



Effect of replacement of buffalo meat with chicken on haematological and serum metabolite profile of captive Indian leopards (*Panthera pardus fusca*)

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Indian leopard (*Panthera pardus fusca*) is protected under Schedule I of WPA of India, 1972 and is categorized as Near Threatened (NT) in IUCN Red Data Book. Information available on feeding and nutrition of captive leopards are meager which mostly pertains to intake and utilization of major nutrients (Pradhan *et al.* 2015). In free range, leopard is an opportunistic predator that preys on several species like Sambar, spotted deer, barking deer, four horned antelope, mouse deer, wild pig, gaur, hare, domestic cattle, and many other mammals and birds (Andheria *et al.* 2007). However, in captivity, they are fed solely on buffalo meat that contains either no or very negligible amount of carotenoids as compared to natural preys of leopards that accumulate moderate to good amount of carotenoids (Slifka *et al.* 1999). Thus, captive diets of leopards were suspected to be deficient of these pigments. Birds, on the other hand, are good accumulator of carotenoids. It is thus possible to increase the dietary supply of carotenoids through incorporation of chicken in the diet. Carotenoids are highly lipophilic and have conjugated double bonds in their chemical structure which enable them to clean up the excessive free radicals produced in the system as an initial response to infection (Pham-Huy *et al.* 2008). Considering that captive animals are continuously exposed to different kinds of stressors (Morgan and Tromborg 2007), it would be desirable to explore ways through which it may be possible to alleviate stress and improve welfare. Replacement of buffalo meat with chicken is one such approach. An index comprising of relative abundance of neutrophil and lymphocytes is the neutrophil/ lymphocyte (N : L), which is used widely used to assess stress in mammals (Davis *et al.* 2008). We hypothesized that increased supply of carotenoids through chicken would

improve the N : L in captive Indian leopards without any adverse impact on serum clinical chemistry profile. Specific objective of this experiment was to study the effect of partial replacement of buffalo meat with chicken on serum clinical chemistry parameters in captive Indian leopard.

All procedures followed in this experiment were approved by the Committee on Protocol, Control and Supervision of Experimentation on Animals (CPCSEA), Ministry of Environment and Forests, New Delhi, India. Twelve adult leopards (7 Male and 5 Female, age 3–14 years, BW ranging from 45–63 kg) of Pandit GB Pant High Altitude Zoo, Nainital were used for this experiment. The experiment was based on replicated Latin square design comprising of 3 treatments, 3 periods (21 days each) and 4 animals and 3 sequences. Leopards in group T₁ were fed normal zoo diet of buffalo meat six days a week without any chicken; whereas, 10 and 20% of buffalo meat was replaced with chicken in groups T₂ and T₃, respectively. Standards, guidelines and protocol with respect to housing, feeding and management as suggested by the Central Zoo Authority were strictly followed during the period of experimentation.

On d 21 of each experimental period at 07:30 h, blood was collected from all the animals by ventral coccygeal vein puncture into 'serum plus' blood collection tubes and was allowed to clot. After one hour, blood samples were centrifuged at 3000 rpm for 10 min to harvest serum. The harvested serum samples were stored at –20°C for further analysis. Three ml of fresh blood was stored separately with heparin for lab use.

Total haemoglobin (Hb) concentrations in blood samples were determined by using Sahli's haemoglobinometer. Blood smears were prepared at the time of blood collection and preserved in absolute methanol to estimate the relative microscopic differential leucocyte count (DLC), stained with May-Grunwald-Giemsa stain. Total erythrocyte count (TEC) and leukocyte count (TLC) were made in Neubauer chamber. Serum biochemical parameters were estimated by using standard diagnostics kits. Data obtained were subjected to analysis of variance (ANOVA) by using

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Table 1. Effect of gradual replacement of buffalo meat with chicken on blood biochemical profile of captive Indian leopard (*Panthera pardus fusca*)

