

Study on hematological and serum biochemical parameters of Pulikulam cattle in a nucleus herd

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Abstract

The Pulikulam cattle breed is a native breed of Tamil Nadu mostly reared in southern districts. In this study normal reference values of hematological and serum biochemical values were determined in Pulikulam cattle reared in Pulikulam Cattle Research Station. The animals were randomly selected and divided into different groups based on their age and physiological status *viz.*, calves, heifers, early lactation, mid lactation, late lactation and bulls. Each group consists of six animals. The hematological parameters and serum biochemical parameters were estimated with the help of the auto analyzer. The values for different parameters during different physiological stages were analyzed. It is concluded that the hematological value of Pulikulam cattle are within the normal referral value of bovine species and in serum biochemical values some parameters are varied. The hematological and serum biochemical values of Pulikulam cattle breed will be useful for academic purpose and helpful to identify the health and nutritional status of Pulikulam cattle breed.

Key words: Hemato-biochemical reference value, Indigenous cattle breed, Native cattle, Pulikulam cattle, Tamil Nadu

INTRODUCTION

The health status of the cow can be evaluated based on the hematological and serum biochemical parameters. In combination with clinical examination, hematological and serum biochemical analysis is used as a diagnostic tool in bovine medicine (Roland *et al.*, 2014). The hematological and serum biochemical values provide baseline information of nutritional, physiological and health status of the animal (Osman and Al- Busadah, 2003). Hematological and serum biochemical parameters of cow were analyzed by many authors at various physiological conditions. Biochemical profile of healthy cows and reproductive disorder were studied by Ruginosu *et al.* (2011). Influence of lactation on biochemical profile of cows was studied by Filipejová, and Kovářek (2009). Changes in the hematological parameters due to environmental effect were studied by Mazzullo *et al.* (2014). Seifi *et al.* (2007) studied the variation in the

biochemical profile of cows during transition period. Cozzi *et al.* (2011) had estimated the references values for blood parameters in Holstein cows. Influence of age on hematology and serum biochemical reference value for female Holstein cattle were studied by Lumsden *et al.* (1980). Sabasthin *et al.* (2012) studied the hematological and serum biochemical parameters in normal cyclic, pregnant and repeat breeding buffalos (*Bubalus bubalis*). Hematological profile of Indian native breed Hallikar cattle at its native tract was studied by Lankesh *et al.* (2015). In general, metabolic profile test will give health status of the cow in herd basis. Nutrition plays a major role in changing the metabolic profile of the animal (Dhiman *et al.*, 1991; Khaled *et al.*, 1999; Ingvarstsen, 2006). National Bureau of Animal Genetic Resources (NBAGR), registered 50 Indigenous cattle breeds (NBAGR website, 2021). The accession number allotted by NBAGR for Pulikulam cattle is

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INDIA_CATTLE_1800_PULIKULAM_03035. Pulikulam cattle breed is primarily a draught breed, small in size and capable of endurance activities and is reared in the southern part of Tamil Nadu, India (Littlewood, 1936). This breed is locally popular for its vigour and can sustain even under feed and fodder scarcity (Srinivasan and Sathiamoorthy, 2020). The population of Pulikulam cattle was about 50,000, of which 20,000 were breeding female (Singh *et al.*, 2012). There is no data available on normal reference value of hematological and serum biochemical parameters of Pulikulam animals. Therefore the present study was designed to study the various hematological and serum biochemical parameters of Pulikulam cattle reared in nucleus herd.

MATERIALS AND METHODS

The blood samples were collected from the Pulikulam animals reared in Pulikulam Cattle Research Station, a constituent unit of Tamil Nadu Veterinary and Animal Sciences University. The study was conducted in the month of October, 2020. The animals were randomly selected and divided into different groups based on their age and physiological status viz calves, heifers, early lactation, mid lactation, late lactation and bulls. Each group consists of six animals. The animals were reared in intensive housing system. All the animals were in good health condition and fed with green fodder, dry fodder and concentrate feed based on the ICAR, 2013 requirement. Blood samples were collected as per the standard procedure during early morning before feeding. The ethylenediamine tetraacetic acid (EDTA) was used as anticoagulant for collection of whole blood (2 mL) and another blood sample was collected without anticoagulant (8 mL) for collection of serum. Serum was harvested after standing the blood for 30 minutes and then centrifuging it at 3000 rpm for 20 minutes. The hematological parameters such as hemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC) count, white blood cell (WBC) count,

