

Original Research Article

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Metabolic Indicators for Pneumonia and Diarrhoea in Holstein Frisian Crossbred Calves

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Present study estimated alteration of certain plasma metabolites (glucose, urea nitrogen, cholesterol, NEFA and NEFA: cholesterol ratio) and minerals (Ca, P, Zn, Cu and Mn) in healthy calves (n=96), calf with diarrhoea (n=32) and calf with pneumonia (n=32). Plasma glucose was significantly lower both in calves with diarrhoea and pneumonia as compared to healthy calves ($P \leq 0.05$). There was lower plasma cholesterol level in pneumonic calves as compared to healthy calves ($P \leq 0.05$). Plasma BUN, NEFA and NEFA: cholesterol ratio remained similar between healthy and diseased calves. Plasma Ca level in diarrhoea and pneumonia condition decreases significantly than the health calves ($P \leq 0.05$). In pneumonia condition, plasma Zn level remained significantly lower than healthy ones ($P \leq 0.05$). Plasma Cu level was observed to be significantly lower in both diarrhoea and pneumonia condition compared to healthy calves ($P \leq 0.05$). The plasma P and Mn values were similar between healthy and diseased calves. Further, Receiver Operating Characteristic analysis (ROC) revealed that among different plasma metabolites and minerals, plasma glucose is 100% accurate to differentiate diseased calves from their healthy counterparts. It may be inferred that the levels of glucose would help in differentiate diseased calves from healthy calves in the herd.

Introduction

Calves are considered as future replacement stocks in a dairy farm, so they are reared economically in a sound manner to ensure early maturity. However, calf hood diseases particularly calf diarrhoea and pneumonia cause considerable economic losses in terms of mortality, morbidity, treatment cost and long term negative consequences on performance in survivors after recovery

(Millemann 2009; Poulsen and McGuirk 2009). In India, incidence of calf diarrhoea and pneumonia within first month has been reported to be 17.77-21.21% and 7.25-8.51%, respectively in crossbred calves (Brahma and Singh 2007). Therefore, accurate diagnosis followed by proper treatment including management at proper time could help early recovery and reduce long term negative effect on performances. Previous studies reported significant alteration of circulating glucose,

urea and cholesterol levels in neonatal calf diarrhoea (Chalupnic *et al.*, 2012; Mansour, 2006; Pekcan *et al.*, 2012) and pneumonia (Mansour, 2006; Ragbetli *et al.*, 2010; El-Bahr and EL-Deeb, 2013; Almujalli *et al.*, 2015). Similarly, alteration of circulating macro (Ca and P) minerals (Cabello and Michel, 1977; Walker *et al.*, 1998) and micro (Cu and Zn) minerals (Ghergariu and Kadar, 1979; Ranjan *et al.*, 2006; Soltesova *et al.*, 2015) in calves during diarrhoea has been reported. Further, Ragbetli *et al.*, (2010) reported altered circulating Ca in pneumonic calves. Hence, there is need to identify the alteration of plasma metabolites and minerals before treatment and supportive therapy of the deficient things would improve recovery of these diseases and would further reduce the negative effect in recovered calves. Present study was designed to estimate certain plasma metabolites (glucose, urea nitrogen, cholesterol, NEFA and NEFA: cholesterol ratio) and minerals (Ca, P, Zn, Cu and Mn) during neonatal calf diarrhoea and pneumonia. Further, we estimated the accuracy of the parameters to differentiate the diseased and healthy calves.

Materials and Methods

Present study was conducted on 160 Holstein frisian crossbred calves (96 healthy, 32 diarrhoea and 32 pneumonia) of age within one month at livestock research center, ICAR-National Dairy Research Institute, Karnal, India over a period of one year.

Calves were weaned after birth on day zero and housed in a well ventilated pen with straw bedding for 0-4 days and fed colostrum @ 1/10th of body weight twice daily by pail feeding per day. Then on 5th day calves were shifted to another pen, where kept upto one month and fed solely with pooled whole milk @ 1/10th of body weight per day twice daily.

Disease diagnosis

Both diarrhoeic and pneumonic calves were diagnosed based on clinical signs. Diarrhoea was diagnosed when a dull and weak calf had frequent defecation, profuse watery faeces with or without mucous/blood, >8% dehydration (sunken eyes, loss of skin elasticity). A calf was diagnosed as pneumonic when he/she showed some or all of the symptoms like fever, lack of appetite, ocular/nasal discharge, dullness, dysponea, increased pulse and respiration, cough, enlarged mandibular lymph node and moderate rales on chest auscultation.

Blood sampling, plasma metabolites and mineral estimation

Approval of Institutes Animal Ethics Committee (IAEC) was obtained for blood collection in the experimental calves. Blood samples were collected using 9 ml blood collection tubes with heparin as anticoagulant (Vacutte®, Griner Bio-one GmbH, Austria) from jugular veinpuncture between 8.30 - 9.30 AM before milk feeding or treatment. Samples were immediately centrifuged at @3000 rpm for 20 minutes at 4°C and plasma samples were separated, stored in 2 ml capacity cryovials (Tarson, India) at -20 °C until further analysis.

