced in the muscle of thigh as compared to the muscles of other cut-up parts. Among internal organs of black group: the gizzard tissues had good degree of melanosis while spleen, and heart had slight degree of melanosis. But, the liver almost appeared normal in appearance.

Chickens of moderately pigmented group possessed intermediate or patchy melanosis in the muscles of thigh, neck, wings and back while the breast muscles were not having any significant degree of melanosis except the presence of few spots. There was no appreciable degree of melanosis in the visceral organs too.

Chickens of non-pigmented group had no melanosis, at all, in their cut-up parts as well as in their visceral organs. The cut-up parts and visceral organs of this group was fully comparable to that of the purebred WLH. The melanosis of meaty cuts and internal organs of Cross-A chickens has been presented pictorially in figures 4.3 and 4.5 respectively.

The melanosis in the shanks of Cross-A chickens corresponded to the melanosis observed in the skin and other organs, however the shanks of the non-pigmented group had slightly green patches, which

KN Purebred | KN-WLH (Black skinned) | KN-WLH (Slaty skinned) | KN-WLH (White skinned) | WLH Purebred

Fig. 4.3 The meaty cut-up parts from the deskinned carcasses from various phenotypes of Cross-A chickens

Note : that the intensity of melanosis from fully pigmented chickens of Cross-A surpassed that of purebred KN chickens

(a) WLH Purebred | (b) KN-WLH (White skinned) | (c) KN-WLH (Slaty skinned) | (d) KN-WLH (Black skinned) | (e) KN Purebred

Fig. 4.4 The intensity of melanosis visible in the shank colour of chickens of Cross-A belonging to 3 different phenotypes alongwith the purebred KN and WLH

Note : that the melanosis visible on the shank at fig. b was the only melanotic part in the carcass of this groups as rest part of body was non-pigmented

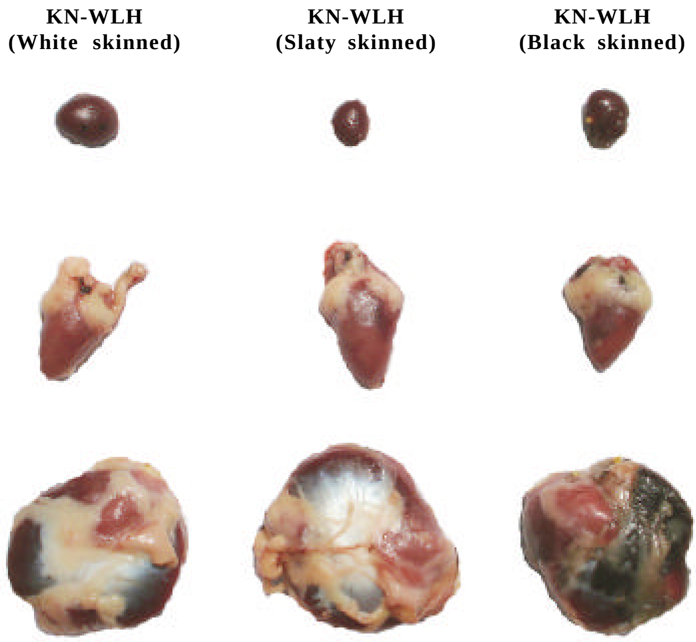


Fig. 4.5 **The trend of melanin deposition on the internal organs of chickens from 3 different phenotypes of Cross-A chickens**

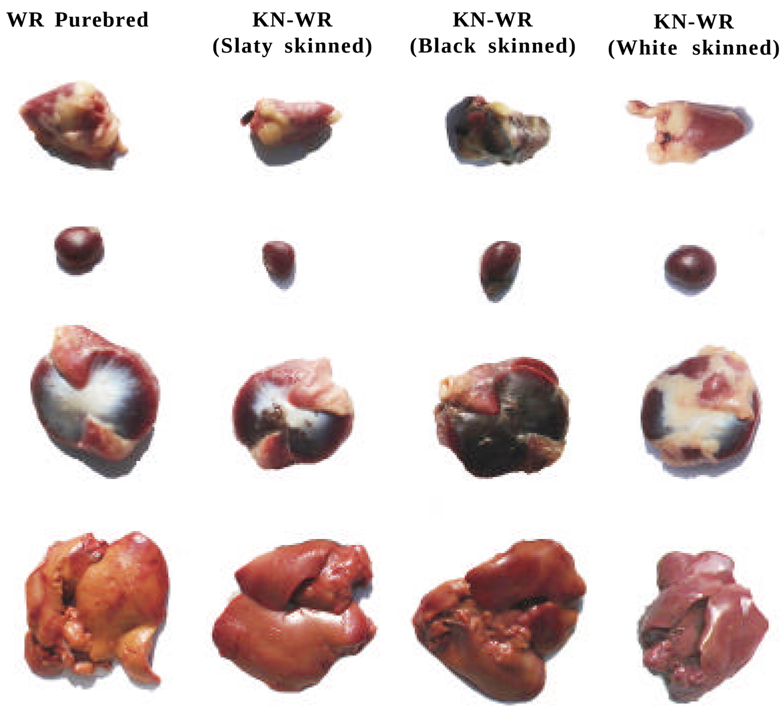


Fig. 4.6 **Details the intensity of pigmentation in the internal organs of the various phenotypes of Cross-B chickens alongwith that of purebred WR chickens**

varied between willow-green to light green while the rest part of body was non-pigmented (white-skinned). The figure 4.4 depicts the intensity of melanosis visible on the shanks of three phenotypes of Cross-A chickens along with that of purebred Kadaknath and White Leghorn.

The melanosis of the KN purebred chickens displayed the intense pigmentation across muscles, meaty cuts and visceral organs. It was observed that the melanosis of the crossbred black (fully pigmented) chickens, at many times, appeared to be more intense than that of purebred KN chickens giving indication to the theory that crossbred completely-pigmented (black-skinned) chickens had more levels of melanins in their body. In variation to the KN, the purebred WLH chickens did not have any degree of melanosis in skin, beak, combs, shanks, meaty cuts and the internal organs and therefore provided a contrast for the comparison of melanotic levels in different phenotypes.

4.2.2 Melanosis assessment in Cross-B (KN-WR) chickens

4.2.2.1 Melanosis assessment of chickens with skin

The major patterns of melanosis visible in cross-B chickens in their respective carcasses are presented in fig. 4.2. As seen from the results all the shades of melanosis (grossly 3 kinds of phenotypes) starting from carcasses devoid of melanin (nearly 99%) to carcasses with carcasses showing slaty pigmentation and intensely black pigmentation were recorded. Thus, 3 distinct phenotypes which almost corresponded to their respective shank coloration were visualized. The intensely-dark pigmented carcasses were comparable to those of KN purebred carcasses. Wherever the carcasses were devoid of pigment (99%) in the skin, there was no black pigment either in comb and or in the shank. For the second category (slaty), the comb’s melanotic pattern was analogous to that of skin showing dusky or smoky appearance. However, in 50% of the cross-B progeny, the pattern of pigmentation was patchy (carcasses) in general. The trend of melanin deposition in the skin of Cross-B chickens has been presented in figure 4.2.