platelet count, neutrophils count, lymphocyte count, monocytes count and eosinophils and serum biochemical parameters such as albumin, globulin, blood urea nitrogen, serum creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (Tbill), direct bilirubin (Dbill), serum calcium, phosphorus, magnesium, sodium, potassium and chloride were estimated. The mean corpuscular value (MCV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) values were calculated (William O Reece, 2004). Hematological parameters were analyzed by auto hematology model Cellenium 21 and Labmate model for serum biochemical parameters. Data were subjected to analysis of variance (ANOVA) and Tukeys test using IBM SPSS Version 20. The statement of significant was considered on a probability of $p < 0.05$.

RESULTS

Hematological parameters

The hematological value for different parameters in the Pulikulam cattle is presented in Table 1. Highest Hb level was noticed in heifers and lowest Hb level was noticed in calves. The mean Hb concentrations during early, mid and late stage of lactation were 13.93 ± 1.08 , 14.45 ± 1.10 and 15.0 ± 1.19 g/dL respectively. The mean values of packed cell volume and RBC were within normal physiological range of bovine. Platelet count was within the normal physiological range but significant difference ($p < 0.05$) was noticed between calves and other groups. The mean leucocytes value was within the normal physiological range of bovine and significant difference ($p < 0.05$) was noticed between calves and heifers. The mean corpuscular value (MCV) was within the normal physiological values of bovine but significant difference ($p < 0.05$) was noticed between calves and bulls. Mean corpuscular hemoglobin concentration (MCHC) values were within the normal range.

Table 1. Mean (Mean \pm SE) hematological value in Pulikulam breed of cattle

Parameters	Calf	Heifer	Early lactation	Mid lactation	Late lactation	Bull
Hb (g/dL)	11.5 \pm 0.56	15.06 \pm 1.36	13.93 \pm 1.08	14.45 \pm 1.10	15.00 \pm 1.19	13.6 \pm 0.55
PCV (%)	27.33 \pm 1.11	37.40 \pm 3.13	33.56 \pm 2.2	37.25 \pm 2.49	36.66 \pm 2.95	34.76 \pm 0.76
RBC (x 10 ⁶ μ L)	7.91 \pm 0.33	8.93 \pm 0.79	7.94 \pm 0.51	7.75 \pm 0.08	7.97 \pm 0.71	7.04 \pm 0.45
WBC (x10 ³ μ L)	10.933 ^b \pm 0.27	6.266 ^a \pm 0.87	9.933 ^{ab} \pm 1.18	7.800 ^{ab} \pm 1.63	7.300 ^{ab} \pm 0.78	7.700 ^{ab} \pm 0.78
MCV(fl)	34.5 ^a \pm 0.09	41.9 ^{ab} \pm 0.17	42.7 ^{ab} \pm 0.43	47.9 ^b \pm 0.32	46.1 ^{ab} \pm 0.15	47.0 ^b \pm 0.28
MCH(pg)	14.52 ^a \pm 0.02	16.88 ^{ab} \pm 0.07	17.51 ^b \pm 0.03	18.61 ^b \pm 0.12	18.86 ^b \pm 0.04	19.38 ^b \pm 0.05
MCHC (%)	42.04 \pm 0.01	40.23 \pm 0.01	42.08 \pm 0.05	38.75 \pm 0.01	40.91 \pm 0.01	39.10 \pm 0.01
Platelets (lakhs)	5.79 ^a \pm 0.61	3.54 ^b \pm 0.78	2.96 ^b \pm 0.29	3.07 ^b \pm 0.35	3.19 ^b \pm 0.40	2.86 ^b \pm 0.10
Neutrophils (%)	26.68 \pm 1.15	28 \pm 1.45	29.68 \pm 0.66	29.34 \pm 1.63	31.68 \pm 2.84	28.34 \pm 2.33
Lymphocytes(%)	67.66 \pm 0.33	66.67 \pm 1.73	65 \pm 0.5	65.5 \pm 2.85	62.66 \pm 2.72	66.33 \pm 2.02
Monocytes (%)	2.66 \pm 0.33	1.33 \pm 0.33	1.66 \pm 0.33	1.66 \pm 0.81	1.66 \pm 0.33	2.0 \pm 0.57
Eosinophils (%)	3.0 ^{ab} \pm 0.57	4.0 ^b \pm 0.57	3.66 ^b \pm 0.33	3.50 ^a \pm 0.40	4.0 ^b \pm 0.57	3.33 ^{ab} \pm 0.33
Basophils (%)	0	0	0	0	0	0