Parameter	Dietary treatments [†]			P value
	T ₁	T ₂	T ₃	
<i>Haematological parameters</i>				
Hb (g/dl)	11.71±0.68	12.97±0.79	13.15±0.41	0.247
RBC × 10 ⁶ /μl	9.98±0.24	9.24±0.31	9.29±0.36	0.271
Platelets × 10 ³ /μl	566±50	530±17	582±45	0.651
WBC × 10 ³ /μl	9.82±0.2	10.25±0.25	10.29±0.33	0.436
Lymphocyte (%)	21.87 ^b ±0.40	19.79 ^b ±0.18	19.42 ^a ±0.62	0.001
Monocyte (%)	8.49±1	8.44±0.23	7.72±0.29	0.630
Neutrophil (%)	70.87 ^a ±0.37	71.76 ^b ±0.21	72.84 ^c ±0.49	0.004
N : L	3.80 ^c ±0.15	3.63 ^b ±0.03	3.25 ^a ±0.07	0.002
<i>Serum metabolites</i>				
Glucose (mg/dl)	77.92±1.2	79.16±1.5	76.19±2.1	0.457
Total protein (mg/dl)	8.78±0.18	8.64±0.28	8.79±0.25	0.885
Globulin (mg/dl)	5.52±0.21	5.61±0.27	5.33±0.39	0.805
A/G ratio	0.60±0.03	0.55±0.03	0.75±0.44	0.240
Triglyceride (mg/dl)	40.3±0.57	40.2±0.31	40.6±0.38	0.824
Cholesterol (mg/dl)	156±3.2	157±4.0	153±4.3	0.216
Urea (mg/dl)	39.37±0.51	38.59±0.53	38.19±0.35	0.776
Creatinine (mg/dl)	1.41±0.01	1.40±0.02	1.40±0.02	0.903
<i>Serum minerals</i>				
Ca (mg/dl)	9.40±0.40	9.39±0.24	10.4±0.40	0.075
P (mg/dl)	5.43±0.27	4.92±0.07	5.45±0.12	0.079
<i>Serum enzymes</i>				
ALT (U/l)	38.5±0.22	38.7±0.36	38.5±0.37	0.897
AST (U/l)	33.13±1.05	33.00±1.16	33.43±2.45	0.983
<i>Hormones</i>				
T ₃ (nmol/l)	1.49±0.21	1.36±0.12	1.35±0.16	0.829
T ₄ (nmol/l)	25.65±1.54	26.15±1.52	25.17±1.44	0.901

[†]Leopards in group T₁ were fed normal zoo diet of buffalo meat without any chicken; whereas, 10 and 20% of buffalo meat was replaced with chicken in groups T₂ and T₃, respectively. Hb, haemoglobin; RBC, red blood corpuscle; g/dl, gram per decilitre; mg/dl, milligram per decilitre; A/G ratio, albumin to globulin ratio; Ca, calcium; P, phosphorus; U/l, unit per litre; ALT, alanine aminotransferase; AST, aspartate aminotransferase; P, level of significance.

statistical software package SPSS 17.0 (SPSS, Chicago, IL, USA). Treatment means were separated by applying Tukey's test and differences were considered as statistically significant at P<0.05.

Feed consumption and calorie intake was similar among the groups. Carotenoids intake increased ($Y = 0.1198X + 0.5321$; $R^2 = 0.9782$) with increased level of chicken in the diet. Data pertaining to Hb, TEC, TLC, lymphocyte count, neutrophil count, monocyte count, and relative abundance

of neutrophil and lymphocyte (N : L) are presented in Table 1. In the present study, the values of Hb, RBC, WBC, monocyte and platelets were within the normal range reported in jaguar (Maas *et al.* 2013) and wild tigers (Shrivastav *et al.* 2011).

In this experiment, relative abundance of neutrophil ranged from 69 to 75%; whereas, lymphocyte ranged from 16 to 22%. In an earlier study, it was reported that relative abundance of neutrophil was 57–75% and the proportion of lymphocyte was 18–35% in tiger (Shrivastav *et al.* 2011). Thus, it seems that the neutrophil and lymphocyte profile of captive Indian leopard was similar to other felids. The N : L decreased ($P < 0.01$) with increased level of chicken in the diet. An inverse relationship between carotenoids intake and N : L ($y = -0.265x + 4.087$; $R^2 = 0.89$) was observed. Living in a stressful condition results in elevation of circulatory concentration of cortisol that may cause redistribution of lymphocyte into tissue namely skin, spleen, bone marrow, and lymph node resulting in decrease concentration of lymphocyte in blood (Dhabhar *et al.* 1996). Increase cortisol also results in mobilization of neutrophils from bone marrow. These two factor might have contributed to increased N : L in control group. In addition of being used as a indicator of stress, N : L is also used to predict survivability and productivity (Davis *et al.* 2008). Thus, a low N : L would be desirable. In this experiment, replacement of buffalo meat with chicken decreased N : L in a dose dependent manner. Thus, it can be inferred that provision of carotenoids through chicken would improve the power to combat stress. The baseline data of haematological parameters of the species that we have generated through this experiment are novel which could be used as baseline data for future research and clinical investigations.