4.2.2.2 Melanosis assessment of meaty cuts and selected visceral organs

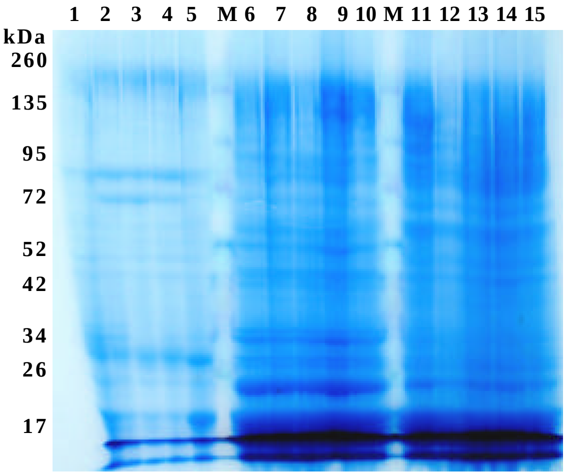
The pattern of pigmentation in the visceral organs (gizzard, liver and spleen) in cross-B carcasses followed the pattern of skin-pigmentation. Analogous pattern of pigmentation was present in the muscles that corresponded to the intensity of pigmentation in the skin of the host. The rate of pigment deposition in the visceral organs was in the order : periostium→bones→gizzard→spleen→liver. It could be seen that the white crossbred chickens did not show even a small trace of pigment in their internal organs. The melanosis pattern in internal organs has been shown in figure 4.6.

4.3 GEL ELECTROPHORETIC ANALYSIS OF MELANOTIC TISSUE PROTEINS

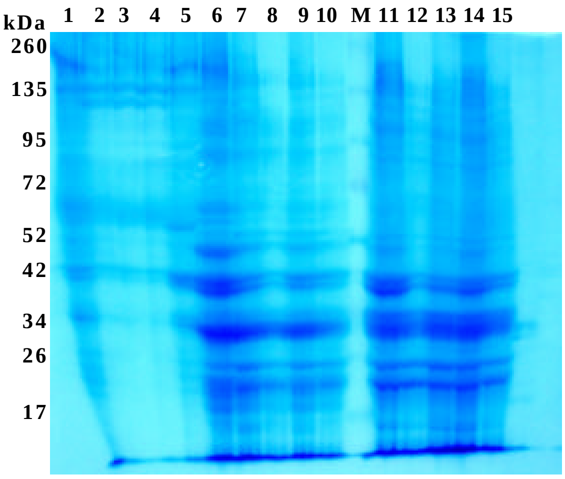
Data regarding the compositional profile analysis of melanotic tissue proteins (skin and breast & thigh muscles) as influenced by the genetic make-up of the chickens in three major phenotypes i. e. completely pigmented (black), moderately pigmented (slaty) and non-pigmented (white) of both Cross-A and Cross-B chickens have been presented pictorially in figures 4.7-4.9.

4.3.1 Gel Electrophoretic Analysis of melanotic skin and meat proteins

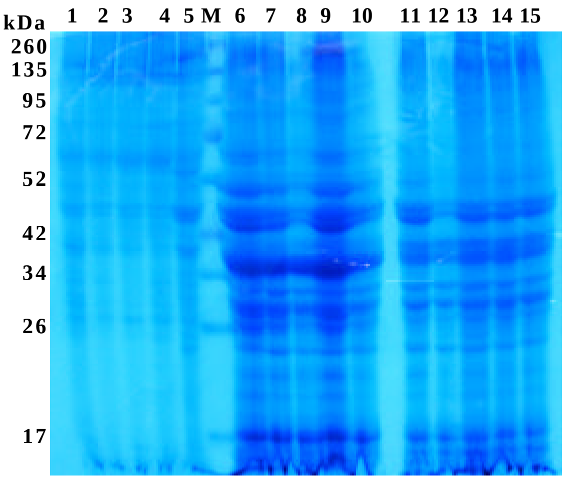
The skin tissue proteins of Cross-A chickens yielded very few bands on SDS-PAGE analysis, that too of poor intensity owing to the presence of smaller quantity of proteins in skin samples. As such, there were no differences in the compositional profiles of the skin samples from the crossbred chickens of Cross-A as well as in the skin samples of purebred KN and WLH chickens.



6% SDS-PAGE



8% SDS-PAGE



10% SDS-PAGE

Fig. 4.7 : The delineation of trends visible on protein profiling of KN & KN crossbred chickens, while optimizing the SDS-PAGE based 1-D electrophoretic separation of proteins

Note : displaying the migratory differences for various protein fragment belonging to skin and muslce-proteins from breast and thigh

Lane M : Molecular weight marker (SM#1841)**; Lane 1-5 :** Skin proteins of WLH, KN-WLH-W, KN-WLH slatty KN-WLH-BL&KN**; Lane 6-10 :** Breast meat proteins of WLH, KN-WLH-W, KN-WLH slatty KN-WLH-BL&KN; **Lane 11-15 :** Thigh meat proteins of WLH, KN-WLH-W, KN-WLH slatty KN-WLH-BL&KN

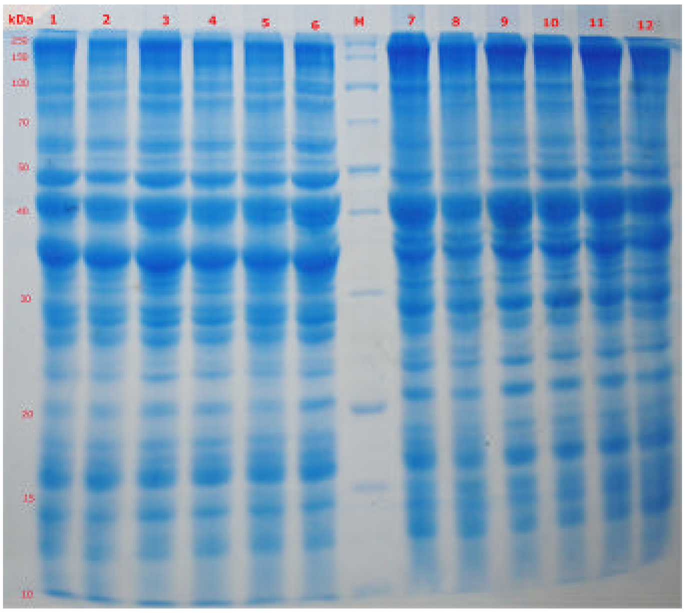


Fig. 4.8 : The SDS-PAGE profile for meat protein samples from breast and thigh of KN-WR crossbred chickens

Lane M : Molecular weight marker (SM#1881)**; Lane 1-3 :** Breast meat proteins of black skinned chickens**; Lane 4-6 :** Breast meat proteins of slaty skinned chickens; **Lane 7-9 :** Thigh meat proteins of black skinned chickens; **Lane 10-12 :** Thigh meat proteins of slaty skinned chickens

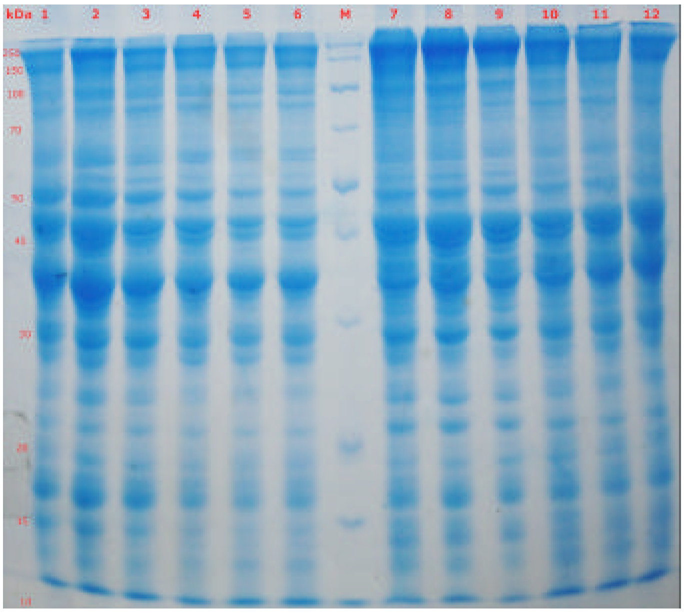


Fig. 4.9 : The SDS-PAGE profile for meat protein samples from breast and thigh of KN-WR crossbred chickens

Lane M : Molecular weight marker (SM#1881)**; Lane 1-3 :** Breast meat proteins of white skinned chickens**; Lane 4-6 :** Breast meat proteins of purebred KN chickens; **Lane 7-9 :** Thigh meat proteins of white skinned chickens; **Lane 10-12 :** Thigh meat proteins of purebred KN chickens

The SDS-PAGE analysis of breast and thigh meat proteins of Cross-B chickens, yielded multi-band profiles for all the analyzed samples, with the protein bands ranging in size from 10 kDa to 250 kDa. On an average, a total of 20 bands could be scored per sample, which remained largely isomorphic across the tested samples among the crossbreds. The higher molecular weight proteins produced thicker bands on comassie blue staining where each thick band appeared to be comprised of multiple protein fragments. The resolution for the smaller size bands was more distinct as compared to the larger ones. The differences between the pigmented versus non-pigmented, for protein profiles appeared to be variant in two low molecular weight protein bands, where the latter tended to possess two additional bands with distinctly variant intensity.