(Values are mean of six replicates)

Mean with different superscript in a row differ significantly (P<0.05)

Mean corpuscular hemoglobin (MCH) values were within the normal physiological range of bovine but significant difference (p<0.05) was noticed in between calf and early, mid and late lactation groups. The mean value of neutrophils was higher in bulls (28.34 \pm 2.33%) than calves (26.68 \pm 1.15%), but highest value was noticed in late lactation group (31.68 \pm 2.84%). However lymphocyte count was higher in calves (67.66 \pm 0.33%) than bulls (66.33 \pm 2.02%). The circulating eosinophils level was higher in late lactation group (4.0 \pm 0.57%) and heifers (4.0 \pm 0.57%) and lower in calves (3.0 \pm 0.57%). A significant difference (p<0.05) was noticed between mid and late lactation group. The mean values of monocytes count were within normal physiological range of bovine, highest value were observed in calves (2.66 \pm 0.33%). No basophil count was reported in this study in different age group of Pulikulam animals.

Serum biochemical parameters

The mean serum biochemical parameters values are given in Table 2. The serum glucose level of Pulikulam cattle ranged from 82 to 117.66 mg/dL. Highest mean serum glucose level was noticed in calves (117.66 \pm 8.68 mg/dL) and lowest mean serum glucose level noticed in early lactation group (82.0 \pm 7.02 mg/dL). The mean total protein was highest (6.43 \pm 0.23 g/dL) in late lactation, the values of early lactation (6.2 \pm 0.3 g/dL) and mid lactation (6.15 \pm 0.15 g/dL) group were almost at par. The serum albumin was higher in early lactation than mid and late lactation. The globulin level was higher in mid lactation (2.75 \pm 0.28 g/dL) and late lactation (2.7 \pm 0.20 g/dL) than early lactation (2.36 \pm 0.20). A significant difference (p<0.05) in globulin level was noticed between calves and mid lactation group. The mean BUN values in heifers, early, mid and late lactation stages were 73.1 \pm 4.13 mg/dL,

