

CHARACTERIZATION OF KADAKNATH BREED OF POULTRY

(FAMOUS BLACK BIRD OF JHABUA)

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## 1. INTRODUCTION

There is a tremendous development in the poultry industry in last few decades, but little attention has been paid for indigenous chicken, due to its poor producing ability. Total poultry population of India was estimated to be 700 million, out of which about 10 to 15% were indigenous or native breeds (Ranganeker and Ranganeker 1999). There are about 20 indigenous breeds/varieties of chicken found in India (Singh and Johari 2000). Back yard poultry farming is a part and parcel of typical rural/tribal household, touching social, cultural and economic aspects in India. The practice of keeping chicken either as native indigenous breeds or crossbreds under scavenging system of back yard farming is still popular in majority of the rural / tribal areas (Sharma and Rao 2001). The birds under rural/tribal conditions are raised under scavenger system of management in small units with little feed supplementation and without any housing or disease control facilities.

Need of conservation and improvement of animal genetic resources has been globally accepted. FAO (1981) recommended the development of a global animal genetic resources. In India National Bureau of Animal Genetic Resources (NBAGR) was established in 1984 at Karnal, which is functioning as a nodal agency for characterization and conservation of animal genetic resources. NBAGR has formulated certain field survey guidelines and standard questionnaires based on FAO recommendations with the objective of identifying the breeding tract, geographical and demographical distributions, proper genetic evaluation in terms of morphological traits, production potential and reproductive status of various indigenous breeds of Livestock and Poultry. Further it has aimed at identification of superior herd / flock / individual animals, which should be specially considered in breed characterization, conservation and improvement programme.

Madhya Pradesh has great bio-diversity of livestock and poultry. Out of many indigenous poultry breeds the state has one well known breed of native origin named as Kadaknath or Kalamasi meaning the fowl having black flesh. The bird is being reared by Bhils & Bhilalas adivasies living in Jhabua District of Western Madhya Pradesh. The commonly available varieties of kadaknath are Jet black, Pencilled and Golden which are found in M.P. The bird is very popular among the tribals / adivasies mainly due to its special capabilities such as adaptability to local environment, resistance to certain diseases, meat quality and many other criteria specific to breed type (Rao and Thomas, 1984).

There is very little information available regarding description, native breeding tract, geographical, demographical distribution, morphological, productive and reproductive traits of Kadaknath breed of poultry. This breed due to pressure from high yielding genetic stock is on the verge of extinction. The systematic evaluation and conservation of this breed using modern technologies is essential for the assessment of genetic potential along with other traits as pure breed. Therefore a planned scientific survey based on questionnaires developed by NBAGR, Karnal is warranted.

Planning of any breeding programme or development strategies for improvement of any livestock or poultry species, its genetic characterization is a mandatory pre-requisite. Similarly evaluation of variability in the stocks help in deciding the procedures to be adopted for their genetic improvement. The genetic variability of indigenous poultry species is reducing through breed substitution. Information on polymorphic loci can be employed to detect population specific alleles to measure the amount of genetic diversity in each species, and to evaluate the change in variation in species over time.

Since last many decades genetic differences between individuals in a population have been identified by unique heritable phenotypic traits. During the past two decades advances in molecular biology, notably the discovery of restriction enzymes, development of nucleic acid hybridization and sequencing techniques, and the automated polymerase chain reaction (PCR) have permitted these differences to be detected at the molecular level.

Highly polymorphic markers are frequently obtained when PCR is used to amplify repetitive sequences. This is the basis for one of the most useful classes of genetic markers, termed microsatellites. Microsatellites are Tandom arrays of short repeats that are subject to great variation in number of repeat units. The application of microsatellites is currently thought to be very important as they are numerous and randomly distributed in the genome, they seem highly polymorphic and they show codominant inheritance (Burke *et al.* 1991). Estimates of genetic uniqueness are usually obtained through measurement of genetic distances.

The main objective of the present study was to characterize the Kadaknath breed of poultry based on physical, growth, reproductive and productive performance traits including estimation of genetic diversity among all the three varieties of Kadaknath breed of poultry using 25 microsatellite DNA markers.

## **2. FIELD SURVEY STUDIES ON DISTRIBUTION OF KADAKNATH BREED OF POULTRY AND SOCIO-ECONOMIC STATUS OF THE FARMERS.**

### **2.1. Name and habitat:**

The original name of Kadaknath is "**Kalamasi**" meaning the fowl having black flesh. However this name is not as popular as the present name Kadaknath (some times spelled as Karaknath) which was suggested by the collector of Jhabua District of Western Madhya Pradesh.

Kadaknath is an important indigenous breed of poultry inhabiting vast area of Western Madhya Pradesh mainly the Jhabua and Dhar Districts and adjoining areas of Gujarat and Rajasthan. But because of indiscriminate crossbreeding with other poultry breeds the pure Kadaknath birds are very rarely available in Dhar and adjoining areas of Gujarat and Rajasthan. At present the Kadaknath birds are mainly available in Jhabua District of Western Madhya Pradesh. This breed has evolved through natural selection in indigenous agro-ecological conditions and are well adapted to local environment. The Kadaknath birds reveals appreciable degree of resistance to diseases than any other exotic breeds of fowl in its natural habitat in free range. Kadaknath birds were also resistant to extreme climatic conditions like summer heat and cold winter stress and can thrive very well under adverse environments like poor housing, poor management and poor feeding etc. The breeding tract of Kadaknath birds along with field survey centres are shown in **Fig.1**.

### **2.2. Field survey plan:**

As per technical programme of first year, breed descriptors/questionnaires for field survey including physical and performance recordings traits were procured from National Bureau of Animal Genetic Resources (NBAGR) Karnal (Haryana). The required numbers of questionnaires No.1, 2, 3 and 4 were got printed. With the help of State Veterinary Department Madhya Pradesh an extensive tour of Jhabua and Dhar District was conducted to decide various field survey centres. Looking to the density of the breed in the areas, two

supervisor and 10 enumerator centres were established, after having detail discussion with Deputy Director / concerned Vety. Assistant Surgeons of Jhabua / Dhar Districts (**Fig. 1**).