There were no visible polymorphisms between the protein profiles of thigh and breast meat samples. It was also not possible to score any intensity shifts for protein fragments between samples which varied in melanotic intensity phenotypically. Largely, it was realized that it was difficult to differentiate the pigmented meat samples from the non-pigmented, based on the combined protein profiles.

4.4 PROXIMATE ANALYSIS OF MELANOTIC TISSUES

Proximate analysis (moisture, crude protein and ether extract only) of melanotic skin and meat tissues of three major phenotypes of Cross-B chickens i.e. completely pigmented (black), moderately pigmented (slaty) and non-pigmented (white) were done and data were compared between the different crossbred phenotypes as well as between the two purebreds.

4.4.1 Moisture content

The means and standard errors for moisture content recorded for both purebreds and KN-WR crossbred chickens are presented in table 4.4. There was no significant difference ($P<0.05$) in the moisture percentage of skin tissues and thigh meat among the three major phenotypes of Cross-B chickens, while the breast meat of slaty group chickens had significantly higher moisture percentage when compared to those of other crossbred groups. As such, significant differences were also evident for the breast meat moisture between the KN and WR samples.

4.4.2 Crude protein (CP) content

There was no significant difference in the CP percentage of skin tissues and thigh meat among the three major phenotypes of Cross-B chickens. The breast meat of black- crossbred chickens had significantly higher ($P<0.05$) CP percentage when compared to the breast meat of chickens from other genetic groups. Even the slaty group chickens, maintained a non-signficant superiority over the non-pigmented chickens for CP percentage.

Comparison between the purebreds showed that: the KN chickens had significantly higher CP percentage in breast meat than the white Rock chickens. However, the same trend was not maintained for the CP of thigh meat or the skin tissues. The means and standard errors for CP content recorded for both purebreds and KN-WR crossbred chickens are presented in table 4.5.

4.4.3 Ether extract content

The ether extract (EE) percentages for breast meat samples were recorded very low as compared to the E.E of the skin and thigh meat. There was no significant difference among all the genetic groups for EE of breast meat in contrast to the same for thigh and skin. The black chicken phenotypes had significantly higher ($P<0.05$) percentages of E.E in the skin than others, while the lowest was recorded for the white-skinned group. The difference between the EE levels of the black and the slaty phenotypes however remained non-significant.

Table 4.4 : Moisture content in percentage (Mean±SE) of KN-WR crossbred, purebred KN and WR chickens

Genetic groups	N	Mean±S.E. Skin	Breast	Thigh
KN-WR BL	5	48.24±2.596	73.29±0.219[b]	73.45±0.604
KN-WR SL	5	48.59±1.769	74.03±0.212[c]	73.79±0.348
KN-WR W	5	49.84±2.626	73.77±0.290[b]	73.42±0.349
Purebred WR	5	47.39±1.005	73.66±0.149[b]	73.59±0.281
Purebred KN	5	46.78±0.542	72.52±0.179[a]	74.40±0.163
Overall	**25**	**48.17±0.805**	**73.45±0.138**	**73.73±0.171**

Means bearing different superscripts column wise are significantly different (P<0.05)

Table 4.5 : CP content in percentage (Mean±SE) of KN-WR crossbred, purebred KN and WR chickens

Genetic groups	N	Mean±S.E. Skin	Breast	Thigh
KN-WR BL	5	42.42±2.716	19.37±0.163[b]	17.60±0.506
KN-WR SL	5	42.34±1.645	18.63±0.169[b]	17.51±0.231
KN-WR W	5	41.67±2.706	18.46±0.278[a]	18.07±0.264
Purebred WR	5	44.74±0.957	18.55±0.129[a]	18.31±0.134
Purebred KN	5	45.93±0.749	19.83±0.464[c]	18.04±0.158
Overall	**25**	**43.420±0.860**	**18.96±0.156**	**17.90±0.134**

Means bearing different superscripts column wise are significantly different (P<0.05)

The difference between the EE levels of thigh meat tissues of all crossbred chickens was non-significant while the purebred KN chickens had significantly lower percentages of E.E. than other genetic groups including WR purebreds. The means and standard errors for E.E. content recorded for both purebreds and KN-WR crossbred chickens are presented in table 4.6.

4.5 BIOCHEMICAL ANALYSIS

The total blood plasma protein was estimated by using Lowry method (1951) in the Cross-B (KN-WR) chickens of three major phenotypes i.e. completely pigmented (black), moderately pigmented (slaty) and non-pigmented (white). The results indicated significantly higher level of protein in the plasma of the black phenotype as compared to the slaty and white-skinned phenotypes. As such, the KN had higher levels of plasma protein in their blood than the purebred WR chickens. The means and S.E. for the plasma protein for these crossbred and purebred groups have been summarized in the Table 4.7.

4.6 SENSORY EVALUATION SCORES

The emulsion based meat product i.e. chicken nuggets prepared from the meat of Completely pigmented (black-skinned) chickens had poor sensory scores in terms of appearance when compared to the nuggets prepared from the meat of slaty and white-skinned chickens. Nuggets prepared from the meat of slaty chickens had significantly higher sensory scores for appearance compared to that of black meat group. Though the slaty-meat nuggets had numerical superiority in appearance over white-meat nuggets but it was not significant. The slaty-meat nuggets had non-significant superiority in appearance compared to the WR meat nuggets.

When juiciness was compared, slaty meat nuggets scored over the black, white and purebred WR meat nuggets and it was

Table 4.6 : E.E content in percentage (Mean±SE) of KN-WR crossbred, purebred KN and WR chickens

Genetic groups	N	Mean±S.E. Skin	Breast	Thigh
KN-WR BL	5	8.11±0.238[a]	6.3±0.063	7.49±0.402[b]
KN-WR SL	5	7.96±0.219[a]	6.25±0.267	7.6±0.240[b]
KN-WR W	5	7.26±0.143[c]	6.50±0.216	7.3±0.273[b]
Purebred WR	5	7.43±0.269[b]	6.44±0.131	7.25±0.147[b]
Purebred KN	5	7.35±0.131[b]	6.62±0.083	6.45±0.128[a]
Overall		**7.624±0.110**	**6.42±0.075**	**7.22±0.134**

Means bearing different superscripts column wise are significantly different (P<0.05)

Table 4.7 : Total plasma protein in blood in g/dl (Means ± SE) of KN-WR crossbred, purebred KN and WR chickens

Genetic groups	N	Mean±S.E.
KN-WR BL	15	4.71±0.0895[a]
KN-WR SL	15	4.42±0.104[b]
KN-WR W	15	4.40±0.109[b]
Purebred WR	15	4.24±0.0465[c]
Purebred KN	15	4.59±0.0525[a]
Overall	**75**	**4.47±0.0413**

Means bearing different superscripts column wise are significantly different (P<0.05) statistically- significant. Slaty meat nuggets also scored higher in terms of overall palatability over the black, white, meat nuggets and which remained significantly different from other groups. The means and standard errors for sensory attributes of all the experimental groups have been summarized in the table 4.8.