Table 2. Mean (Mean \pm SE) serum biochemical value in Pulikulam breed of cattle

Parameters	Calf	Heifer	Early lactation	Mid lactation	Late lactation	Bull
Glucose (mg/dL)	117.66 \pm 8.68	97.0 \pm 6.65	82.0 \pm 7.02	93.5 \pm 26.53	89.66 \pm 1.76	106.0 \pm 1.0
Albumin (g/dL)	3.63 \pm 0.12	3.73 \pm 0.14	3.83 \pm 0.18	3.4 \pm 0.16	3.73 \pm 0.08	3.6 \pm 0.15
Globulin (g/dL)	1.46 ^a \pm 0.31	2.46 ^{ab} \pm 0.31	2.36 ^{ab} \pm 0.20	2.75 ^b \pm 0.28	2.7 ^{ab} \pm 0.20	2.46 ^{ab} \pm 0.35
Total Protein (g/dL)	5.1 \pm 0.37	6.2 \pm 0.11	6.2 \pm 0.30	6.15 \pm 0.15	6.43 \pm 0.23	6.06 \pm 0.23
BUN (mg/dL)	68.8 \pm 6.55	73.1 \pm 4.13	66.7 \pm 4.25	83.2 \pm 0.73	68.73 \pm 4.71	69.90 \pm 1.10
Creatinine (mg/dL)	1.70 ^a \pm 0.05	2.1 ^b \pm 0.05	2.0 ^{ab} \pm 0.03	2.0 ^b \pm 0.16	2.3 ^b \pm 0.05	2.03 ^{ab} \pm 0.06
Cholestrol (mg/dL)	178.66 \pm 26.19	147.33 \pm 3.17	147.33 \pm 6.74	169.0 \pm 15.51	147.66 \pm 2.60	139.0 \pm 9.29
Triglycerides (mg/dL)	55.7 \pm 3.30	52.53 \pm 2.40	63.23 \pm 8.87	62.25 \pm 3.22	63.56 \pm 4.40	36.56 \pm 5.26
ALT (U/L)	26.66 ^{ab} \pm 2.96	29.0 ^{ab} \pm 2.0	23.33 ^b \pm 1.76	26.5 ^{ab} \pm 0.40	21.66 ^b \pm 1.20	23.33 ^a \pm 1.66
AST (IU/dL)	66.0 \pm 4.50	66.0 \pm 2.08	63.33 \pm 1.85	69.5 \pm 2.04	65.66 \pm 1.66	69.66 \pm 4.97
ALP (U/L)	223.66 \pm 49.41	140.0 \pm 7.09	189.33 \pm 18.70	124.50 \pm 1.22	90.66 \pm 17.33	210.33 \pm 56.0
Dbill (mg/dL)	0.05 \pm 0.01	0.04 \pm 0.01	0.02 \pm 0.01	0.05 \pm 0.00	0.05 \pm 0.01	0.05 \pm 0.01
Tbill (mg/dL)	0.26 ^a \pm 0.12	0.41 ^{ab} \pm 0.05	0.35 ^{ab} \pm 0.10	0.43 ^b \pm 0.11	0.37 ^{ab} \pm 0.05	0.44 ^{ab} \pm 0.05

(Values are mean of six replicates)

Mean with different superscript in a row differ significantly (P<0.05)

66.7 \pm 4.25 mg/dL, 83.2 \pm 0.73 mg/dL and 68.73 \pm 4.71 mg/dL respectively. The mean serum creatinine values of Pulikulam cattle during early, mid and late lactation were 2.03 \pm 0.03 mg/dL, 2.0 \pm 0.16 mg/dL and 2.3 \pm 0.05 mg/dL respectively. But significant difference (p<0.05) was noticed between calves and mid, late lactation groups. The highest serum cholesterol level was noticed during mid lactation (169.0 \pm 15.5 mg/dL) followed by late lactation group early lactation group, and heifers viz 147.66 \pm 2.60 mg/dL, 147.33 \pm 6.74 mg/dL and 147.33 \pm 3.17 mg/dL respectively. The significant difference (p<0.05) in triglyceride value was noticed between bulls and early lactation group. The ALT value reported in this study was within the normal physiological range of cattle. Highest value was observed in heifers (29.0 \pm 2.0 U/L)

followed by calves (26.66 \pm 2.96 U/L). The lowest value was noticed in late lactation group (21.66 \pm 1.2 U/L).

The mean AST values noticed in this study was within the normal physiological range of bovine in all stage of lactation. The mean ALP value of Pulikulam cattle in the present study was within the normal physiological range. Highest ALP values noticed in calves (223.66 \pm 49.41 U/L) followed by bulls (210.33 \pm 56.0 U/L) and early lactation (189.33 \pm 18.70 U/L). Both mean total billurubin and direct billurubin values were within the normal physiological range of cattle (Kahn, 2010) but significant difference (p<0.05) in total billurubin level was noticed between calves and mid lactation group.

The mean serum calcium values of

Table 3. Mean (Mean \pm SE) serum minerals level in Pulikulam cattle breed

Parameters	Calf	Heifer	Early lactation	Mid lactation	Late lactation	Bull
Calcium (mg/dL)	7.8 \pm 0.20	7.36 \pm 0.17	7.63 \pm 0.26	7.6 \pm 0.40	7.73 \pm 0.12	7.06 \pm 0.12
Phosphorus (mg/dL)	8.23 \pm 0.78	6.86 \pm 0.41	5.56 \pm 1.39	7.7 \pm 0.32	5.9 \pm 0.20	5.66 \pm 0.44
Magnesium (mg/dL)	3.93 \pm 0.03	3.96 \pm 0.06	3.93 \pm 0.06	3.95 \pm 0.12	4.0 \pm 0.05	3.86 \pm 0.03
Sodium (mmol/L)	162.49 \pm 2.93	170.01 \pm 2.24	162.49 \pm 6.12	159.89 \pm 0.57	164.56 \pm 0.87	161.52 \pm 2.31
Potassium (mmol/L)	5.09 \pm 0.08	4.39 \pm 0.23	4.65 \pm 0.13	4.87 \pm 0.89	5.06 \pm 0.52	4.02 \pm 0.20
Chloride (mmol/L)	115.71 \pm 0.89	114.09 \pm 3.0	107.62 \pm 1.37	111.11 \pm 3.15	113.12 \pm 2.70	111.43 \pm 1.10