Serum concentrations of different metabolites, Ca, P and enzymes and hormones (T₃ and T₄) were similar among the group. An earlier report from ICAR-IVRI indicated that serum concentration of glucose, total protein, albumin, globulin, creatinine, urea, cholesterol and triglyceride were 79.6±0.41 mg/dl, 6.92±0.10 g/dl, 3.47±0.09 g/dl, 3.44±0.14 g/dl, 1.25±0.05 mg/dl, 39.7±0.50 mg/dl, 166.9±2.18 mg/dl, and 39.55±0.62 mg/dl, respectively (Pradhan *et al.* 2015), which was similar to the values that was observed in this experiment. As the dietary concentration of Ca and P and their ratio in the diet was similar, no change in their serum profile was expected. Further, serum concentration of Ca is tightly regulated under hormonal control and does not change much under normal circumstances. Serum concentration of Ca and P that was observed in this experiment was within the normal range reported in wild felids (Depauw *et al.* 2011). Activity of AST and ALT is widely used as marker of liver damage. Replacement of 20% of buffalo meat with chicken does not cause any change in concentration of these enzyme. So it can be deduced that replacement of buffalo meat with chicken had no adverse impact on liver health. Similarly, unaltered urea and creatinine level would indicate about normal kidney

function. Thus, it can be inferred that the replacement of 20% of buffalo meat with chicken had no adverse effect on serum metabolic profile.

Even though, thyroid hormones perform such versatile function as regulation of basal metabolic rate and immune responses, there are only few reports that indicate the status of the hormones in captive felids, and apparently there is no report in captive Indian leopard. Thus, it would be desirable to generate baseline data in this regard. In this experiment, serum concentration of T_3 ranged from 0.3 to 2.4 (mean 1.49 ± 0.21) nmol/l. Serum concentration of T_3 ranged from 0.77 to 1.38 nmol/l in cheetah (Depauw *et al.* 2012). In another study, it was reported that serum concentrations of T_3 were 0.86, 1.03, 0.66 and 1.01 nmol/l, in jaguar, puma, lion and tiger, respectively (Rodini *et al.* 2007). Even though the mean values of T_3 were within the range reported in other felids, considering the large individual variation it would be desirable to generate more data so as to fix the baseline data for the species. Serum concentrations of T_4 were 12.48, 14.41, 7.33 and 16.22 nmol/l, in jaguar, puma, lion and tiger, respectively. In this experiment, serum concentration of T_4 ranged from 13.6 to 36.7 nmol/l. An inter-species comparison would indicate that the value observed in leopards were within the higher range that is given for other felids. However, still higher concentration (18.9 to 57 nmol/l) of T_4 had been reported in domestic cat (Skinner *et al.* 1998). From the results and the review of literature, it is apparent that serum concentration of thyroid hormone varies among different species of felids. Replacement of buffalo meat with chicken showed no adverse effect on the functioning of thyroid hormones. Thus, 20% of buffalo meat can be replaced safely with chicken.

SUMMARY

In order to study the effect of gradual replacement of buffalo meat with chicken on haematology and serum metabolite profile, an experiment was conducted on twelve adult leopards. The experiment was based on replicated Latin square design comprising 3 treatments, 3 periods (each comprising 21 days), 4 animals and 3 sequences. Diets of the leopards in group T_1 comprised solely of buffalo meat, however, buffalo meat was replaced with chicken at 10 and 20% on DM basis in the diets of animals in groups T_2 and T_3 , respectively. Blood was collected from all the animals by puncturing the ventral coccygial vein on d 21 of each experimental period at 07:30 h. Lymphocyte count decreased and neutrophil count increased with increased level of chicken in the diet. Other haematological and serum metabolites were similar among the groups. It was concluded that replacement of 20% of buffalo meat with chicken increased the ability of the leopards to combat stress

without any adverse effect on other haematological and serum metabolite profile.