Plasma metabolites; glucose (O-Toluidine end point method, Hultman, 1959), urea nitrogen (Rahmatullah and Boyde, 1980), cholesterol (ferric chloride-sulphuric acid method, Zlatkis *et al.*, 1953), NEFA (modified copper soap extraction method, Shipe *et al.*, 1980) were estimated. Plasma minerals except phosphorus were estimated with the help of Atomic Absorption Spectrophotometer (Philips PU 9100X). Plasma inorganic phosphorus was estimated by standard method given by Fiske and Subbarow (1925).

Statistical analysis

The variation of plasma metabolites and minerals were compared by one way analysis of variance (ANOVA) and mean difference between two groups was compared by 'Duncan' post hoc test and considered as significant when $P \leq 0.05$. Further, accuracy of discrimination power of plasma metabolites and minerals to differentiate healthy and diseased calves was estimated by Receiver Operating Characteristic (ROC) analysis (Patbandha *et al.*, 2013). The accuracy in ROC analysis is interpreted based on the area under curve (AUC), which is a 2 dimensional graph (Sensitivity and 1 - Specificity plotted in Y-axis and X-axis, respectively). The AUC must be >0.5 , otherwise it is called non-discriminative or non-informative. If the AUC is 0.5-0.7 then the diagnostic test has low accuracy, if 0.7-0.9 then moderate accuracy, if 0.9-1.0 then high accuracy and if 1 then it is considered perfect (Swets, 1988; Patbandha *et al.*, 2013).

Results and Discussion

Plasma metabolic profiles

Plasma metabolic profile of healthy, diarrhoeic and pneumonic calves is presented in Table 1. There was significantly lower glucose level in calves with diarrhoea and pneumonia as compared to health calves ($P \leq 0.05$). Hypoglycaemic condition observed in our study is in agreement with previous reports in calf diarrhoea (Mansour 2006; Saleh 2006; Chalupnic *et al.*, 2012; Pekcan *et al.*, 2012) and pneumonia (Sayed *et al.*, 2002; Soltesova *et al.*, 2015). Hypoglycemia condition in diseased calves might be attributed to anorexia condition or less mobilization of glucose from glycogen due to less storage or lower synthesis of glucose by gluconeogenesis (Klein *et al.*, 2002; Pekcan *et al.*, 2012). In diseased animals, anorexia is an

adaptive mechanism to combat the disease (Hart 1988). We observed lower plasma cholesterol level in pneumonic calves only but in diarrhoeic calves the concentration was similar to that of healthy calves ($P \leq 0.05$) which is in accordance with the previous reports (Civelek *et al.*, 2007; El-Bahr and EL-Deeb, 2013; Almujalli *et al.*, 2015). The concentration of plasma cholesterol might be associated with inflammatory process or lower secretion in live owing to liver dysfunction (Civelek *et al.*, 2007). Lower glucose level in diseased calves may reduce secretion of cholesterol (Mapiye *et al.*, 2010). In consonance with Soltesova *et al.*, (2015), we did not observe any variation of BUN and NEFA in diseased calves than healthy calves. Although NEFA: cholesterol ratio is a sensitive indicator of energy status in dairy cows but did not show any variation in neonatal calf diarrhoea and pneumonia.

Plasma mineral profiles

Plasma mineral profile of healthy, diarrhoeic and pneumonic calves is depicted in Table 2. Similar to plasma glucose, Ca level in diarrhoea and pneumonia condition decreases significantly compared to health calves ($P \leq 0.05$). Lower plasma Ca observed in diarrhoea and pneumonia condition is in agreement with other reports (Cabello and Michel 1977; Walker *et al.*, 1998; Ragbetli *et al.*, 2010). Cabello and Michel (1977) reported lower concentration of Ca in diarrhoeic calves compared to healthy counterparts. However, Walker *et al.*, (1998) observed lower level of Ca in experimentally induced diarrhoeic calves after 48 hours of induction than before induction. Although, information regarding circulating mineral profiles in calf diarrhoea condition is available, in case of calf pneumonia condition such informations are very less. Ragbetli *et al.*, (2010) stated lower Ca level in pneumonic calves compared to healthy. Impaired placental transfer of Ca

before birth or absorption through intestine after birth may lower plasma level in diseased calves (Cabello and Michel 1977). In pneumonia condition, plasma Zn level remained significantly lower than healthy ones ($P \leq 0.05$) which is in consonance with Soltesova *et al.*, (2015), who observed lower Zn in pneumonic calves. In addition, in human children pneumonia is associated with lower level of Zn (Arora *et al.*, 2007). Further,

plasma Cu level was significantly lower in diseased calves compared to healthy counterparts ($P \leq 0.05$). Ghergariu and Kadar (1979) and Ranjan *et al.*, (2006) reported lower Zn level in diarrhoeic calves than healthy calves. However, plasma P and Mn values were similar between healthy and diseased calves. Lower circulating minerals might be attributed to lower intake due to anorexia condition (Soltesova *et al.*, 2015).