(Values are mean of six replicates)

Pulikulam cattle are given in Table 3. The serum calcium level was similar in all stages of lactation. The highest serum calcium level was notice during late lactation stage (7.73 \pm 0.12 mg/dL) followed by early and mid lactation cows. The serum phosphorus level was lower during early lactation (5.56 \pm 1.3 mg/dL) as compared to mid (7.7 \pm 0.32 mg/dL) and late lactation (5.9 \pm 0.20 mg/dL). The mean serum magnesium level was almost similar in all stages of lactation. The mean serum sodium, serum potassium and serum chloride levels were higher in late lactation group as 164.56 \pm 0.87, 5.06 \pm 0.52 and 113.12 \pm 2.70 mmol/dL, respectively.

DISCUSSION

Hematological parameters

The mean Hb concentration was within the normal physiological range of cattle as reported by Jain (1986) ranged from 11.5 \pm 0.56 to 15.06 \pm 1.36 g/dL. The mean haemoglobin level was in agreement with the values reported by Raghunandan *et al.* (1977) and Mahima *et al.* (2013) in Haryana Cattle, Pyne and Maitra (1981) in Sahiwal cattle and Deshpande *et al.* (1986) in Red Kandhari cattle. The findings of packed cell volume, MCV, MCH and MCHC

values were within the normal physiological range of cattle as reported by Jain (1986). Jacob *et al.* (2019) reported that there was no significant difference in PCV value between lactating Gir cattle. The observation was in agreement with the findings of various workers in different indigenous breeds such as Raghunandan *et al.* (1977) and Mahima *et al.* (2013) in Haryana Cattle, Pyne and Maitra (1981) in Sahiwal cattle, Deshpande *et al.* (1986) in Red Kandhari cattle. The mean value of TEC was within the normal range of cattle as reported by Jain (1986). Mahima *et al.* (2013) reported that RBC and TLC of Haryana cattle were within the normal physiological range. Variation in the reference values of TLC was reported by various authors due to difference in age, breed, environmental temperature, physiological status, stress prior to handling and parasitic infection (Kapale *et al.*, 2008; Farooq *et al.*, 2011). The circulating TLC generally represents the dynamic production by bone marrow, the release of the cells to the peripheral circulation and their storage in different organs or pools. Jacob (2012) reported that Gir bulls were found to have higher TLC than male calves with different age. Shil *et al.* (2012) found that

lymphocyte count was higher in calves than bulls which were similar to the findings of this study. Canfield *et al.* (1984) reported that eosinophil count was higher in adult buffalos than calves. The increase in eosinophils with advancement of age might be adoptive response of the body to various parasitic load or other pathogens exposed to over the period of time. The mean value of monocytes count was within normal physiological range as reported by Jain, 1986. The finding of this study was in conformity with the findings of Abd Ellah *et al.* (2014) in water buffalo heifers and Ali and Shukla (2012) in normal cyclic post partum buffalos. The finding of this study on basophil level was in agreement with Mayengbam *et al.* (2018) who reported that basophil level was in the range of 0-1% in both Sahiwal and Karan Fries cattle breeds at different intervals of thermal exposure. Jacob *et al.* (2021) reported that basophil count (%) differed non-significantly between all the ages and physiological stages studied in Gir cattle and Jaffarabadi buffaloes and also between the two species.