Stratified two stage sampling design was adopted for field survey studies. Looking to the availability of the Kadaknath breed, Jhabua District was divided in two centres that is Jhabua – 1 (Meghnagar) and Jhabua-II (Jobat). Each district was further divided into five zones / strata and from each zone 7 to 25 villages were randomly selected for survey. Villages within a strata / zone constitute the first unit and houses within the village, the second unit.

### **2.3. Description of District, Climate, Soil and Feed:**

The Jhabua Hills zone which covers whole Jhabua District, is situated in the Western part of the Madhya Pradesh in between the latitudes (N) 20° 50' and 23° 40' and longitude (E) 74° 30' and 75° 16'. The mean altitude ranges from 450 to 700 meter above mean sea level. The District covers 0.68 m ha geographical area. The climate is hot semi arid with an annual rainfall of 500 to 1000 mm, The rainfall is erratic and annual variation of rainfall is a constant feature. The minimum average temperature is 10°C and the maximum average temperature rises upto 41 to 43°C.

In Kharif, maize occupies the largest area followed by Jowar, groundnut, black gram and Kulthi (horse gram) (In Rabi Bengal gram is a major choice of the farmers. The cultivation of cotton is limited to black soils. Soils of the area are medium deep black clay soils, more than 50 percent of the soils are shallow to very shallow prone to erosion due to steep slopes of land. The heavier soils are 40 percent with clay and clay loam texture.

### **2.4. Demographical distribution of the breed:**

The questionnaire No.1 included general description about family members, literacy percentage, land holding, annual income, family members involved in poultry rearing and poultry keeping as backyard poultry or commercial chick rearing. The questionnaire No.1 also included age-wise & sex-wise information for cockerels, pullets, hens and cocks for all breeds/desi poultry available in the breeding tract including Kadaknath. The questionnaire No.2 included birds vaccination schedule and treatment of specific poultry diseases. The questionnaire No.2 also included information on flock size, farm type, housing, feeding and management of birds, mortality and stress tolerance against many infections

The sex-wise & age-wise population dynamics pooled for both the districts are shown in **Table 1**. As shown in Table 1 almost equal population of Desi birds (20292) and Kadaknath birds (20305) was found in both the Districts, whereas population of other breeds were less. Similarly the age-wise and sex-wise Kadaknath breed population dynamics of Jhabua-I Meghnagar and Jhabua-II Jobat are presented in **Table 2 and 3**.

As shown in **Table 2 & 3 and Fig. 2**, it was observed that more number of Kadaknath birds of all age groups were found in Jhabua-II (Jobat) as compared to Jhabua-I (Meghnagar) .

**Table 1.** Demographic Distribution of (sex-wise and age-wise) total population in District Jhabua-I (Meghnagar) and Jhabua II (Jobat)

S. No.	Name of Species/Breed	Age upto 0-5 months		Age above 5 months		Total
		Cockrel	Pullet	Cock	Hen	
1	Kadakhnath	5879	7296	1911	5219	20305
2	Desi	6451	6574	2550	4817	20292
3	R.I.R.	667	566	75	585	293
4	W.L.H.	28	44	21	67	160
5	Black minorka	3	2	8	12	25
6	Ducks	2	3	3	5	13
Grand Total		12930	14485	4968	10705	43088

**Table 2.** Demographic Distribution of Kadakhnath Birds for District Jhabua – I (Meghnagar)

S. No	Name of zone (Code No.)	No. of villages covered	No. of houses covered	Age upto 0 – 5 months		Age above 5 months		Total
				Cockrel	Pullet	Cock	Hen	
1	Jhabua-I (S <sub>1</sub> )	14	97	19	33	39	139	230
2	Thandla (E <sub>11</sub> )	20	637	185	317	251	246	999
3	Kalyanpura(E <sub>12</sub> )	17	516	152	412	63	236	863
4	Jhabua (E <sub>13</sub> )	17	325	168	276	2	825	1539
5	Para (E <sub>14</sub> )	34	387	372	339	72	284	1067
6	Rama (E <sub>15</sub> )	13	345	97	95	12	39	243
G. Total		115	2307	993	1472	707	1769	4941

(S<sub>1</sub>) Supervisor center (E<sub>11</sub> .....E<sub>15</sub>) Enumerator centers

**Table 3.** Demographic Distribution of Kadakhnath Birds for District Jhabua – II (Jobat)

S. No	Name of zone (Code No.)	No. of villages covered	No. of houses covered	Age upto 0 – 5 months		Age above 5 months		Total
				Cockrel	Pullet	Cock	Hen	
1	Jhabua-II (S <sub>2</sub> )	9	137	148	268	40	148	604
2	Bhawra (E <sub>21</sub> )	13	743	1463	1564	415	1091	4533
3	Ambua (E <sub>22</sub> )	13	802	1151	1411	173	929	3664
4	Udaygarh (E <sub>23</sub> )	14	580	1684	2077	285	716	4762
5	Bori (E <sub>24</sub> )	13	413	221	283	193	286	983
6	Ranapur (E <sub>25</sub> )	18	474	219	221	98	280	818
G. Total		80	3149	4886	5824	1204	3450	15364

(S<sub>2</sub>) Supervisor center (E<sub>21</sub> .....E<sub>25</sub>) Enumerator centers

## 2.5. Socio-economic status of the farmers:

The results of general information regarding the Socio-economic status of farmers under random field survey of District Jhabua-I (Meghnagar) and District Jhabua-II (Jobat) are presented in **Table 4**.

As shown in **Table 4** the total agricultural land holding was found to be 4.1 and 4.2 (acres) for District Jhabua-I and Jhabua-II respectively, with an average of 4.15 for both the Districts. The average irrigated land holding pooled for both the Districts were almost same (2.20 acres). The maximum percentage of farmers in both the Districts belongs to small land holding group. The average number of literate family members in both the districts that is Jhabua-I and Jhabua-II were almost similar with an overall average of 2.25. The average family size of both the Districts were almost similar with an overall average of 8.1. The number of family members engaged in poultry rearing activities were 1.3, which was same in both the districts. In terms of average annual income the farmers of District Jhabua-II (Jobat) were having higher income (Rs. 9920.9) as compared to District Jhabua-I (Meghnagar) (Rs.8814.8).

