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A Comparative Study of Meat Quality Traits in Different Improved Varieties of Chicken

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ABSTRACT

The present experiment was designed to study meat quality traits in improved varieties of chicken. Six different varieties (CARI Shyama, Vanaraja, Kalinga Brown, Black Rock, Caribro Dhanraja and Kuroiler) used in Chhattisgarh for backyard poultry were studied. The meat quality parameters like pH, total lipid, total cholesterol, ash, ether extract and sensory evaluation were studied. The pH values of meat was significantly (P<0.01) influence by varieties. The overall mean pH values were recorded to be 6.40 ± 0.50, 6.35 ± 0.05, 6.39± 0.05, 6.17± 0.06, 6.38± 0.06 and 6.26± 0.05 for CARI Shyama, Vanaraja, Kalinga Brown, Black Rock, Caribro Dhanaraja and Kuroiler respectively. Significantly (P<0.05) higher pH value of leg meat as compared to breast meat was recorded. Older birds had more lipid content than younger birds. The cholesterol content of meat was significantly (P<0.01) affected by age and body parts. Cholesterol content of meat increased with increase of birds' age. Leg meat had high cholesterol content as compared to breast meat. Higher protein and total ash content as well as lower ether extract content was observed in CARI Shyama. Highest overall acceptance was recorded for meat of CARI Shyama in sensory evaluation.

Keywords: Poultry, improved varieties, backyard, meat, quality traits

The success of chicken meat production has been strongly related to improvement in growth and carcass yield, mainly by increasing breast proportion and reducing abdominal fat. Though intensive selection of meat type chicken for growth for more than 50 years has increased growth rate, simultaneously rapid growth has been accompanied by increase in body fat deposition (Griffin, 1996). Deposition of body fat in chicken differs markedly in different carcass parts and its pattern of accumulation varies with species, variety, sex and age. Similarly, the proportion of different cuts also shows considerable variation. Therefore, in present scenario it is customary to study all these aspects in backyard poultry varieties. Meat quality, determined by several factors markedly affects the consumers demand. The aroma, tenderness, juiciness and flavour of cooked meat must meet the expectations of consumers (Thu, 2006). Factors like species, breed, variety, gender and age affect meat tenderness. Meat pH value gives clue about keeping duration as well as technical processing characteristics and pH of animals prior to slaughter must be 7.1. Moreover, after slaughter some amount of meat glycogen converted in to lactic acid, resulting in lower value of pH. Increasing activity of maturing carcass varies in its speed depending upon a number of factors such as species, breed, variety, sex, age, rearing characteristics, treatment of birds prior to slaughter etc. The pH of meat also influences its colour and water holding ability. Lower meat pH increases drip losses which negatively affect the appearance and thereby the purchase intent. Further the pH also impacts eating quality characteristics such as juiciness, tenderness and taste (Ismail and Joo, 2017). Nutritional value of chicken meat can be assessed based on the parameters such as

contents of dry matter, protein, lipid, ash and cholesterol. Hence, the present study was designed to evaluate meat quality traits in improved varieties of chicken used in Chhattisgarh for backyard poultry.

MATERIALS AND METHODS

Six hundred chicks were selected for this study with 100 birds from each variety at Veterinary College, Anjora, Durg, C.G. All the birds were maintained under similar managerial conditions. Representative meat samples measuring 100 to 150 g were collected from different cut parts like breast and leg separately from six birds each (3 male and 3 female) at 8th, 9th and 10th week with total of 18 birds from each variety. The meat samples were minced, then packed properly and stored in -20^{o}C. Sensory evaluation of cooked meat was conducted using score sheet developed by Peryan and Pilgrim (1957). The pH was determined by pH meter make No. 768 Analogue (Systronic). Total lipid for meat (leg and breast) was determined as per the procedure of Bligh and Dyer (1959) with minor modification, whereas total cholesterol was determined by the method as described by Allain *et al.* (1974). Proximate compositions such as moisture, crude protein, ether extract and total ash contents of meat were estimated following the procedure of AOAC (1995). The meat quality parameters among the different chicken varieties were compared by one-way analysis of variance. The results are presented as mean ± error and the mean difference between the groups were considered significant if P≤0.05.