Figure 4.10 shows the various shades of melanosis observed during the processing of chicken meat from three phenotypes of Cross-B (from minced meat to nugget preparation).

☑☑☑

Table 4.8: Sensory scores (Mean ± SE) for various sensory attributes of chicken nuggets of crossbred KN-WR chickens (N=21)

Genetic groups	Mean±S.E.					
	Appearance	Flavor	Binding	Texture	Juiciness	Overall palatability
KN-WR BL	6.4286±0.12[b]	6.64±0.11	6.45±0.10	6.73±0.10	6.4524±0.12[a]	6.4286±0.11[a]
KN-WR SL	6.9405±0.06[c]	6.56±0.12	6.38±0.15	6.61±0.12	6.7024±0.13[b]	6.7619±0.09[b]
KN-WR W	6.7738±0.09[c]	6.61±0.15	6.49±0.13	6.51±0.15	6.4405±0.14[a]	6.5952±0.11[a]
Purebred WR	6.75±0.09[c]	6.45±0.14	6.50±0.13	6.51±0.13	6.4167±0.17[a]	6.7857±0.12[b]
Overall	**6.26±0.10**	**6.55±0.05**	**6.48±0.05**	**6.60±0.05**	**6.58±0.06**	**6.64±0.05**

Means bearing different superscripts column wise are significantly different ($P<0.05$)

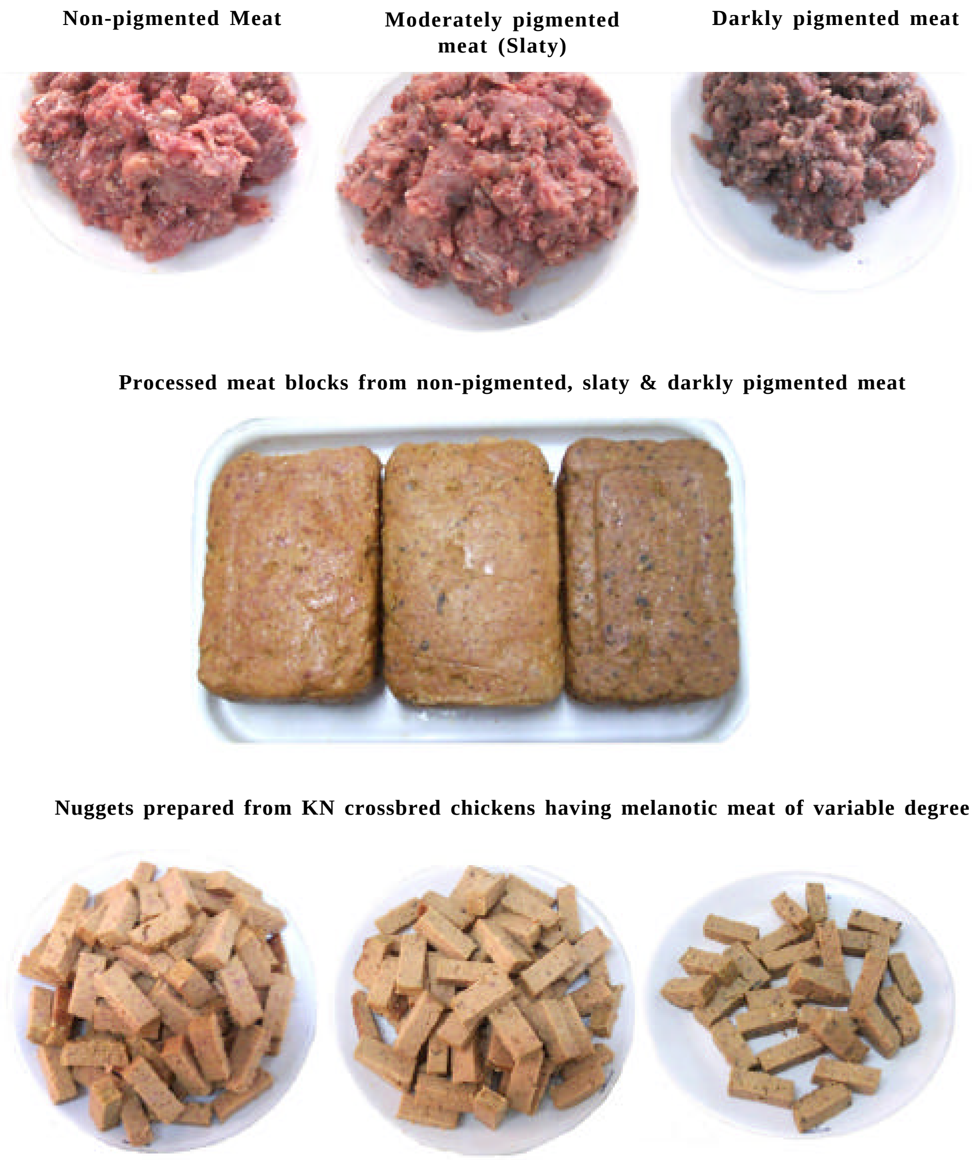


Fig. 4.10 : Highlights the shades of melanosis during the processing of meat from chickens of various phenotypes from Cross-B (from minced meat to nugget preparation)

Discussion

5.1 GROWTH PERFORMANCE

5.1.1 Bi-weekly BW of purebreds

All the three purebreds i. e. KN, WR and WLH chickens showed statistically significant difference ($P<0.05$) in body weights. As expected, the KN and WLH being slow growing breeds showed slow growth and were comparable to each other throughout the juvenile phases i.e. from 4^{th} week till 12^{th} week of age. WR being from broiler background exhibited a superior linear growth curve than other purebreds. The present study concludes that the purebred KN chickens are having slow-growth potential in comparison to the latter two breeds. This slow growth potential for purebred KN chickens has been well documented in earlier studies (Chatterjee *et al.*, 2007; Arora, 2010 and Pratap *et al.*, 2010).

5.1.2 Bi-weekly growth of Cross-A chickens

The non-pigmented (white-skinned) crossbred chickens exhibited significantly better growth potential than the completely-pigmented (black) and moderately pigmented (slaty) chickens. The melanosis of the skin tended to have no advantageous influence on the body weight. Our findings of having no special advantages of skin-melanosis attached with KN-WLH black chickens over others are further supported by the findings of Pratap *et al.* (2010).

5.1.3 Bi-weekly growth of Cross-B chickens

In general, the crossbred KN-WR chickens had a better growth potential than the purebred KN chickens. Within the cross-B chickens, the completely pigmented (black-skinned) chickens had higher body weights than the moderately pigmented (slaty) and non-pigmented (white) chickens during 4 to 8 weeks of age, following which the superiority in the growth remained numerical only. This fact of recording higher body weight for the black skinned and black meat chickens over the ones having slaty and non-pigmented skins are however contrary to the findings of Arora *et al.* (2010) who recorded only non-significant superiority for black skinned KN-WR crossbred chickens over the white skinned ones in a similar study. The reasons for such variation could be due to the season, where the authors (Arora *et al.*, 2010) had raised many hatches of chickens which were grown in a variable ambiences including winter and spring. As such, no comparable literature on KN-crossbred chickens involving meat type breeds have been published so far, which could be cited to discuss this fact, as witnessed in our study.