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REFERENCES

- Andheria A P, Karanth K U and Kumar N. 2007. Diet and prey profiles of three sympatric large carnivores in Bandipur Tiger Reserve, India. *Journal of Zoology* **273**: 169–75.
- Davis A K, Maney D L and Maerz J C. 2008. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. *Functional Ecology* **22**: 760–72.
- Dhabhar F S, Miller A H, McEwen B S and Spencer R L. 1996. Stress induced changes in blood leukocyte distribution – role of adrenal steroid hormones. *Journal of Immunology* **157**: 1638–44.
- Depauw S, Hesta M, Whitehouse Tedd K, Stagegaard J, Buyse J and Janssens G P J. 2012. Blood values of adult captive cheetahs (*Acinonyx jubatus*) fed either supplemented beef or whole rabbit carcasses. *Zoo Biology* **31**: 629–41.
- Maas M, Keet D F and Nielen M. 2013. Haematologic and serum chemistry reference intervals for free-ranging lions (*Panthera leo*). *Research in Veterinary Science* **95**: 266–68.
- Morgan K N and Tromborg C T. 2007. Sources of stress in captivity. *Applied Animal Behaviour Science* **102**: 262–302.
- Pradhan S K, Das A, Kullu S S, Saini M, Pattanaik A K, Dutta N and Sharma A K. 2015. Effect of feeding Jerusalem artichoke (*Helianthus tuberosus*) root as prebiotic on nutrient utilization, fecal characteristics and serum metabolite profile of captive Indian leopard (*Panthera pardus fusca*) fed a meat on bone diet. *Zoo Biology* **34**: 153–62.
- Pham-Huy L A, He H and Pham-Huy C. 2008. Free radicals, antioxidants in disease and health. *International Journal of Biomedical Science* **4**: 89–96.
- Rodini D C, Felipe E C G and Oliveira C A. 2007. Measurement of thyroid hormones (thyroxine, T_4 ; triiodothyronine, T_3) in captive nondomestic felids. *Journal of Zoo and Wildlife Medicine* **38**: 125–26.
- Shrivastav A B, Singh K P, Mittal S K and Malik P K. 2011. Haematology and biochemical studies in tigers. *European Journal of Wildlife Research* **57**: 103–06.
- Skinner N D. 1998. Thyroid hormone levels in cats: Colony average and the decrease with age. *Journal of Nutrition* **128**: 2636S–2638S.
- Slifka K A, Bowen P E, Stacewicz-Sapuntzakis M and Crissey S D. 1999. A survey of serum and dietary carotenoids in captive wild animals. *Journal of Nutrition* **129**: 380–90.

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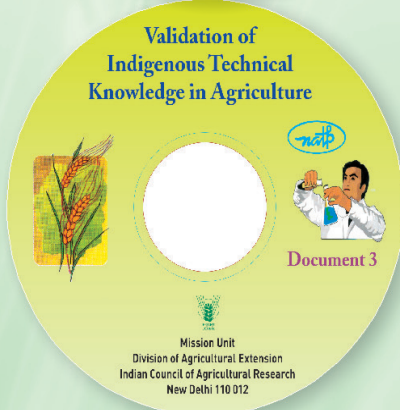
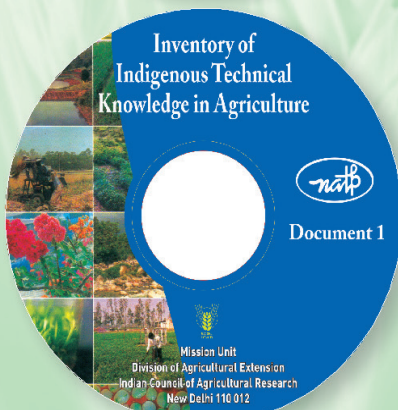
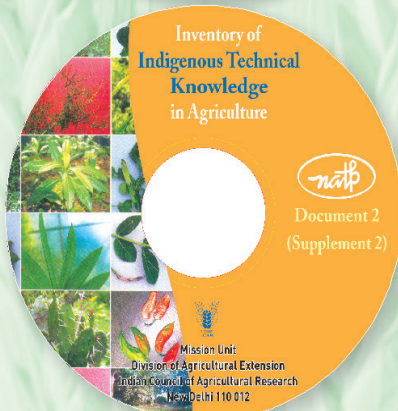
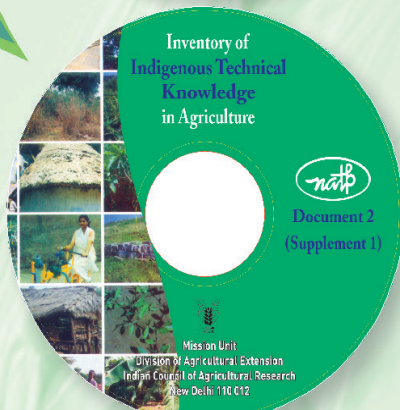
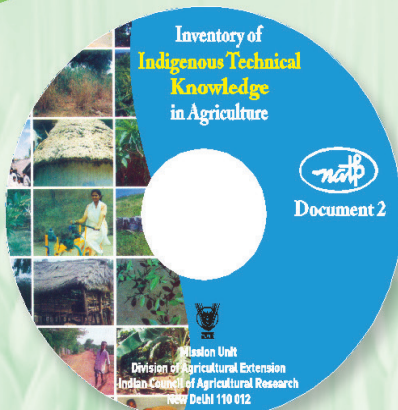
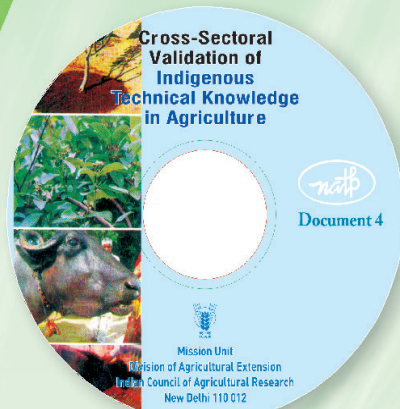