RESULTS AND DISCUSSION

Meat pH profile

Overall meat pH values at different weeks are presented in table 1. Results showed that meat pH values were significantly (P<0.05) influenced by the varieties but sex and age did not show any significant effect. On the other hand, significant (P<0.01) influence of body parts was observed in the present investigation. The mean values for leg meat was found higher as compared to the breast meat. Though the influence of sex was found to be statistically insignificant, but the overall trend showed that the meat of the males have lower pH values than the females. Amongst the variety studied in the present investigation, the higher values of pH was observed with CARI Shyama, which also ranked top for juiciness by the panel of sensory characteristics evaluation which supported the findings of this investigation itself. In the present finding neither any significant difference nor trends could be observed for age variations. The highest pH value was observed to be of CARI Shyama followed by Kalinga Brown,

Table 1: pH profile of meat in improved varieties of backyard chicken

Parts	Age in weeks	CARI Shyama	Vanaraja	Kalinga Brown	Black Rock	Caribro Dhanraja	Kuroiler
Leg	8	6.56±0.05	6.56±0.12	6.80±0.06	6.55±0.04	6.56±0.13	6.54±0.05
	9	6.07±0.07	6.23±0.07	6.48±0.05	6.17±0.10	6.57±0.08	6.31±0.06
	10	6.78±0.08	6.57±0.08	6.65±0.04	6.37±0.06	6.77±0.06	6.56±0.04
	Total	**6.47±0.08**	**6.45±0.06**	**6.65±0.04**	**6.36±0.05**	**6.63±0.06**	**6.47±0.04**
Breast	8	6.18±0.06	6.16±0.12	6.28±0.11	6.17±0.08	6.06±0.11	6.12±0.06
	9	6.46±0.12	6.58±0.08	6.07±0.05	6.33±0.10	6.14±0.07	5.87±0.17
	10	6.35±0.06	6.02±0.04	6.16±0.08	5.45±0.48	6.18±0.09	6.13±0.03
	Total	**6.33±0.05**	**6.25±0.07**	**6.17±0.05**	**5.98±0.18**	**6.13±0.05**	**6.04±0.07**
Total	8	6.38±0.07	6.36±0.10	6.54±0.10	6.36±0.07	6.31±0.11	6.33±0.07
	9	6.26±0.09	6.40±0.07	6.28±0.07	6.25±0.07	6.35±0.08	6.09±0.11
	10	6.56±0.08	6.29±0.09	6.41±0.08	5.91±0.27	6.48±0.10	6.35±0.07
	Total	**6.40^{b}±0.05**	**6.35^{b}±0.05**	**6.41^{b}±0.05**	**6.17^{a}±0.10**	**6.38^{b}±0.06**	**6.26^{ab}±0.05**

Means with different superscript within a row differed statistically (P≤0.05).

Caribro Dhanraja, Vanaraja and Black Rock. The results also revealed a significantly higher pH value of leg meat (6.505) as compared to breast meat.

The pH of the meat influences the meat quality in terms of water holding capacity, colour and tenderness. Present results exhibited that sex and age did not have any significant effect on the meat pH; however pH was affected by the variety. The results corroborate with Demarchi *et al*. (2005) who also documented that there was no effect of sex on breast meat pH. However in the present investigation, thigh pH was found to be significantly ($P<0.01$) higher in females as compared to males. On the other hand, a significant ($P<0.01$) effect of body parts on meat pH was also registered. The mean values for leg meat was found higher as compared to the breast meat. Similar findings have been reported by Demarchi *et al*. (2005) in Padovana breed of chicken found in Italy. They reported breast pH ranging from 5.29 to 5.83 and thigh pH in the same experiment ranged from 6 to 6.17, though the significance in the mean differences has not been mentioned. In the present finding, age difference could not produce any visible trends for the meat pH, the probable reason for the insignificant effect of age may be due to lower age difference between the age groups slaughtered for the experiment.

Meat total lipid profile

Total lipid per cent of different backyard varieties of chicken are presented in table 2. The result revealed that age and body parts significantly ($P<0.01$) influenced the total lipid. However, sex of the birds did not influence meat total lipid content. Our results demonstrated that older birds had higher total lipid content than younger ones and the meat cholesterol values between leg and breast differed significantly ($P<0.05$). The breast meat contained less total lipid as compared to the leg meat. The highest total lipid content was recorded for Caribro Dhanraja variety which is a broiler type variety developed for backyard production. Vanaraja was observed to be next to Caribro Dhanraja followed by Kuroiler, Black Rock, CARI Shyama and Kalinga Brown.