5.2 CARCASS MELANOSIS ASSESSMENT

5.2.1 KN as a population hosting major gene 'Fm'

Hyperpigmentation, the unique breed characteristic of KN chickens involving the darkening of the skin and other body tissues due to excess deposition of melanin pigments is the subject matter of the current study. A Chinese chicken breed: Silkie is also well-documented for harboring the same 'hyperpigmentation' character, technically called 'fibromelanosis' (Stanhope, 1995; Li and Luo, 2003; Dorshost *et al.*, 2010). Hutt (1949) suggested the gene symbol 'Fm' for this character. The KN is also reported to have possessed the same gene: Fibromelanosis (Fm) which is the basis for expressing the black coloration of the skin in KN purebred and KN crossbred chicken. In this study both the cross A and B expressed good amount of expression of the Fm gene in the respective crosses. While the Cross A exhibited the Fm's expression in nearly 75% of its progeny (Black and Slaty) and the cross B exhibited the Fm's expression in nearly 50% of its progeny (Black and Slaty) in our study clearly indicated the Fm's expression as a single major gene inheriting in the Simple Mendelian fashion. As the KN remained the contributor of the Fm gene to both these crossbred population, the impact of the Fm gene on the skin and meat tissue of the progeny resulted from introduction of Fm into non-melanotic breeds (WR and WLH) was only natural to be expected. Witnessing the intense black skin color in both KN purebreds and many of the Black-skinned crossbred chickens of Cross A and B alike, only confirms the hyperpigmentation potential of the Fm gene harbored in KN chickens, similar to that of Fm gene of Silkie breed. This finding of pinpointing the Fm gene as the primary causative basis of melanosis in the KN-crossbred chickens and KN purebred chickens, as revealed from our study is well supported by many published literature (Mishra *et al.*, 2008; Dorshost *et al.*, 2010 and Arora *et al.*, 2010).

5.2.2 Carcass melanosis assessment in cross-A (KN-WLH) chickens

The three skin phenotypes showed melanosis in skin, comb, beak and shanks as well as in the meaty cuts along with the internal organs as per the expectation of Mendelian ratio. Sometimes the crossbred completely-pigmented (black-skinned) chickens showed a greater degree of melanosis in their external and internal tissues exceeding the level of melanosis witnessed in the purebred KN chickens. Similar findings were reported by Pratap *et al.* (2010) when the authors compared the extent of melanosis in the back-crosses of KN and WLH chickens. However, these authors have also reported an additional fourth skin phenotype having non-pigmented skin with few melanotic spots, which were not revealed in our study. The basic fact that the Cross-A was an advanced intercross from the parents generated by Pratap *et al.* (2010) only refines the findings of the said authors that the intensity of hyper pigmentation was itself a result of involvement of more genes than the Fm alone, which was surmised by the said authors. As such by design, this cross is not likely to have the ‘Id’ gene in any of its progeny and therefore, the alternate Id genotypes (id/_ and id/id) are only natural in these chickens. In view of these facts, the more intense black color visible in some of our Cross-A chickens stands validated, which hypothesizes a basic difference in the ‘Id’ locus alleles present in KN versus WLH. Similar hypotheses on ‘Id’ alleles like the ones presumed in our study, have also been made by Arora, (2010) based on the author’s study involving more non-melanotic breeds like Aseel, Rhode Island Reds, besides the White Rock chickens used against Kadaknath chickens as the parents.

5.2.3 Carcass melanosis assessment in cross-B (KN-WR) chickens

The Cross-B (KN-WR) chickens showed variable degree of melanosis in skin ranging from the chickens totally devoid (~99%) of melanin to moderately-pigmented (slaty) and completely-pigmented (black-skinned) chickens. A corresponding pattern of melanosis was observed in these chickens, in their meaty cuts as well as in the internal organs, wherever the melanotic (black and slaty skinned chickens) chickens were slaughtered. These findings, “The correspondence between the intensity of skin-melanosis to melanosis of internal organs” are in concurrence with the report of Arora, (2010). The variation in intensity of blackness among the internal organs in our study remained in the order: periostium→bones→gizzard →heart→spleen→liver. It was also evident from our study that the white-skinned crossbred chickens did not show, even a small trace of pigment in their internal organs, though they were generated from the same set of parents which produced the fully-black and slaty progeny. The order of melanotic intensity among different internal organs and non-pigmentation in the internal organs of white skinned cross-bred chickens, as recorded in our study is agreement with earlier puglished reports (Mishra *et al.*, 2008b and Arora, 2010).

5.2.4 Melanosis of the Shank in both Cross-A and Cross-B crossbred chickens

In Cross-A (KN-WLH) crossbred chickens, the intensity of melanosis in the shank corresponded to their respective skin-phenotype. While the black-skinned chickens sported a fully-black shank color, akin to that of purebred KN chickens, the moderately-pigmented (slaty) chickens had greenish-black (slaty) shank-color. Interestingly, in the non-pigmented (white-skinned) chickens the shank color appeared as the only-pigmented portion of their body. In majority of such (white-skinned cross-A) chickens, the shank-color appeared pale green or white skinned with faint-green spots [which varied from yellowish-green to willow-green] across the shank (See figure 4.4 for clear distinction). It was also noticeable that in most of such white-skinned chickens, the greenish shank developed in the late neonatal period, (through 2^{nd} to 4^{th} week), as most of such chicks from this phenotype appeared white-shanked at the hatch.

In Cross-B (KN-WR) crossbred chickens, the degree of shank-melanosis was found to be corresponding to their respective skin-phenotypes. The non-pigmented (white-skinned) chickens had no melanosis in their shanks and resembled to those of purebred WR chickens. However, for the slaty and fully-black skinned phenotypes, the shank color remained slaty-black (greenish black) and dark-black in color respectively.

5.3 GEL ELECTROPHORETIC CHARACTERIZATION OF THE SKIN AND MEAT PROTEINS

Variable electrophoretic approaches through use of varying-density poly-acrylamide matrices, were adopted to resolve the possible differences between the protein-composition in the skin of WLH compared to crossbred samples of Cross-A [melanotic vs slaty vs non-melanotic phenotypes], which are summarized in fig. 4.7. It can be clearly elucidated from this figure that: as the percentages of PAGE-matrix increased from 6 to 10%, the resolution of the protein fragments differed correspondingly between the experiments. The 10% PAGE matrix composition appeared to be the best and hence were used in subsequent gels.

The compositional protein-profiles of respective meat tissues however, were not distinct from each other, through the 1-Dimensional electrophoretic patterns. High molecular weight proteins produced thicker bands with poor resolution among the major bands. In contrast, the low molecular proteins had comparatively better resolution. The meat protein profiles were found to be similar among the various phenotypes in the crossbred chickens of the Cross-B. There appeared to be few additional protein bands in the non-pigmented versus pigmented samples, which gives rise to some speculations that melanin-inhibitory proteins could be there in such samples, discussion of which remains inconclusive. As much as our study only had scopes for one dimensional protein profiling, more could not be revealed out about the presence of highly polymorphic proteins which were possibly present in the melanotic samples in variation to non-melanotic samples. The lack of any published literature on the melanotic meat-protein profiling makes it really difficult to verify the trend of such differential electrophoretic patterns between the profiles from black and slaty skin-phenotypes (see in figures 4.8&4.9).

The comparison among the protein profiles of skin, thigh and breast (figures 4.7) for the cross-A chickens revealed that the skin had less number of protein fragments than the thigh or breast. This feature of differential protein fragments between the skin and thigh/ breast remained constant between the breeds, involving KN, WLH and the crossbreds alike. This fact of having additional protein fragments in the breast meat or otherwise compared to the skin could be easily reasoned out, considering the presence of muscle proteins in the latter over the former.

5.4 PROXIMATE ANALYSIS OF MELANOTIC TISSUES OF CROSS-B (KN-WR) CHICKENS

Proximate analysis for the moisture, Crude protein, and Ether extract were carried out for the chickens of Cross-B. The results presented in the previous chapter, have been discussed hereunder.