**Table 4.** General information regarding the Agricultural Status under random survey of District Jhabua I (Meghnagar) and Jhabua II (Jobat)

Trait	Jhabua-I (Meghnagar) No. of houses covered 2306	Jhabua-II (Jobat) No. of houses covered) 3149	Pooled (No. of houses covered) 5455
Agricultural holding (acres)	4.1	4.2	4.15
Irrigated Land (acres)	2.1	2.3	2.20
Annual Income(Rs.)	8814.8	9920.9	9367
Family members	7.9	8.3	8.1
Literate members	2.2	2.3	2.25
Family members engaged in Poultry	1.3	1.3	1.3

Singh *et al.*(2000) reported socio-economic status of farmers in three districts, that is North Baster and South Baster in M.P. and Khaman in Andhra Pradesh in the breeding tract of Aseel fowl. They reported the status of farmers in terms of average family size, average land holding and the percentage distribution of farmers into different categories on the basis of size of land holding. The average number of family members in three districts ranged from 5 to 6. The average number of literate members per family are 66% in North Baster and only 40% in South Baster and Khaman Districts. The farmers of North Baster have better land holding (6 acres) than those of South Baster and Khaman Districts.

## 2.6. Housing, feeding and managerial practices:

Both the Districts Jhabua-I (Meghnagar)and Jhabua-II (Jobat) falls under tribal region, where Bhils and Bhillala Adavasies does not follow the scientific methods of housing, feeding and rearing of the birds. The farmers of the area mostly follow backyard poultry farming. Generally all the tribals keep some birds along with animals like Buffalo, Cattle and Goat etc. The birds are kept in Kachha houses mostly during night time only. During day time birds are let loose and are free to move anywhere in the field (**Fig.4**). The birds reared under different feeding systems are presented in **Table 5**.

**Table 5.** Different feeding systems and mortality percentage distribution of poultry population in Jhabua I (Meghnagar) and Jhabua II (Jobat).

Feeding System	CENTRES		Pooled
	Jhabua- I (Meghnagar)	Jhabua-II (Jobat)	
Scavenging / Free ranging (%)	37.3	38.0	37.6
Scavenging with local supplement feed/ kitchen waste (%)	58.8	58.2	58.5
Scavenging and formulated feed (%)	2.3	3.2	2.75
Scavenging, kitchen waste, feed (%)	1.5	0.1	0.8
<b>Mortality %</b>			
0 – 1 week	16.6	24.9	20.72
1 – 4 weeks	10.2	18.4	12.1
4 – 8 weeks	6.7	12.1	9.4

As shown in **Table 5** scavenging with local supplement feed/kitchen waste feeding system was most common in both the Districts most of the farmers feed their birds with maize, paddy, & wheat, but their amount is quite low . Singh *et al.* (2000) conducted field survey studies in Aseel breed of poultry at Baster District of Chattisgarh State. They found that scavenging with supplementation of kitchen waste and free ranging with supplemental feeding of grains are the most common feeding systems in Aseel breed of poultry. The field survey studies were conducted at Buster District of Chatishgarh State. In both the Districts very few farmers avails the facility of vaccination of their birds. Hence most of the birds were not vaccinated. Due to poor vaccination and treatment facilities of birds, the mortality percentages were quite higher in the field. As shown in **Table 5** the mortality percentages from 0 to 8 weeks of age were higher for Jhabua – II (Jobat) as compared to Jhabua-I (Meghnagar). Farmers generally eat eggs/ meat of Kadaknath birds and keeps them for hatching .and further propagation of their flocks. Some farmers sell the eggs / birds in the market.

Singh *et al.* (2000) reported percentage of birds reared under different feeding systems in the breeding tract of Aseel fowl. The free ranging with supplemented feeding of grains was found to be most common (65%) in North Baster, which was in close agreement with the results reported in the present study for Kadaknath breed of poultry.

### 3. BREED CHARACTERISTICS OF KADAKNATH:

The detail breed characteristics for various traits namely physical, growth, reproductive, productive performance, feed utilization and carcass traits are described below.

#### 3.1. Physical characteristics

Since long Kadaknath breed of poultry was reared by tribals/ adivasies (Bhils, Bhillalas and others) and therefore through many generations of selection and fixation of genes some of the important breed characteristics had been established. There were three main varieties of Kadaknath breed which are found in Jhabua District. They were Jet black,



Pencilled and Golden Kadaknath. The Jet black adult males and females were black in colour, the Golden adult male and females were basically black in colour with Golden feathers on head and neck, whereas in Pencilled variety adult male and female plumage were black with white feathers on neck (**Fig.,4& 5**). In all the three varieties, skin, beak, shanks, toes and soles of males as well as females were dark gray coloured, whereas tongue was dark gray or light black colour. Comb, Wattles and earlobes were light gray to dark gray coloured. However, in comb, Wattles and earlobes purple hue coloured were also observed. In all the three varieties of Kadaknath breed most of the internal organs exhibit intense black colouration which is due to the deposition of melanin pigment in the connective tissue of organs and in the dermis (Rao *et al.*, 1980). Although Kadaknath breed is poor in egg production potential, but their black flesh is very delicious and popular. Its flesh is of higher value and is being used for the treatment of many diseases in human beings by tribals / adivasies living in Jhabua District of (M.P.). However, this needs proper scientific evaluation. In the whole of the breeding tract Kadaknath birds are in great demand and are very costly. The meat and eggs are also reckoned to be a rich source of protein (25.47 in flesh) (Rao and Thomas, 1984).

There are variations in physical traits of different varieties of Kadaknath breed. The different physical traits observed during field survey of second year and third year using questionnaire no.3 were calculated (percentages) using MS Excel computer software. The prominent characteristics of the breed on overall basis are as follows (**Fig 5& Table 6**).