In this study, total lipid was significantly affected by age and body parts but not sex. In a similar line, Demarchi *et al*. (2005) reported that effect of sex was not significant on lipid percentage while effect of age was observed. However, our results are contrary to Suchy *et al*. (2002), who reported that due to different growth intensities of females and males, the females accumulate more fat than males. They also reported that thigh muscle contains more fat as compared to breast muscles. We also observed higher total lipid in leg meat as compared to breast

Table 2: Total lipid (%) profile of meat in improved varieties of backyard chicken

Parts	Age in weeks	CARI Shyama	Vanaraja	Kalinga Brown	Black Rock	Caribro Dhanraja	Kuroiler
Leg	8	0.33±0.07	0.50±0.22	0.67±0.21	0.45±0.26	0.48±0.28	0.16±0.05
	9	1.09±0.18	1.17±0.20	0.54±0.16	0.91±0.25	1.09±0.58	1.13±0.16
	10	1.09±0.15	1.15±0.29	0.47±0.10	1.08±0.27	1.38±0.18	1.45±0.23
	Total	**0.83±0.12**	**0.94±0.15**	**0.56±0.09**	**0.82±0.17**	**0.99±0.23**	**0.92±0.16**
Breast	8	0.60±0.22	0.72±0.28	0.29±0.13	0.38±0.19	0.62±0.21	0.33±0.20
	9	0.34±0.05	0.81±0.13	0.51±0.15	0.55±0.06	0.62±0.25	0.39±0.08
	10	0.65±0.22	0.73±0.31	0.77±0.14	0.72±0.34	1.32±0.20	0.97±0.16
	Total	**0.53±0.10**	**0.75±0.14**	**0.52±0.09**	**0.55±0.13**	**0.85±0.14**	**0.57±0.11**
Total	8	0.47±0.11	0.60±0.17	0.48±0.13	0.42±0.16	0.55±0.17	0.25±0.09
	9	0.71±0.14	0.99±0.13	0.53±0.10	0.73±0.13	0.85±0.31	0.76±0.14
	10	0.86±0.14	0.94±0.21	0.62±0.09	0.90±0.21	1.35±0.13	1.21±0.15
	Total	**0.68^{ab}±0.08**	**0.85^{b}±0.10**	**0.54^{a}±0.06**	**0.69^{ab}±0.10**	**0.92^{b}±0.13**	**0.74^{ab}±0.10**

Means with different superscript within a row differed statistically ($P\leq0.05$).

meat. Similarly, Rondelli *et al.* (2003) reported higher percentage of fat in leg as compared to breast muscle. The mean comparison shows significant difference and might be attributed to the genetic variation prevailing amongst the different varieties.

Meat cholesterol profile

Cholesterol profile of meat in improved varieties of chicken is presented in Table 3. The cholesterol content was significantly (P<0.01) influenced by age and body parts of the bird. However, the variety and sex had no significant influence on the cholesterol content of the meat. The cholesterol content was significantly higher at the age of 10 weeks as compared to 8 and 9 weeks. The leg meat contained higher cholesterol than the breast meat and the mean difference was found statistically (P<0.01) significant. Though the mean difference amongst the two sexes was observed to be statistically non-significant, the overall value for males (28.44mg per 100g meat) was recorded higher than the females (26.84 mg per 100g meat).In the present investigation, the backyard chickens studied had slow rate of growth.

The cholesterol content was significantly higher at the age of 10 weeks as compared to 8 and 9 weeks, which is similar to Raj *et al*. (2005). However, the mean values observed in present investigation were much lower than those observed by the other workers, which might be attributed to the genetic differences in experimental birds. Higher cholesterol content in leg meat than breast meat was similar with the findings of Rondelli *et al*. (2003) and Raj *et al*. (2005), who also reported high cholesterol values for leg meat as compared to breast meat. In the present study, a non significant difference was observed in both sexes although the cholesterol value was slightly higher in male birds as compared to females. The cholesterol level in the present study was found to be much lower than those observed by other investigators, probably due to their genetic differences. Backyard chicken varieties have slower growth rate as observed in the present study. The rapid growth of present day commercial broiler chicken accompanied by a number of negative consequences including an increase in fat deposition (Griffin, 1996) which may be attributed to higher cholesterol value of commercial broiler varieties as compared to backyard varieties.