5.4.1 Moisture content

The breast meat of the moderately pigmented (slaty) chickens showed a statistically significant (P<0.05) difference in moisture percentages as compared to those of other two crossbred groups i. e. completely-pigmented and non-pigmented (white skinned) chickens. Significant differences were also recorded for the breast meat moisture content between the purebred KN and WR chickens. However, no such trends were observed in case of skin and thigh tissues for the crossbred chickens of Cross-B or between the purebred chickens (KN versus WR). In general, the breast and thigh meat moistures were recorded in normal range as reported in published literature (Lawrie, 1979 and Hedrick *et al.*, 1993). As such, the WR being a meat type chicken breeds, the higher moisture content present in it compared to the KN meat is very much in the expected lines considering the published literature in this regard (Hedrick *et al.*, 1993).

5.4.2 Crude protein content

The completely-pigmented (black-skinned) crossbred chickens of cross-B had significant differences in crude protein (CP) content of the breast meat than the other two groups. The purebred KN chickens possessed significantly higher percentages of CP in breast-meat than both crossbred and purebred WR chickens. Similar findings have been reported by Mati and Verma (1983). However, these authors have reported a higher level of CP content in the meat of purebred KN chickens than has been recorded in our present study. The reason behind this variation could be that: their study had used adult KN chickens in contrast to ours, where all CP estimations were performed on 12 weeks-old chickens. The degree of melanosis appears to have a correlation with the CP content of the breast meat, since the slaty meat remained intermediate in CP-content, lying between the black and white-skinned phenotypes (non-pigmented). However, the pattern of CP difference for breast-meat between the crossbreds, revealed in our study could not be confirmed from the C.P profiles of skin and thigh tissues. Kawalkar and Bhambal (1996) in their comparative crude-protein analysis of two chicken breeds, have reported protein content of 91.94% on DM basis, in the Black meat chicken(BMC), compared to the routine table chickens. The authors also valued the BMC over the RTC, due to their higher composition of essential amino acids. The findings of these authors, though not exactly supportive of our observations in the current study, largely confirms our basis assumption that the KN chicken and their crosses displaying higher melanosis in the skin and meat tissues tended to record more CP in their meat compared to those of non-melanotic chickens.

5.4.3 Ether Extract content

Higher ether extract (EE) content in the skin tissues of completely-pigmented (black) and moderately pigmented (slaty) chickens were witnessed in comparison to white-skinned phenotypes. This would probably suggest that 'Fm' gene had no role in accumulation of fat content in the skin tissues, leading to differential

E.E-content in the meat of the crossbred chickens. The general trend of "lower E.E. values being recorded for breast meat than that of the skin and thigh tissues" was uniformly visible in all the crossbred group of Cross-B and the purebred KN and WR chickens, alike. The breast meat E.E. contents differed numerically among the chickens of three crossbred groups (Cross-B), showing no statistical significance. However the E.E content of thigh meat of purebred KN chickens had lower values with significant difference ($P<0.05$) in comparison to the three crossbred phenotypes of Cross-B as well as the purebred WR chickens. The findings of the present study about the E.E contents of the thigh and breast meat are well supported by the reports of Mati and Verma (1983), where the authors reported a further-low ether extract values estimated from the purebred KN meat tissues.

Kawalkar and Bhambhal (1996), in their studies, viewed it highly-enigmatic that the concentration of cholesterol, (184.75 mg/ 100g) recorded in the BMC meat was significantly lower ($P<0.01$) than that of Routine table chicken (RTC) meat (218.12 mg/100g), in spite of recording significantly ($P<0.01$) higher blood cholesterol (352.37 mg/dL) in BMC than RTC (253.12 mg/dL). Thus, it was inferred that: Kadaknath meat was richer in certain proteins, and was lower in cholesterol [while its fat possessed higher degree of unsaturation in terms of linoleic acid] than the routine table chicken. In view of such contrasting trend with respect to Ether extract content, especially which covers the cholesterol content of the meat, the KN meat deserves an elaborate and detailed study from the healthy-nutrition point of view.

5.5 BIOCHEMICAL ANALYSIS

The completely pigmented (black-skinned) crossbred and purebred KN chickens had significant differences in total blood plasma protein in comparison to the other crossbred chickens of Cross-B. The plasma samples from all the crossbred groups had however, normal and similar color intensity in visual scoring (colorimetric scale). In our study, apart from the minor changes in the plasma protein concentration recorded between the pigmented and non-pigmented samples, largely the total plasma protein content remained in the normal bio-chemical ranges for all crossbred chickens of Cross-B as well the purebred WR chickens. This might suggest just an adjunct role of fibromelanosis on the plasma protein content, and therefore, no drastic influence could be attributed to the 'Fm' gene, *per se*. As such, there is no published literature on the impact of 'Fm' gene on total plasma proteins in the crossbred KN chickens, till date, which poses a difficulty in discussing any such trend visible, in our findings.

5.6 SENSORY EVALUATION SCORES

Sensory evaluation studies were done in order to explore the possibility of boosting the consumption of KN-meat as emulsion-products, in phenotypically-diluted forms (through its crosses to meat type chickens). With this aim, through our study, suitable crosses of KN were raised against the WR broiler breed, so as to dilute the intense black phenotype of the resultant crossbreds of KN chickens. As such, globally, the demand for restructured meat products, such as nuggets, has increased significantly in the last 20 years (Resurreccion, 2003). This process has been cited to offer many opportunities to the food industry (Mandigo, 1986) and therefore, this deserved a trial in our experiment.

Our results showed that: nuggets prepared from the meat of moderately pigmented (slaty) chickens of Cross-B had statistically-significant ($P<0.05$) difference in appearance than those prepared from the meat of completely-pigmented (black) chicken. This is quite obvious considering the aesthetic properties of black or melanotic-meat and somewhat-repulsive appearances revealed in the black-meat nuggets (see figure 4.10 for clear distinction of blackness in the final product). As a welcome trend, the slaty-meat nuggets scored higher over the meat nuggets prepared from the other two crossbred chickens (both intense-black and non-pigmented), in terms of juiciness and overall palatability.

The present study concludes that addition of some artificial colors to mask the color of melanotic-meat while making a meat-emulsion in the course of nugget preparation, would certainly add more aesthetic value to the final product, thus making it more acceptable to the consumers. As a general trend, the urban consumers who might be hesitant to buy meat nuggets prepared from KN or its crosses having intense black-color could be offered a nugget with minimum mealanotic tinge, for their acceptability. Thus, considering the fact that melanotic meat products have significantly higher protein content, a nugget prepared from such meat, in diluted proportion (rendering it at least to moderately-pigmented level), would offer a distinct value- addition by offering a more-juicy and more proteincious product (nugget) in the market. As such, there is no literature available so far, on this aspect which could be cited vis a vis with our findings.

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Summary & Conclusions

Kadaknath is an important indigenous chicken breed of India, which has evolved through the centuries of natural selection and is well adapted to the local environment. The Kadaknath (KN) breed possesses a special characteristic like extensive melanin deposition in its skin as well as in the internal organs including bones, muscles, heart, spleen, gizzard, ovary etc. a condition which is termed as "Fibromelanosis". Literature available on this breed of chicken suggests that the fibromelanosis of KN is caused by an autosomal gene: 'Fm' gene fixed in this breed. The literature also has few reports, where the involvement of an incompletely dominant, sex linked gene 'id' i.e Non-inhibitor of dermal melanin is suspected in rendering melanin-deposition in the skin of such chickens. However, exact mode of Fibromelanosis expression in the KN chickens vis a vis these two causative genes: 'Fm' and 'id' is not clearly elucidated, till date. The meat and eggs of Kadaknath chickens are believed to have nutritional superiority over the other chickens which are likely to be triggered by the 'Fibromelanosis' character of this breed, due to which, the KN meat and eggs have long been valued in traditional medicine. There is only another such well-descript Internationally-documented chicken breed, named Silkie, which developed and migrated from China, possessing a similar characteristics like KN: 'Fibromelanosis'. As such, the Fibromelanosis studied and reported internationally from the Silkie breed attributes this character to the gene action from 'Fm' which is presumably the same gene held by the KN breed. Since, phenotypically, the KN and Silkie are wide apart, the fibromelanosis as a character warranted detailed studies.