### **3.1.1. Plumage Colour and Pattern**

**Plumage Colour:** In all the three varieties i.e. Jet black, pencilled and golden the predominant plumage colour observed was black (54.25 %). However golden with black (15.86 %) and pencilled with black had also been observed (29.89 %).

**Pattern :** The dark /solid black pattern (37.32 %) was generally more common in both sexes, however some light /dull black (22.62%), stripped (31.61%) and patchy (8.36%) pattern had also been observed .

### **3.1.2. Colour of shank, skin and ear lobes**

**Shank colour :** Grey (30.84%), dark grey (57.95%) or black shank (11.21%) were generally prevalent .

**Skin colour :** The black (28.41%), grey (19.12%) and dark grey (52.41%) skin colours were observed in all the three varieties

**Ear lobe colour:** Moderate in size fitting close to the head. Light to dark grey colour had been observed.

### **3.1.3. Comb colour, comb type and wattle**

**Comb colour :** The comb colour observed in all the three varieties of Kadaknath bird was grey (40.01%) to dark grey (49.36%), however purple hue colour (10.61%) was also observed.

**Comb type:** The single comb type was predominantly (69.73%) observed in all the three varieties, however pea comb (33.24) and mixed comb (2.60%) were also observed.

**Wattle:** The size of the wattle was smaller in females as compared to males, which was of light gray colour.

#### **3.1.4. Beak, eyes and neck**

**Beak:** Black, stout and well curved

**Eyes:** Moderately large, round and were of black coloured (74.35%). However few light black/grey eye coloured (25.67%) birds were also observed

**Neck:** Moderately long, nicely arched.

#### **3.1.5. Breast, wings, legs and toes**

**Breast:** Well rounded and carried forward.

**Wings:** Large, well folded and carried without drooping.

**Legs and Toes:** Legs set well apart, straight lower thighs, shanks moderately long and the toes were straight and well spread. Lower thighs were having black feathers. Shanks and toes were black or bluish black in colour.

**3.1.6. Other specific characteristics:** Naked neck, frizzled birds, multiple spurs and rare variety (Kabra) had also been observed in very few cases.

### **3.2. Growth performance**

The body weight of birds at the specific age interval starting from 0 to 20 weeks and from 6 to 12 months of age were recorded from various survey centres located in District-I (Meghnagar) and District-II (Jobat) using questionnaire No.4. The body weights were recorded at weekly intervals from 0 to 4 weeks of age, at fortnightly intervals after 4<sup>th</sup> week to 20 week of age and at monthly interval thereafter up to 12 months of age. The body weights were recorded for males and females separately from 6<sup>th</sup> week onwards.

#### **3.2.1. Body weight from 0 – 12 months of age**

The pooled overall average body weights for Jhabua-I (Meghnagar) and Jhabua-II (Jobat) from 0 week to 12 months of age ranged from 27.99 to 1497.53g. As shown in **Table 7**. It was observed that birds attain 1 kg body weight between 6 to 7 months of age and the weight reached around 1.5 kg at one of age. It was also observed that the above growth trend was under field conditions, where the birds are mostly raised under free ranging system with very little supplementation of kitchen waste or locally produced food grains. Thus the birds failed to express their full potential of growth in different periods.

#### **3.2.2. Comparative growth performance for Jhabua-I(Meghnagar) and Jhabua-II (Jobat).**

The growth trend in both the districts i.e. Jhabua-I (Meghnagar) and Jhabua-II (Jobat) were similar upto 16 weeks of age and thereafter Jhabua-I (Meghnagar) centre showed superiority in growth over the Jhabua-II (Jobat) upto 52 weeks of age. Fast growth trend was observed for birds in both the districts upto 20<sup>th</sup> week of age and thereafter the growth trend became slower upto 52<sup>nd</sup> week of age.

### 3.2.3. Linear regressions for estimation for gain in body weight

Linear regressions were plotted taking of the body weights as dependent variable and the age in weeks as independent variable to assess the weekly gain in body weight. The 48 weeks period was divided into three phases 0 – 4 (Phase – I), 4 – 20 (Phase – II) and 20 – 48 weeks (Phase – III). This classification is based on the growth curve . The weekly gain in body weights were obtained separately for the three phases for both districts and also on pooled basis. The results are presented in **Table 8**.

From the **Table 8** it as observed that weekly gain in body weights during growth phase – I (0 – 4week) ranged from 17.87 to 24.68 g with an overall (Pooled over two districts) gain of 21.28g. During phase – II (4 – 20 weeks) the weekly gain ranged from 46.99 to 47.13 and overall gain of 47.06g. During the third phase (20 – 48 weeks) the body weight gain ranged from 17.50 to 18.15 with an overall gain of 17.82. It was concluded that during the first growth phase (0-4 weeks), the difference among the two districts was significant. However, during the II<sup>nd</sup> and III<sup>RD</sup> phase (4 – 48 weeks) no significant differences among the districts was observed.

Gurung and Singh (1999) reported the pooled body weights in Aseel breed of poultry based on data collected through field survey in three districts of Bastar division. The body weights for 0 to 48 weeks of age ranged from 29 to 2590g. The higher body weights from 0 – 48 weeks of age were observed in Aseel breed of poultry as compared to Kadaknath breed, may be due to its larger body size. Singh *et al.* (1999) reported that there was a linear increase in body weight in both the sexes, however they reported that males were heavier than females from 0 – 21 weeks of age in Aseel bred of poultry.

### 3.3 Reproductive and Productive Performance: -

The reproductive and productive performance traits includes broodiness, hatchability percent on total egg basis, age at first egg and egg production during first, second, third and fourth laying cycles. Under natural setup, after the attainment of sexual maturity, a hen starts laying eggs which continues for a period of 15 to 20 days. This period may be termed as a "**laying period**", after this the bird becomes broody and incubates the eggs for 21 to 26 days. This period may be termed as "**incubation period**". After the hatching of chicks hen broods them for 35 to 70 days, this period may be termed as "**brooding period**". This completes one laying cycle and then the birds enter next laying cycle. Each laying cycle comprise of 3 to 4 months duration, which results in 3 or 4 laying cycles per year (**Fig. 7**). Although the birds lay eggs throughout the year but the frequency is extremely low during summer, particularly in the month of May and June. Even if eggs are laid, the hatchability was very poor.