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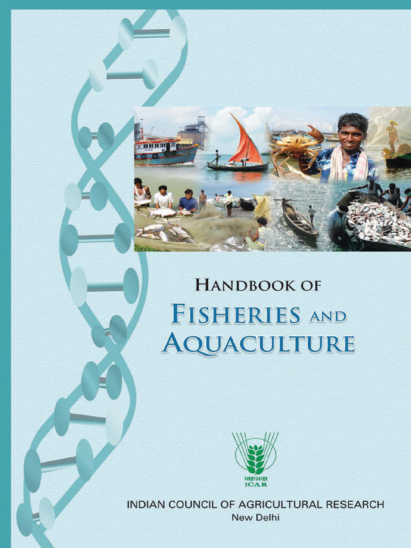
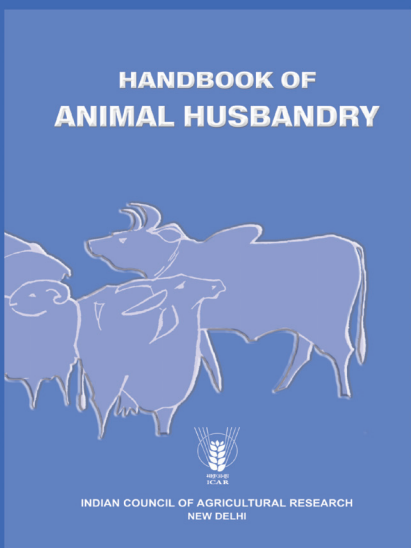
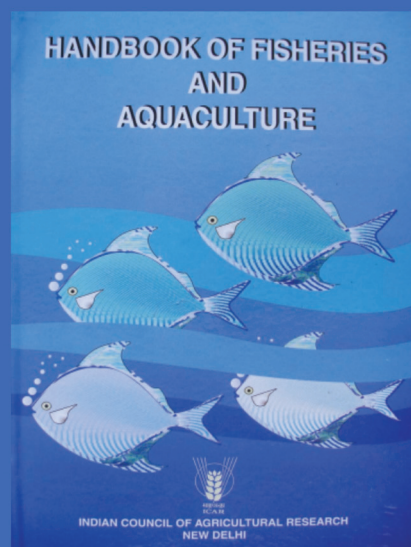
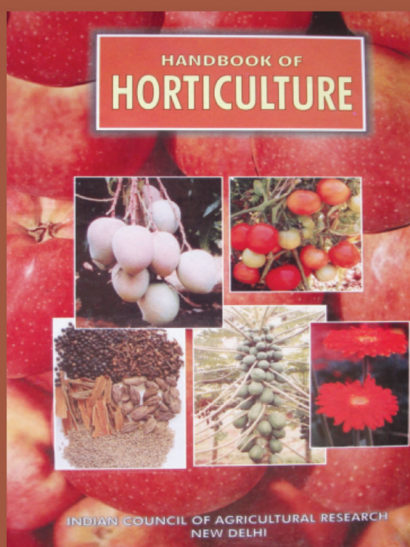
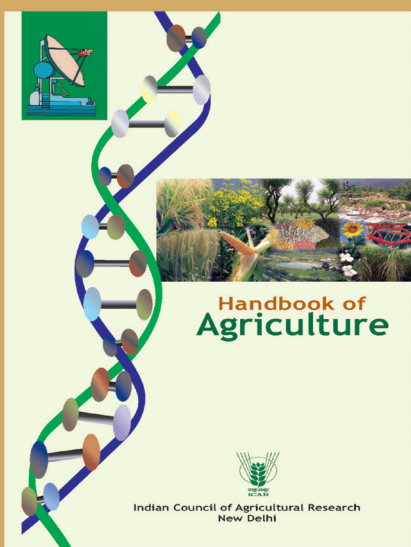


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