The present study attempted to explore the impact of 'Fm'gene hosted in the KN chickens, through introduction of the same into two chicken breeds (White Leghorn [WLH] and White Plymouth Rock [WR] possessing non-melanotic phenotypes (lacking 'Fm' gene), which made it easy to study the effect of this gene in detail, in crossbred backgrounds. Therefore, the objectives of the study were:

(1) To characterize the melanosis of skin and meat from crossbred-Kadaknath chickens involving non-melanotic chickens, and analyze selected meat-quality parameters in Kadaknath-White Rock crossbred chickens.

(2) To subject the melanotic tissue-protein from above crosses to gel-electrophoretic analyses for differentiating their compositional profiles.

To have an idea about the pigmentation potential and pattern of melanosis influenced by the 'Fm' gene in KN crossbreds, two genetic groups were raised via mating of KN parents with chickens of WR and WLH respectively, in customized manners. Our study proposed to use the existing resources of crossbred KN chickens (available at experimental native-fowl farms, CARI, Izatnagar). Two types of crossbred resources were used: (A) a KN-WLH advanced inter-cross which segregated for alternative alleles of Fibromelanosis at 'Fm locus' (Fm vs. fm) but remained fixed for the 'Id' genotypes [males being 'id/id' and females as 'id/_' ; (B) a KN-WR F_2 resource which segregated for alleles of both the loci i. e. Fm, fm and Id, id alleles.

Using the above two customized crosses were raised. The 1st cross-A was generated by the mating of moderately pigmented (slaty looking) males (Fm/fm) and females (Fm/fm) of KN-WLH intercross, resulting into the three distinct skin phenotypes in the progeny i.e.

completely-pigmented (black), moderately pigmented (slaty), non-pigmented (white-skinned) chickens[which presumably were: Fm/Fm, Fm/fm^{+} and fm^{+}/fm^{+} genotypes respectively]. The Cross-A chickens were used in the qualitative analysis of skin-proteins and carcass melanosis assessment studies.

The 2nd customized cross-B was generated by the mating of non-pigmented KN-WR F_1 males [(putative genotype: Fm/fm; Id/id), being largely white-skinned with few black spots in body and shanks) with slaty looking KN-WR F_1 females [(putative genotype: Fm/fm; id/_) being moderately-pigmentd (slaty-skinned)], to generate all possible combinations of genotypes from the 'Fm' and 'Id' locus. The cross-B primarily resulted in generation of three skin phenotypes in their progeny i.e. completely-pigmented (black), moderately pigmented (slaty), non-pigmented (white-skinned) chickens with or without occasional pinpointed spots). The chickens of Cross-B were mostly used in the carcass-melanosis assessment, meat-proteins profile analysis, proximate-analysis of selected parameters, biochemical parameter (total protein in blood plasma) and sensory-evaluation studies of a chicken-product [nuggets] made out of their deboned meat. The study also utilized the purebred KN, WLH and WR chickens as control groups, besides evaluating the crossbred chickens of Cross-A and Cross-B to conclude on the respective objectives.

Analyses of the results of the growth-performance data revealed that: WR purebred chickens possessed higher body weight than the KN and WLH purebred chickens. Among the crossbreds of Cross-A, the non-pigmented (white-skinned) chickens had superiority in growth over the moderately pigmented (slaty) and non-pigmented chickens. In Cross-B, the completely pigmented (black-skinned) chickens possessed better growth potential than the chickens of other two phenotypes of this crossbred group.

The crossbred chickens of Cross-A, exhibited carcass melanosis in the external and internal tissues as per the expectation where the chickens of black-skinned group ranked higher in internal tissue-melanosis as compared to the other two groups of crossbred chickens. The trend of melanosis in the skin, dressed carcass and internal organs of chickens of Cross-B followed the pattern of melanosis as evident in progeny of Cross-A. The study also highlighted that the intensity of the fully-black skinned progeny of Cross-A in some proportion, tended to exceed the intensity of melanosis of the purebred KN, giving rise to assumptions that the 'id' alleles worked differentially in the KN purebred and the KN-WLH crossbred black-skinned chickens.

The one-dimensional protein profiles of skin tissues of Cross-A chickens yielded few protein bands and it was largely difficult to interpret any conclusive findings regarding the polymorphism of the skin proteins of melanotic vs non-melanotic phenotypes from the Poly-acrylamide gel electrophoresis patterns. The breast and thigh meat proteins of Cross-B chickens also showed no visible polymorphisms in their protein profiles except for the variability in intensity of two low-molecular-weight proteins or their differential-presence in non-pigmented phenotypes. In general, the meat proteins tended to produce more number of protein fragments as compared to skin proteins, leading to inference that the meat-protein obviously had additional protein-components drawn from the constituent muscle proteins in contrast to that of skin. It however, remained difficult to resolve the total number of protein fragments from this 1-D electrophoresis profiles and this calls for at least 2-Dimensional electrophoresis experiments to differentiate the variable protein constituents of melanotic versus the non-melanotic meat from KN crossbred chickens.

The Proximate analysis (for moisture, crude protein and ether extract contents) carried out on the chickens of cross-B, for the skin, breast and thigh meat samples showed that the breast meat of slaty chickens had significantly higher moisture percentage than others. The completely-pigmented chickens exhibited significantly higher crude protein content in breast meat as compared to slaty and non-pigmented samples. This therefore, indicated about a good correlation between degree of melanosis of meat tissues and their protein content. The ether extract (E.E) content was significantly higher in the skin tissues of completely-pigmented chickens than the white phenotypes giving speculations to the enigmatic impacts of melanosis on ether-extract content of tissues. In general, thigh meat had higher E.E. content than breast meat for all the crossbred chickens. The biochemical analysis revealed significantly higher plasma-protein content in black-skinned chickens than less-pigmented or non-pigmented chickens. However, these plasma protein values fell in the normal ranges considering the literature-reports for such values and therefore, no special impact of melanosis on plasma protein could be interpreted.

The sensory evaluation of meat-products generated from chickens of variable melanotic-phenotypes (cross-B) that considered three product groups: intensely-pigmented (black); moderately pigmented (slaty) and un-pigmented (white) meat-nuggets, provided interesting results. The slaty meat nuggets scored significantly higher on the juiciness and appearance scores than the other two groups, which led to conclusions that the presence of melanin pigments in moderate quantity in one such speciality-chicken product had higher consumer acceptance and appeal. This led to a general conclusion that product quality of slaty-meat nuggets were better than the other two category by distinct value-additions since it was proven that melanin-pigmentation led to protein-enriched product (due to higher CP content) and simultaneously, such a product was likely to gain more consumer-appeal due to higher juiciness and overall palatability.

CONCLUSIONS

(1) The non-pigmented chickens of the KN-WLH background had better growth potential than the pigmented phenotypes. In contrast, for the meat type chickens (KN-WR crossbred), the completely-pigmented chickens exhibited superior growth patterns in comparison to the less and non-pigmented phenotypes.

(2) The study evidenced that: in KN-WLH intercrosses, which were devoid of 'Id' genes in their genetic-make-up, the intensity of melanosis in completely-pigmented skin-phenotypes often surpassed the usual-level of melanosis visible in purebred KN chickens. Thus a differential expression of 'Fm' gene was hypothecated in KN-WLH crosses.

(3) In general, muscle tissues recorded larger number of protein fragments, than the skin tissues, in the 1-dimensional protein profiling study and no significant polymorphic protein profiles could be visualized for the melanotic meat proteins over non-melanotic samples.

(4) The Crude protein content in breast meat of completely-pigmented (intense-black) crossbred chickens was found to be higher in comparison to that of less-pigmented or non-pigmented phenotypes.