The overall (pooled for two districts) productive and reproductive performances are given in **Table 9** The **broodiness** was 70.96 percent in usual cases and 29.05 percent in sometime cases. Average egg weight for Jhabua-I (Meghnagar) and Jhabua-II (Jobat) was observed to be 41.14 and 41.77 g respectively with an **average overall egg weight** of 41.46 g. Overall **hatchability percentage on total egg basis** was found to be 71.10 percent. The **average age at first egg** was 187.19 days whereas the overall **average egg production** was found to be 67.91 eggs per year. Singh *et al.*(2000) studied various reproduction and production performance traits of Aseel birds under field conditions. The overall broodiness was 58 percent in usual cases and 42 percent in occasional cases. The overall hatchability on total egg basis was found to be 73.97 percent. The average egg weight was recorded as 41 g, whereas age at first egg was 29 weeks. The average egg production was found to be 33.17 eggs per year.

### 3.4. Egg quality traits:

The overall averages for various egg quality traits pooled for both Districts are presented in **Table 10**. As shown in **Table 10** dark brown **shell colour** was most frequent (67.87%) followed by light brown colour (32.12%). The maximum **number of eggs** (65.39%) were strong shelled, (32.06%) were medium shelled and (2.56%) were brittle shelled. The mean **shell thickness** pooled for both the districts was found to be 0.309 mm. The overall mean shape index pooled for both the district was found to be 73.95, whereas overall average mean **albumin index** was found to be 7.03. The overall **haugh unit** pooled for both the district was found to be 73.77, whereas over all average for **yolk index** was observed to be 37.07. The overall average **yolk weight** pooled for both the districts was found to be 14.77g, whereas overall **albumin weight** was found to be 20.74gm

### 3.5. Feed Efficiency:

Feed efficiency of the Kadaknath birds was studied by raising birds under farm condition. The weekly body weight and feed consumption of individual birds were recorded from 0 to 20 weeks of age, which are presented in **Table 11**. Total of 60 day-old chicks were randomly divided into 6 groups of 10 birds each. The birds of each group were raised separately under deep litter system from 0 to 20 weeks of age. The birds were weighed individually at weekly intervals and the body weight and feed consumption was recorded from 0 to 20 weeks of age.

The regressions of body weight on age (weeks) were fitted to estimate the average weekly gain in body weights. The average weekly gain in body weight from 0 to 8 weeks of age was estimated as 24.17, whereas for the period from 9 to 20 weeks this estimate was 69.80g.

The average weekly feed consumption per bird was 27.56 g during first week, 69.25g during 4<sup>th</sup> week, 133.63g during 8<sup>th</sup> week, 290.50g during 12<sup>th</sup> week, 366.87g during 16<sup>th</sup> week and 458.68g during 20<sup>th</sup> week. The feed efficiency (feed consumed /gain in body weight) during different weeks ranged from 2.04 to 7.61.No definite trend was observed for feed efficiency, however it can be observed that feed efficiency increased with increase in age of the birds. The overall feed efficiency was observed as  $4.26 \pm 0.40$ . Gupta *et al.*(2000) studied feed efficiency from 1 to 21 weeks of age in Aseel breed of indigenous chicken. The feed efficiency during different weeks ranged from 2.45 to 5.29 with an overall feed efficiency was observed as 5.00.

**Table 11.** Feed consumption and feed efficiency in Kadaknath birds

Weeks	Body weight (g)	Feed consumption (g)	Feed efficiency (FE)
0	29.97	-	-
1	41.55	27.58	2.38
2	61.12	49.57	2.53
3	85.80	68.15	2.76
4	105.92	69.25	3.44
5	126.90	76.30	3.64
6	146.38	76.92	3.95
7	174.39	90.83	3.24
8	239.95	133.63	2.04

9	280.25	153.92	3.82
10	360.85	175.50	2.18
11	458.75	278.63	2.85
12	500.50	290.50	6.96
13	571.73	295.62	4.15
14	666.73	301.50	3.17
15	735.78	365.75	5.30
16	802.30	366.87	5.52
17	850.75	368.63	7.61
18	915.25	397.53	6.16
19	985.85	440.17	6.23
20	1048.47	458.68	7.32
<b>Overall = 4.26 ± 0.40</b>			

### 3.6. Carcass traits:

In order to study the carcass traits a total of 16 birds (8 males & 8 females) raised under farm conditions were sacrificed at 23 weeks of age. The results of various carcass traits, sex wise and overall are presented in **Table 12**. There is no fixed age for slaughter of Kadaknath birds under field conditions. The tribals usually slaughter the birds after 7 to 8 months, that too during some festival, religious events or marriages only. The average live body weights at the time of slaughter of males and females were found to be 1212.5g and 1111.25g, whereas average dressed weight of males and females were 862.63g and 825.88g respectively. The dressing percentage in males and females were found to be 71.14% and 74.32% respectively with an overall percentage of 72.73%.

The percentage yield of different cuts had also been compared among the male and female birds. As shown in **Table 12**, the percentage of breast muscles is more in males than in females. The percentages of drumstick and thigh cuts are almost similar in both sexes. The overall percentage of different cuts viz neck, wings, back, breast, drum stick, thigh, heart, gizzard and liver were recorded as 4.9, 11.88, 19.43, 26.12, 13.10, 17.11, 0.67, 26.15 and 31.63 per cent respectively. Gupta *et al.* (2000) reported higher dressing percentage in Aseel birds (74.76%) as compared to Kadaknath birds, where the dressing percentage was found to be 72.73%.