Serum biochemical parameters

Serum glucose value was increased along with the progress of lactation. During early lactation more glucose is needed for lactose synthesis and thus lower glucose level was present in the circulation. The mean serum glucose level in the present study was higher than that reported by Gopalakrishna Rao *et al.* (1981) and Sharma and Quadri (2001). The finding of this study was in accordance with the findings of Rawat *et al.* (2006) who reported higher value for indigenous cows. Maintenance of stable glucose concentration depends on hormones like insulin, glucagon, epinephrine, glucocorticoids and thyroid hormones which control regulatory mechanism (Rawat *et al.*, 2006). The mean plasma total protein in our study was lower than that reported by Prakash *et al.* (2018) in Kankrej and Gir cattle. Prakash *et al.* (2018) reported that serum albumin levels in cross bred cattle was increased during

progression of lactation. The physiological status or lactation stage can change the serum plasma protein level. Serum protein level indicates the balance between anabolism and catabolism of protein in the body and it depends on nutritional status, water consumption and health status (Samanta and Dass, 2007). With advancement of lactation albumin level is decreased, merely because of decreased biosynthesis by the liver in order to cope up from lactation induced metabolic stress (Kulkarni *et al.*, 1983). Serum albumin level of Trinket cattle reported by De *et al.* (2020) was similar to the finding of this study. The increased BUN values in various breeds have been reported by various authors in various studies. The elevated BUN level is due to enhancement of metabolic activities leading to increased protein catabolism (Dar *et al.*, 2019). The mean creatinine value of Pulikulam cattle found from this study seems to be higher than the creatinine value of Hariana cattle reported by Kumar *et al.* (2018). In contrast to the present study on serum creatinine, Sreedhar *et al.* (2013) reported that a non significant difference was observed in serum creatinine levels between Sahiwal heifers and Sahiwal cows. Finding of this study on cholesterol was similar to the value reported by Ramakrishnan *et al.* (2012) in Gir cows and Mahima *et al.* (2013) in Hariana cattle. In contrast, Rawat *et al.* (2006) and Jacob (2012) reported higher cholesterol values in indigenous cattle. The higher value during mid lactation in the present study was due to physiological adjustment to meet lactation requirement (Rawat *et al.*, 2006). Milorad *et al.* (2013) reported that total cholesterol and triglycerides levels were significantly affected by physiological status.

The average ALT value reported in this study was within the normal physiological range of cattle as 11 to 40 U/L (Herbert, 1984). The average ALT value of Pulikulam cattle was lower than the Sahiwal cows as reported by Chandra *et al.* (2012). The mean AST values Pulikulam cattle was lower than the Hariana cattle (Muthukumar *et al.*, 2004), Sahiwal and

Hardhenu cattle (Kumar *et al.*, 2018). Gomes *et al.* (2016) reported that mean ALP value of crossbred Holstein with Gir cross bred calves was 303.82 U/L which was higher than the value noticed in the present study. The mean ALP value of Haryana heifer as reported by Muthukumar *et al.* (2004) was lower than the Pulikulam animal. The mean total billurubin value of Pulikulam cattle was higher than Haryana cattle and Sahiwal cattle. The mean direct billurubin values of Pulikulam cattle were lower than Haryana and Sahiwal breeds (Kumar *et al.*, 2018).

The finding of this study on serum calcium level was similar to Kumar *et al.* (2018) who reported the serum calcium level of Haryana cattle was 7.8 mg/dL. Increased serum calcium level during progress of lactation was reported by Rowland *et al.* (1980) and Nale (2003). The result of this study was similar to findings of Hagawane *et al.* (2009) who reported the serum phosphorus level in early stage of lactation was lowered than the normal healthy control and other group of buffaloes. The high demand of phosphorus during early stage of production may be the reason for lower level in serum. The average serum magnesium

level of Haryana cattle reported by Mahima *et al.* (2013) was lower than Pulikulam cattle. Mamun *et al.* (2013) reported that there was no significant variation ($p > 0.05$) in serum magnesium and phosphorus levels observed between male and female native cattle in hilly area of Bangladesh which was similar to the observation of this study.

The hematological values of Pulikulam cattle were within the normal referral value of bovine species and values of some parameters in serum biochemical were varied than normal referral values of bovine. The hematological and serum biochemical values of Pulikulam cattle breed will be useful for academic purpose and helpful to identify the health and nutritional status of Pulikulam cattle breed.

Conflict of interest: Authors have no conflict of interest in this study.

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