Proximate Composition

Proximate compositions of meat of improved varieties of chicken are presented in Table 4.

Moisture

The moisture content of the chicken meat was found to be significantly (P<0.01) influenced by the varieties but no

Table 3: Cholesterol (mg/100g meat) profile of meat in improved varieties of chicken

Parts	Age in weeks	CARI Shyama	Vanaraja	Kalinga Brown	Black Rock	Caribro Dhanraja	Kuroiler
Leg	8	27.87±1.29	30.92±3.91	28.04±6.12	31.64±5.49	25.88±2.44	24.80±2.20
	9	30.92±2.00	33.72±2.94	32.39±6.54	36.70±0.55	26.34±1.94	36.21±2.64
	10	36.88±3.27	33.26±1.51	38.31±4.27	33.41±2.95	32.66±1.47	36.76±4.24
	Total	**31.89±1.56**	**32.63±1.63**	**32.91±3.27**	**33.92±2.02**	**28.29±1.31**	**32.59±2.17**
Breast	8	22.80±0.51	22.62±0.57	33.60±1.89	21.28±1.06	21.60±0.49	22.02±0.15
	9	24.19±1.40	21.10±2.45	21.61±2.89	21.08±3.58	18.1±1.73	25.43±2.12
	10	24.65±3.68	23.84±2.30	29.13±3.86	21.88±2.63	26.15±2.29	27.34±1.90
	Total	**23.88±1.26**	**22.52±1.10**	**24.78±1.79**	**21.41±1.43**	**21.95±1.21**	**24.93±1.04**
Total	8	25.33±1.01	26.77±2.26	25.82±3.13	26.46±3.09	23.74±1.35	23.41±1.13
	9	27.56±1.54	27.41±2.64	27.00±3.77	28.89±2.92	22.22±1.75	30.82±2.29
	10	30.77±2.98	28.55±1.93	33.72±3.07	27.65±2.56	29.41±1.63	32.05±2.63
	Total	**27.89±1.20**	**27.58±1.29**	**28.85±1.96**	**27.66±1.62**	**25.12±1.03**	**28.76±1.35**

Means with different superscript within a row differed statistically (P≤0.05).

Table 4: Proximate analysis of chicken meat from improved varieties

Content	Parts	CARI Shyama	Vanaraja	Kalinga Brown	Black Rock	Caribro Dhanraja	Kuroiler
Moisture	Leg	76.15±0.12	76.01±0.13	78.06±0.24	79.93±0.02	78.07±0.12	78.04±0.12
	Breast	75.82±0.16	75.79±0.23	79.69±0.74	77.75±0.04	78.09±0.10	78.06±0.09
	Total	**75.98^{a}±0.12**	**75.90^{a}±0.13**	**79.15^{c}±0.42**	**77.84^{b}±0.04**	**78.08^{b}±0.07**	**78.05^{b}±0.06**
Protein	Leg	22.28±0.13	21.39±0.17	16.85±0.19	18.99±0.09	17.84±0.35	17.92±0.69
	Breast	22.76±0.18	20.31±0.42	17.57±0.52	18.67±0.03	18.17±0.17	17.79±0.24
	Total	**22.52^{d}±0.15**	**20.85^{c}±0.39**	**17.21^{a}±0.30**	**18.83^{c}±0.08**	**18.00^{b}±0.19**	**17.86^{ab}±0.33**
EE	Leg	2.27±0.12	4.22±0.23	2.72±0.20	1.79±0.09	2.77±0.40	2.55±0.48
	Breast	1.78±0.05	3.12±0.50	1.71±0.17	1.91±0.03	2.25±0.04	2.55±0.37
	Total	**2.03^{ab}±0.12**	**3.67^{c}±0.35**	**2.22^{ab}±0.25**	**1.85^{a}±0.05**	**2.50^{b}±0.21**	**2.55^{b}±0.27**
TA	Leg	1.27±0.03	1.11±0.02	0.94±0.02	0.88±0.02	0.86±0.01	0.88±0.02
	Breast	1.30±0.09	1.22±0.07	1.06±0.06	1.05±0.02	1.07±0.02	0.99±0.08
	Total	**1.29^{c}±0.05**	**1.16^{b}±0.04**	**1.00^{a}±0.04**	**0.96^{a}±0.04**	**0.96^{a}±0.05**	**0.94^{a}±0.05**