**Table 12.** Mean values of carcass traits sex wise and overall

Traits	Males (8)	Females (8)	Overall (16)
Live body weight ( g)	1212.5	1111.25	<b>1161.88</b>
Dressed weight ( g)	862.63	825.88	<b>844.26</b>
Dressing percentage	71.14	74.32	<b>72.73</b>
Percentage yield of different cuts			
i. Neck	4.58	5.22	<b>4.9</b>
ii. Wings	11.64	12.11	<b>11.88</b>
iii. Back	18.04	20.81	<b>19.43</b>
iv. Breast	27.42	24.82	<b>26.12</b>
v. Drum stick	13.4	12.79	<b>13.10</b>
vi. Thigh	16.74	17.48	<b>17.11</b>
vii. Heart	0.77	0.56	<b>0.67</b>
viii. Gizzard	27.13	25.16	<b>26.15</b>

ix. Liver	31.88	31.38	<b>31.63</b>
x. Giblet	8	7.4	<b>7.7</b>
xi. Fat	0.13	0.12	<b>0.13</b>

#### 4. GENETIC CHARACTERIZATION OF KADAKNATH BREED USING SPECIFIC MICROSATELLITE DNA MARKERS

The methodology used for microsatellite DNA typing was as follows

##### 4.1. DNA extraction from blood samples

The DNA will be extracted as per the method described by Hillel *et al.* (1989) and Sambrook and Russel (2000) with minor modifications. Incubation with proteinase-K and sodium dodecyl sulphate (SDS) was followed by phenol-chloroform extractions.

##### 4.2 Quality checking and quantification of DNA

The quality and quantification of DNA was carried out by UV-Spectrophotometry and agarose gel electrophoresis respectively.

The 20 µl of DNA samples diluted 1:50 in distilled water were used for Spectrophotometry. Optical density (OD) value at 260 nm and 280 nm were measured using distilled water as reference. DNA concentration was estimated as under:

$$\text{Concentration of DNA } (\mu\text{g/ml}) = \text{OD at 260 nm} \times \text{dilution factor} \times 50$$

Where, 50 is the concentration (µg/ml) of double stranded DNA at OD 1.

DNA sample with OD<sub>260</sub>: OD<sub>280</sub> (λ) ratio of 1.7–2.0 were retained for typing. The quality was verified on agarose gel electrophoresis. The 0.8 percent agarose in 0.5X Tris-Borate buffer (TBE) (pH 8.0) buffer was used for submarine gel electrophoresis. Ethidium bromide (0.1%) was added at the rate of 0.5 µl/100ml of agarose gel. The wells were charged carefully with 2 µl DNA in gel loading buffer. Electrophoresis was carried out at voltage 80V for 25 minutes at room temperature. On completion of electrophoresis, the gel was visualized under UV Transilluminator. Samples with single compact bands were uniformly diluted to 30ng/µl in distilled water and stored at -20°C.

##### 4.3 Microsatellite loci

Twenty five chicken specific microsatellite primers recommended in the FAOs, MODAD programme list for chicken microsatellite genotyping (Crooijmans *et al.*, 1995, Cheng *et al.*, 1995, Cheng, 1997 and Wimmers *et al.*, 2000) were custom synthesized from Sigma, Aldrich foreign holding Co., USA. Some of the recommended primers did not give distinct PCR amplifications, therefore changed with other poultry primers. The details of the primers are given in **Table 13**

Initially the primers were dissolved in TE buffer (1.0µg/µl). The primers (1.0µg/µl) were reconstituted in distilled water to give final concentration 1pmol /µl and stored at -20°C until used.

**Table 13** Characteristics of chicken microsatellite markers.

Primer (Local code)	Sequences (5' – 3')		Repeat	Length (bp)
1	2	3	4	5
ADL 23	FP	CTT CTA TCC TGG GCT TCT GA	(CA)5 (CG)4 (CA)9	164
	RP	CCT GGC TGT GTA TGT GTT GC		
ADL 34	FP	AAC CTA AAA ACT CCT GCT GC	(AC)11 (AT)2	126
	RP	GGG AAC CTG TGG GCT GAA AG		
ADL44	FP	AAG TGG TTT ATT GAA GTA GA	(AC)9	168
	RP	CTG TGG TGT TGC GTT AGT TG		
ADL 39	FP	GCT ACA ACG CTT CAA ACC TG	TS(TG)8	154
	RP	ACA AAC AAA CCA AAA AAC CT		
ADL 176	FP	TTG TGG ATT CTG GTG GTA GC	(GT)12	191
	RP	TTG TGG ATT CTG GTG GTA GC		
ADL 158	FP	TGG CAT GGT TGA GGA ATA CA	(CA)12	216
	RP	TAG GTG CTG CAC TGG AAA TC		
ADL210	FP	ACA GGA GGA TAG TCA CAC AT	(AC) 15	130
	RP	GCC AAA AAG ATG AAT GAG TA		
ADL 267	FP	AAA CCT CGA TCA GGA AGC AT	(CA)12	117
	RP	GTT ATT CAA AGC CCC ACC AC		
HUJ 1	FP	CCC TTT GTT AAC ACC TAC TGC A	(CA)	192
	RP	CAT CCG GCT TAT ACA GAG CAC A		
HUJ 2	FP	CAT CTC ACA GAG CCA GCA GTG	(AC)	125
	RP	GAA TCC TGG ATG TCA AAG CC		
HUJ 12	FP	GTCTCATGCTATGAGAGTGG	(AC)n	116 – 140
	RP	CCTCTGGTTGAATCAGTCTG		
MCW 1	FP	ACT GTC ACA GTG GGG TCA TGG ACA	(TG)9	161
	RP	ACA CGT CCT GTG TCA CAT GCC TGT		
MCW 7	FP	AGCAA AGAAG TGTTTCTGT TCAT	(TG)5	313 – 349
	RP	ACCCT GCAA CTGGA AGGGT CTCA		
MCW 73	FP	TCACCCACGGGGACGAATAC	(GAG) 10	250
	RP	TGCTGAGAGCTGCCAATGTC		
ADL 136	FP	TGTCACCTTTCTTTTTTATT	(TG) 10	145
	RP	CCACCTCCTTCTCCTGTTCA		
ADL-102	FP	TTCCACCTTTCTTTTTTATT	(GT) 18	122