(5) The Ether extract content was higher in the skin tissues of black-skin phenotypes than the less pigmented lot, which warranted further studies.

(6) Chicken nuggets prepared from the moderately-pigmented meat got more consumer preference and acceptability. Therefore, the emulsion-based meat products like nuggets prepared from moderately pigmented chickens (Kadaknath-crossed to meat type chickens) would yield a distinct value-addition due to higher protein content, and thus recommended for marketing in larger scales.

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Mini Abstract

The present study was conducted to understand the impact of 'Fm' gene fixed in the Kadaknath chickens, in suitable crossbred backgrounds involving White Leghorn and White Plymouth Rock chickens. For this purpose, an advanced intercross of Kadaknath-White Leghorn and an F_2-cross of Kadaknath-White Plymouth Rock, designated as Cross-A (KN-WLH) and Cross-B (KN-WR) respectively, were used. Both Cross-A and Cross-B yielded three distinct types of progeny based on their skin color i.e. Completely-pigmented (black), moderately-pigmented (slaty) and non-pigmented (white) chickens. While the Cross-A chickens served as study materials for carcass-melanosis assessment without genetic-implications from sex-linked 'Id' gene. The Cross-B (KN-WR) chickens were utilized for an SDS-PAGE based protein profiling for breast and thigh meat proteins, proximate-analysis for: moisture, crude protein and ether extract contents for estimation of total plasma protein and a sensory-evaluation study on chicken-meat nuggets prepared from meat of varying melanotic-intensity, besides validation of carcass-melanosis. The carcass melanosis in the skin, meaty cuts and internal organs in all the three crossbred phenotypes of both Crosses (A&B) was found as per the expectation. The gel-electrophoretic profiles of breast and thigh meat proteins showed no visible polymorphisms between the melanotic and non-melanotic types except for the differential-intensity of the two low-molecular weight proteins in the latter. The breast meat of the moderately-pigmented chickens possessed significantly ($P<0.05$) higher moisture percentages than other phenotypes. The crude protein percentage was significantly higher in breast meat of black-skinned chickens than other phenotypes. The ether extract content was significantly higher in skin-tissues of black-skin chickens than other phenotypes. The total plasma-proteins in blood were significantly higher in the black-skinned chickens among all crossbreds. The chicken-nuggets from moderately-pigmented chickens got better scores in terms of appearance, juiciness and overall-palatability over other two phenotypic-groups and fetched more consumer-acceptability and preference. It was concluded that melanotic meat could yield better protein-value and can add value to the meat-products made from crossbred-Kadaknath chickens.

लघु सारांश

प्रस्तुत अध्ययन कडकनाथ मुर्गो में स्थित 'एमएम' जीन का प्रभाव सफेद लेगहार्न एवं सफेद प्लाईमाऊथ रॉक की उपयुक्त संकर पृष्ठभूमि वाली मुर्गियों में समझने हेतु किया गया। इस उद्देश्य–पूर्ति हेतु कडकनाथ–सफेद लेगहार्न का एक प्रगामी अन्तः संकर एवं कडकनाथ–सफेद प्लाईमाऊथ रॉक का एक द्वितीय पीढी अन्तः संकर क्रास किया गया जिन्हें क्रमशः क्रास–ए एवं क्रास–बी का नाम दिया गया। दोनो क्रास–ए एवं क्रास–बी से त्वचा के रंग के आधार पर तीन विभेदित प्रकार की संताने पूर्णतः कृष्ण, मध्यमकृष्ण एवं पूर्णतः श्वेत उत्पन्न हुई। क्रास–ए के मुर्गो, जोकि लिंग–सलंग्न 'आईडी' जीन के आनुवंशिक प्रभाव से विमुक्त थे, अनुप्रयोगिक सामग्री के रूप में उपयोग में लिये गये तथा उनके मृत शरीर में कृष्णता (मिलेनोसिस) का निर्धारण किया गया। क्रास–बी के मुर्गो के मृत शरीर में कृष्ण्ता–सत्यापन के अतिरिक्त, उनका उपयोग वक्ष एवं जाँघ की माँस प्रोटीन की एसडीएस–पी0ए0जी0ई0 पर आधारित प्रोटीन रूपरेखा बनाने हेतु, प्राक्सिमेट विश्लेषण जैसे आद्रता, अपरिष्कृत प्रोटीन एवं ईथर सार तत्व, सम्पूर्णरक्त प्लाज्मा प्रोटीन एवं भिन्न–भिन्न कृष्ण तीव्रता युक्त मुर्गमाँस से तैयार किये हुये नगेट्स के संवेदी–मूल्यांकन के अध्ययन में किया गया। दोनो क्रास–ए एवं बी के तीनो संकर फीनोटाइप मुर्गो की त्वचा, माँसल हिस्सो तथा आंतरिक अंगो में कृष्णता (मिलेनोसिस) आकांक्षा के अनुरूप पाई गई। वक्ष एवं जाँघ की कृष्णता युक्त एवं कृष्णतारहित माँस प्रोटीन की जैल इलेक्ट्रोफोरेटिक रूपरेखा ने कोई दृश्य बहुरूपता प्रदर्शित नही की किन्तु कृष्णता रहित प्रोटीन में अल्प आणविक भार युक्त दो प्रोटीनों ने विभेदित तीव्रता प्रदर्शित की। मध्यम–रंजित मुर्गो के वक्ष माँस में आद्रता प्रतिशत सार्थक रूप से उच्च थी। अपरिष्कृत प्रोटीन प्रतिशतता कृष्णता युक्त मुर्गो के वक्ष माँस में अन्य फीनोटाइपो की तुलना में सार्थक रूप से उच्च थी। ईथर–सार तत्व कृष्णा त्वचा वाले मुर्गो के त्वचा अतको में अन्य फीनोटाइपो की तुलना में सार्थक रूप से उच्च थी। सभी संकरो के मध्य में कृष्ण्ता युक्त त्वचा वाले मुर्गो में सम्पूर्ण रक्त प्लाजमा प्रोटीन सार्थक रूप से अधिक पायी गयी। मध्यम–रंजित मुर्ग–माँस से बने हुये नगेट्स को अन्य फीनोटाइपो की तुलना में दृश्यता, सरसता एवं सकल स्वादिष्टता में बेहतर प्राप्तांक, उपभोक्ता स्वीकृति एवं प्राथमिकता मिली। यह निष्कर्षित किया गया कि कृष्णता युक्त माँस बेहतर प्रोटीन मूल्य की लब्धिकर सकता है एवं कडकनाथ संकर मुर्गे–माँस से बने उत्पादो की मूल्य वृद्धि कर सकतौ है

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Appendix

(A) REAGENTS FOR SDS-PAGE

(1) 30% Acrylamide monomer solution

Acrylamide	29.0 g
N, N'- Methylene bis acryl amide	1.0 g

Volume made up to 100 ml with distilled water.

(2) 10% Resolving gel (50 ml)

D.W.	19.8 ml
30% acrylamide solution	16.7 ml
1.5 M Tris (pH-8.8)	12.5 ml
10% SDS	500 µl
10% APS	500 µl
TEMED	20 µl

(3) 5% Stacking gel (6 ml)

D.W.	4.1 ml
30% acrylamide solution	1 ml
1 M Tris (pH-6.8)	750 µl
10% SDS	60 µl
10% APS	60 µl
TEMED	6 µl

(4) 1X SDS electrode buffer (Tris Glycine buffer, pH-8.3)

Tris	3 g
Glycine	14.4 g

SDS	1.0 g
D.W.	1.0 l

(5) Staining solution

Coomassie Blue R-250	1.2 G
Methanol	500 ml
Glacial Acetic acid	200 ml
D.W.	500 ml

(6) Destaining solution

Methanol	300 ml
Glacial acetic acid	100 ml
D.W.	1 liter

Vitae

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