Means with different superscript within a row differed statistically (P≤0.05).

significant effect of parts could be observed in the present investigation. In the present study the highest moisture per cent was observed in Kalinga Brown variety followed by Caribro Dhanraja, Kuroiler, Black Rock, CARI Shyama and Vanaraja. Significant difference between parts could not be observed but the trend obtained indicated higher moisture content of breast meat (77.53%) as compared to leg meat (77.46%).

Moisture content was significantly impacted by the varieties of chicken but not by their body parts. These values observed in backyard chickens are comparable with the values reported by other authors, however different authors worked on different varieties, strains and breeds reported different values (Suchy *et al*., 2002; Demarchi *et al*., 2005; Almeida *et al*., 2006). The differences in the moisture contents of different verities may be attributed to the genetic difference among the varieties studied as similar managerial practices were provided for the birds. Higher moisture content in breast meat than leg meat was observed in this study, which is in corroboration with Suchy *et al*. (2002) who documented a higher moisture content of thigh as compared to breast. In the present experiment, whole leg meat was taken for analysis not only the thigh. This could be the probable reason for contradictory findings. However, Khan *et al*. (2003) and Demarchi *et al*. (2005) have reported moisture content ranging between 75 to 76 per cent, but they have not mentioned the age of the birds for chemical composition which may be the probable cause for lower moisture content of the meat.

Crude protein

Highly significant (P<0.01) effect of variety on crude protein content of the chicken meat was observed in the present investigation. Further, meat protein content of different body parts also differed significantly (P<0.05). Out of the 6 varieties studied CARI Shyama had the highest and Kalinga Brown had the lowest protein contents. Significantly (P<0.05) high crude protein observed for CARI Shyama.

The crude protein content of the chicken meat was significantly influenced by the variety and different body parts. Similarly, considerable genetic variation has been reported by many workers in different chicken varieties. Other researchers reported higher values of protein for breast meat as compared to leg meat of the broiler birds (Suchy *et al*., 2005; Pragati *et al*., 2007). The differences amongst the variety might be attributed to genetic variation of the different varieties under study. Khan *et al.* (2003) also reported significant differences between different genotypes they studied. Research conducted at Central Avian Research Institute, Izatnagar, Bareilly, India also documented high crude protein (25.47 per cent in flesh) in CARI Shyama variety. The higher crude protein per cent

as compared to present investigation reported by them might be due to different age groups or strains used by them in their study. The difference might also be attributed to feeding and environmental conditions management and other factors. The higher crude protein content of breast muscle as compared to leg muscle is well in accordance to Suchy *et al*. (2002).

Ether extract

The ether extract content of the chicken meat varies significantly (P<0.01) amongst different varieties and the body parts studied. The mean differences were also found to be significant amongst the varieties and body parts. In the present study, it was demonstrated that leg meat contained more ether extract (2.72%) as compared to the breast meat (2.22%). The highest ether extract content was observed with Vanaraja followed by CARI Shyama, Kuroiler, Caribro Dhanraja, Kalinga Brown and Black Rock. The ether extract content of the chicken meat varied significantly between different varieties and the body parts studied. In this study, we found that leg meat contained slightly higher ether extract as compared to the breast meat. Higher fat percentage for leg meat as compared to breast meat has been demonstrated by many other workers (Suchy *et al*., 2002; Pragati *et al*., 2007). The different ether extract contents for different genotypes were also observed by Khan *et al.* (2003). In their experiment ether extract varied between 0.81 to 2.49 per cent in different genotypes. Suchy *et al*. (2002) reported fat content ranging from 2.39 to 9.04 per cent in broilers. Higher fat per cent of broilers meat up to 4.36 per cent has also been reported by Pragati *et al*. (2007) however, Demarchi *et al.* (2005) reported much lower than our findings (1.39 to 1.65 per cent lipids). The varieties of chicken studied in this experiment were much similar to those studied by Khan *et al.* (2003) at Central Avian Research Institute, Izatnagar because they studied crosses between necked neck indigenous fowl with synthetic male line. In respect to the variation amongst the body parts Suchy *et al*. (2002) also reported significantly (P<0.01) higher fat contents for thigh meat (9.044%) as compared to breast meat (2.399%), their finding is in accordance to the results obtained in this study.