	RP	GCTCCACTCCCTTCTAACCC		
MCW-5	FP	ACCTCCTGCTGGCAAATAAATTGC	(TG) <sub>14</sub>	189-259
	RP	TCACTTTAGCTCCATCAGCATTCA		
MCW-16	FP	TTCTGAAGCAGTTGCTATGG	(TG) <sub>16</sub>	125-178
	RP	CGCAGAAGGCAAAGCGATAT		
MCW-43	FP	TGACTACTTTGATACGCATGGAGA		
	RP	CACCAAGTAGACGAAAACACATTT		
MCW-49	FP	GCGTTGAGTGAGAGGAGCGA		116-144
	RP	CAACCCGCGGAGAGCGCTAT		
MCW-51	FP	CAAGCTCTTTCTTCTTCCCG		180
	RP	GGAGGTGCTGGTACAAAGAC		
MCW-59	FP	TCTATTGTGCAGCAGCTTAT		147-176
	RP	GCCTTTGCTATCCTGATTGG		
LEI-146	FP	TCAAGCCACCAAAGTGCTTGG	(AG) <sub>22</sub>	258-276
	RP	GATCACTCTGCTCATAGCAGT		
LEI-71	FP	TCAGGTTAGTCTGACCATTGC		281-330
	RP	TGAGTGTAAGATTGCTAATGGA		
ADL-172	FP	CCC TAC AAC AAA GAG CAG TG	(AC) <sub>18</sub> 1	154
	RP	CTA TGG AAT AAA ATG GAA AT		

Repeat = The microsatellite repeat amplified using designated primers in original clones.

Length = The predicted length of PCR product.

Where, FP = Forward Primers  
RF = Reverse Primers

#### 4.4. Setting up of PCR reaction

PCR was carried out on 90ng of genomic DNA in 25µl reaction mixture. The reaction mixture consists of 200 µM of dATP, dCTP, dGTP and dTTP, 1.5 mM MgCl<sub>2</sub>, 1 unit of Taq DNA polymerase and 5 pmol of each primer (Sambrook and Russel, 2000).

#### A typical cocktail for PCR reaction consist of:

Template DNA (30ng/µl)	----	----	----3.0 µl
10X PCR buffer with NH <sub>2</sub> SO <sub>4</sub>	----	----	----2.5 µl
15mM MgCl <sub>2</sub>	----	----	----1.5 µl
dNTPs mix (10mM each)	----	----	----0.5 µl
Forward primer (10pmol/µl)	----	----	----1.0 µl



Reverse primer (10pmol/ $\mu$ l)	----	----	----1.0 $\mu$ l
Taq DNA polymerase (5U/ $\mu$ l)	----	----	----0.2 $\mu$ l
Autoclaved HPLC water	----	----	----15.3 $\mu$ l
<b>Total</b>			<b>25 <math>\mu</math>l</b>

#### 4.5. PCR condition

All reactions were carried out in 200  $\mu$ l thin walled PCR tubes. PCR tubes containing reaction mixture were tapped gently and spin at 10000 rpm. The PCR amplification was carried out in Eppendorf thermal cycler in a final reaction volume of 25  $\mu$ l. The PCR protocols for all the twenty five microsatellite markers namely HUU-2, ADL-267, MCW-7, MCW-73, ADL-210, HUU-12, ADL-39, ADL-44, ADL-158, ADL-176, MCW-1, HUU-1, ADL-23, ADL-34, ADL-136, ADL-102, MCW-5, MCW-16, MCW-43, MCW-51, MCW-49, MCW-50, LEI-146, LEI-71 and ADL-172 were same except the annealing temperature, which varied between primers used in the study. The annealing temperature for various microsatellites loci ranged from 46°C 60°C.

**Table 14** eneral PCR programme for microsatellites amplification

	<b>Step</b>	<b>Temperature (°C)</b>	<b>Duration</b>
1	Initial denaturation	95	5minutes
2	Denaturation	94	45 seconds
3	Annealing	T <sup>A</sup> (46-60)	45 seconds
4	Extension	72	45 seconds
5	30 times to step 2		
6	Final extension	72	5 minutes
7	Hold	10	

Where, T<sup>A</sup> = optimum annealing temperature

#### 4.6. Electrophoresis on agarose gel

The PCR products were electrophoresed on 4% agarose gel at 80V for 2 hours at room temperature along with 100bp ladder as size marker (Sambrook and Russel, 2000). On completion of electrophoresis, the bands were visualized under UV light and documented in Syngene gel scanner. The band sizes were judged by Syngene's Genetool software comparing with molecular size markers and images were recorded.

#### 4.7 Urea-Polyacrylamide Gel Electrophoresis (PAGE)

Polyacrylamide gels were used for analysis of DNA with specialized buffer systems and denaturants. Urea was used as denaturant in DNA gels. The urea-polyacrylamide gel electrophoresis (PAGE) was performed as per procedure described by Sambrook and Russel (2000) with minor modifications. The components used to synthesize matrix were acrylamide monomers, N, N-methylene bisacrylamide (Bis), ammonium persulphate (APS) and N, N, N', N'-tetramethylenediamine (TEMED). Ammonium persulphate (APS) when dissolved in water generates free radicals, which activate acrylamide monomers inducing them to react with other acrylamide molecules forming long chains. These chains cross-linked with Bis. TEMED act as catalyst for gel formation because of its ability to exists in free radical form.

#### Composition of the 6% Urea PAGE (for 500 ml)

10 X TBE	-----	50.0 ml
Acrylamide	-----	28.5 gm
Bis	-----	1.5 gm
Urea	-----	210 gm

The autoclaved distilled water was added to make 500 ml. The solution was warmed at 60°C until urea dissolved completely and filtered through Whatman filter paper. In 70 ml of this solution, 300µl of freshly prepared 10 percent ammonium persulphate (APS) and 35µl TEMED were added and mixed just before pouring the gel into the glass plates.

#### 4.8. Detection through silver staining

The detection through silver staining was done as per the technique described by Gustavo and Gresshoff (2000). The staining procedure consisted of three primary steps.

1. Fixing the DNA bands on the gel by acetic acid.
2. Incubation of the gel in the silver nitrate solution and
3. Developing the DNA bands with the help of developer.

#### 5. Results of Microsatellite data analysis

Genetic variation of microsatellite loci in three different varieties of Kadaknath breed of poultry (jet black, golden and pencilled) were assessed by means of PCR amplification with 25 poultry primers namely ADL-23, ADL-34, ADL-39, ADL-44, ADL-158, ADL-176, ADL-210, ADL-267, ADL-136, ADL-102, ADL-172, MCW-1, MCW-7, MCW-73, MCW-5, MCW-16, MCW-49, MCW-59, MCW-43, MCW-51, -HUU-1, HUU-2, HUU-12, LEI- 71 and LEI-146. Silver staining was used to reveal the PCR products after electrophoresis on urea Polyacrylamide gel (PAGE). Among microsatellite loci tested all loci were polymorphic. In all the samples of different varieties of Kadaknath fowl. The number of alleles per microsatellite locus varied from 3 to 10. The allele size of polymorphic loci were ranged from 98-356 bp and showed large variation across loci. The total heterozygosity and PIC value for all the microsatellite loci ranged from 0.416 to 0.817 and 0.373 to 0.786 respectively.

#### 5.28. Measurement of genetic diversity.

The main objective of the present study was to evaluate genetic diversity and phylogenetic relationship among all the three varieties of Kadaknath breed of poultry (Jet black, Golden and Pencilled) originating from the same genetic background.

The Nei,s standard genetic distance (DS) values between Jet black and Golden, Jet black and Pencilled varieties of Kadaknath were found to be 0.1678, 0.0951 and 0.1943 respectively. The highest genetic distance was observed between Golden and Pencilled and smallest between Jet black and Pencilled varieties. All the three varieties of Kadaknath breed had small genetic distances among each other. The phylogenetic consensus tree constructed using mighbor joining tree and the bootstrapped data method grouped all the three varieties in to one cluster **Fig.10**. As shown in figure the Golden and Pencilled varieties were found to be closer as compared to Jet black variety. Thus the results obtained in the present study conclude that all the three varieties belongs to Kadaknath breed of poultry having almost similar genetic base.

**Table-67** Genetic distance (Nei, 1978) of the three varieties of Kadaknath breed.

	<b>Jet black</b>	<b>Golden</b>	<b>Pencilled</b>
Jet black	0.0000	-	-
<b>Golden</b>	0.1678	0.0000	-
<b>Pencilled</b>	0.0951	0.1943	0.0000

### **5.29. Conservation of Kadaknath breed of Poultry:**

During random field survey studies, it was felt that the population of Kadaknath birds declining rapidly and the breed is under threat of extinction. Therefore there was an urgent need of *in-situ* conservation and selective improvement not only at recognized farm but also at farmers door. For *in-situ* conservation of the breed effects were made by the Principal Investigator of the project through Dean, Vety. College, Jabalpur. In response to Dean, Vety. College, Jabalpur letter No.358/AB&G/2002, dated 26.2.2002, Director Veterinary Services, Bhopal (M.P.) has asked the Deputy Director, Veterinary Services, Jhabua (M.P.) to submit the proposal arising the foundation stock of Kadaknath birds from 600 to 1000 at Govt. Poultry Breeding Farm, Jhabua on the basis of proposal submitted by Deputy Director Veterinary Services, Jhabua letter No. 2360, dated 15.4.02 to Director Vety. Services, Bhopal. The Director Veterinary Services, Bhopal had sanctioned the additional budget for raising the founding stock of birds from 600 to 1000 (Director Vety. Service Bhopal letter No. 5474/P/A-5/02-03, Bhopal, dated 6-5-2002). Increase of foundation stock of Kadaknath birds at Govt. Poultry breeding farm, Jhabua will met the demand of farmers to supply more number of chicks to field areas.

During the field survey studies it was observed that most of the farm are keeping only minimum if 5 and maximum of 30 birds and raising the birds under backyard poultry system. None of the farmers was found raising the Kadaknath birds on commercial basis at large scale. It is therefore recommended that for the *in-situ* conservation of the breed the farmers of the breeding tract should be encouraged and financed through Govt. and other agencies for the propagation of the breed.

### **5.30. Recommendations / Suggestions for future work:**

Kadaknath is an important indigenous breed of poultry famous for its delicious meat quality. It has an excellent power of heat tolerance and disease resistance. It can thrive well under free ranging conditions with little grain supplementation/kitchen waste and can be housed in cheap katcha houses. The breed has a natural instinct of broodiness, which makes it a natural incubator and hatcher, a desirable trait for the villagers.

On account of poor egg production and growth potential, as compared to the modern day commercial breeds, this breed is threatened due to the Government schemes which supply commercial chicks to the farmers on subsidized rates. The breed in pure form is therefore found in remote villages away from roadsides. Despite the State Vety. Department initiatives, the proper vaccination and disease control measures for Kadaknath breed were also lacking. In view of the above mentioned factors and the threats to its existence, the following recommendations are proposed.

**(A) At Germ plasm centre:**

1. Establishment of germ plasm centre for conservation of Kadaknath breed by Government agencies or by Agricultural Universities.
2. The growth, reproduction and production potential of the breed should be studied under optimum feeding and management conditions at the germ plasm centre, so that its full potential is explored.
3. The genetic parameters for various economic traits should be established by planned experiment.
4. Selection experiments should be conducted for atleast 10 generations to see the genetic gain of production and carcass traits.
5. For improving the egg production the unproductive period of broodiness and brooding period can be reduced and its effect on egg production should be studied.

**(B) In the breeding tract**

1. Proper vaccination of birds for important diseases.
2. The parasitic infestation and ecto-parasitic problem is very common. Therefore, farmers should be educated to observe regular deworming practices and control of ecto-parasites.
3. Extension programme for educating the farmers about proper feeding and management of birds should be undertaken.
4. Disease diagnostic facilities should be created in every Tahsil / Block level.

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