Total ash

In the present study, total ash content of meat ranged from 0.94 to 1.29 per cent. The effect of variety and body parts were found to be highly significant (P<0.01) on total ash content of the chicken meat. Out of the six varieties studied CARI Shyama was observed to have highest total ash content in the meat. The highest total ash content was observed for CARI Shyama variety followed by Vanaraja, Caribro Dhanraja, Black Rock and Kuroiler. The total ash content of the chicken meat was found to be significantly affected by the variety and body parts and was found highest for the CARI Shyama variety. These observations are comparable with those reported by Demarchi *et al*. (2005). Suchy *et al*. (2002) also reported significantly (P<0.01) higher values of ash content in breast meat as compared with thigh muscle. Similar to the other chemical components variation in total ash can be simply attributed to the genetic differences amongst the varieties studied. The values for total ash reported by Suchy *et al*. (2002) and Demarchi *et al.* (2005) are not much different from the values obtained in the present investigation. Suchy *et al.* (2002) reported total ash per cent ranging from 1.19 to

Table 5: Sensory characteristics in improved varieties of chicken

Attributes	CARI Shyama	Vanaraja	Kalinga Brown	Black Rock	Caribro Dhanraja	Kuroiler	Overall
Colour	7.83±0.32	7.13±0.29	7.07±0.33	7.17±0.53	6.83±0.34	6.92±0.34	7.16±0.15
Flavour	8.89^{b}±0.20	7.35^{a}±0.38	7.11^{a}±0.38	7.10^{a}±0.60	7.50^{a}±0.39	6.98^{a}±0.35	7.49^{a}±0.18
Juiciness	9.08^{b}±0.22	7.25^{a}±0.35	7.73^{a}±0.46	7.06^{a}±0.70	7.83^{a}±0.26	7.79^{a}±0.29	7.79^{a}±0.19
Tenderness	8.20±0.45	7.08±0.38	7.93±0.34	7.02±0.78	7.64±0.39	7.89±0.37	7.63±0.19
Texture	8.20±0.38	7.85±0.24	7.93±0.44	7.29±0.47	7.22±0.40	8.24±0.31	7.79±0.16
Acceptance	8.38^{b}±0.23	7.67^{ab}±0.19	7.62^{ab}±0.38	6.91^{a}±0.70	7.77^{ab}±0.28	7.84^{ab}±0.34	7.70^{ab}±0.16

Means with different superscript within a row differed statistically (P≤0.05).

1.24, whereas Demarchi *et al*. (2005) reported the range of total ash per cent to be 0.97 to 1.17. They have also reported ash per cent to be significantly (P<0.01) higher for breast meat as compared to leg meat.

Sensory Characteristics

Sensory characteristics of improved variety of chicken meat are presented in Table 5. Chicken meat from the improved varieties was liked by the taste panellists and was rated good to very good for different sensory characteristics like colour, flavour juiciness tenderness texture and overall acceptability. Out of the sensory attributes, only flavour and juiciness scores were found to be significantly (P<0.05) influenced by variety. Amongst all the varieties studied, CARI Shyama scored highest in flavour as well as juiciness scores. The mean values for sensory scores of overall acceptance differed significantly (P<0.05) amongst the varieties. The predominant order of preference for overall acceptance was Black Rock < Kalinga Brown < Vanraja < Caribro Dhanraja < Kuroiler < CARI Shyama.

For sensory characteristics of improved variety of chicken, a lower value as compared to present investigation has been reported by Raj *et al*. (2005) for all sensory scores in commercial broiler birds. They also reported that all sensory attributes including overall acceptability increased with the age of the birds. This may be one of the reasons for more acceptability of backyard varieties because they are marketed at later ages as compared to commercial broiler birds.

CONCLUSION

The results revealed variation of meat pH among different varieties of backyard chickens as well as different body parts. Meat lipid and cholesterol content increased with increase in age. Leg meat had higher cholesterol content irrespective of varieties. Among the different varieties, meat of CARI Shyama had higher protein and total ash content, but lower content of ether extract. The overall acceptance was recorded higher for meat of CARI Shyama in sensory evaluation.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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