Table.1 Plasma metabolic profiles of healthy, diarrhoeic and pneumonic calves

Plasma minerals	Healthy (n=96)	Diarrhoea (n=32)	Pneumonia (n=32)
Glucose (mg/dl)	$83.61^a \pm 0.63$	$51.91^b \pm 1.25$	$52.77^b \pm 1.40$
BUN (mg/dl)	$28.24^a \pm 0.36$	$27.75^a \pm 0.54$	$28.10^a \pm 0.69$
Cholesterol (mg/dl)	$99.88^a \pm 1.29$	$95.52^a \pm 1.80$	$90.46^b \pm 1.55$
NEFA (μ mol/L)	$222.61^a \pm 9.61$	$195.49^a \pm 15.86$	$194.19^a \pm 17.33$
NEFA/Chol	$0.02^a \pm 0.00$	$0.02^a \pm 0.00$	$0.02^a \pm 0.00$

Means with different superscript within a row differed statistically ($P \leq 0.05$)

Table.2 Plasma mineral profiles of healthy, diarrhoeic and pneumonic calves

Plasma minerals	Healthy (n=96)	Diarrhoea (n=32)	Pneumonia (n=32)
Ca (mg/dl)	$12.39^a \pm 0.07$	$12.03^b \pm 0.11$	$11.75^c \pm 0.10$
P (mg/dl)	$5.74^a \pm 0.12$	$5.53^a \pm 0.19$	$5.30^a \pm 0.15$
Zn (ppm)	$1.37^a \pm 0.02$	$1.29^a \pm 0.03$	$1.18^b \pm 0.04$
Cu (ppm)	$1.07^a \pm 0.02$	$0.93^b \pm 0.03$	$0.86^b \pm 0.04$
Mn (ppm)	$0.35^a \pm 0.01$	$0.36^a \pm 0.02$	$0.35^a \pm 0.02$

Means with different superscript within a row differed statistically ($P \leq 0.05$)

Figure.1 Area Under Curve (AUC) of plasma metabolites during calf diarrhea

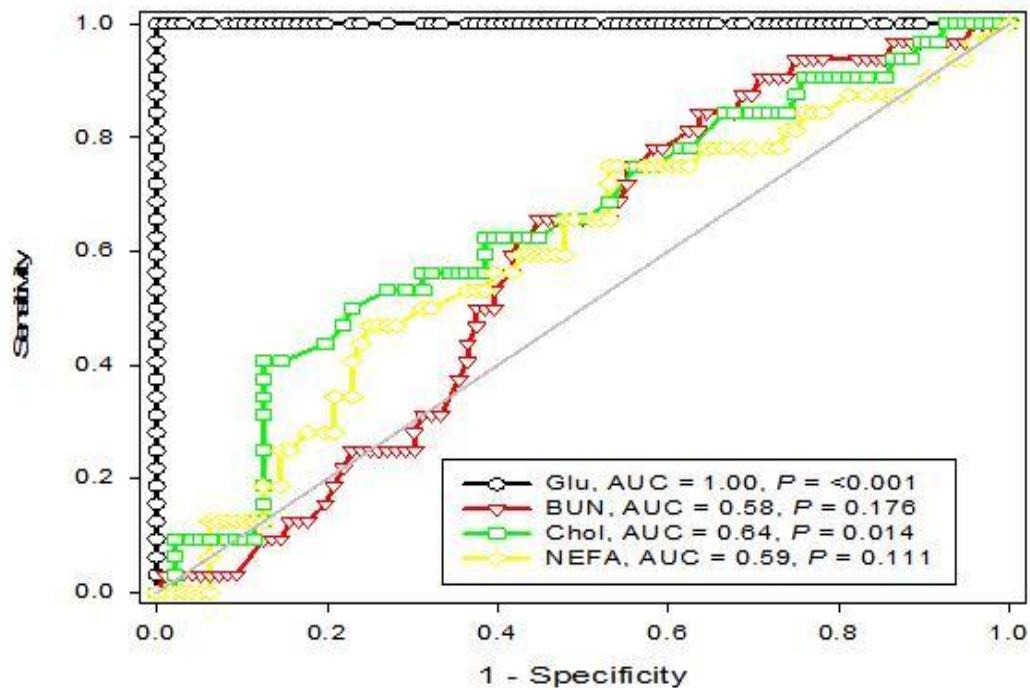


Figure.2 Area Under Curve (AUC) of plasma metabolites during calf pneumonia

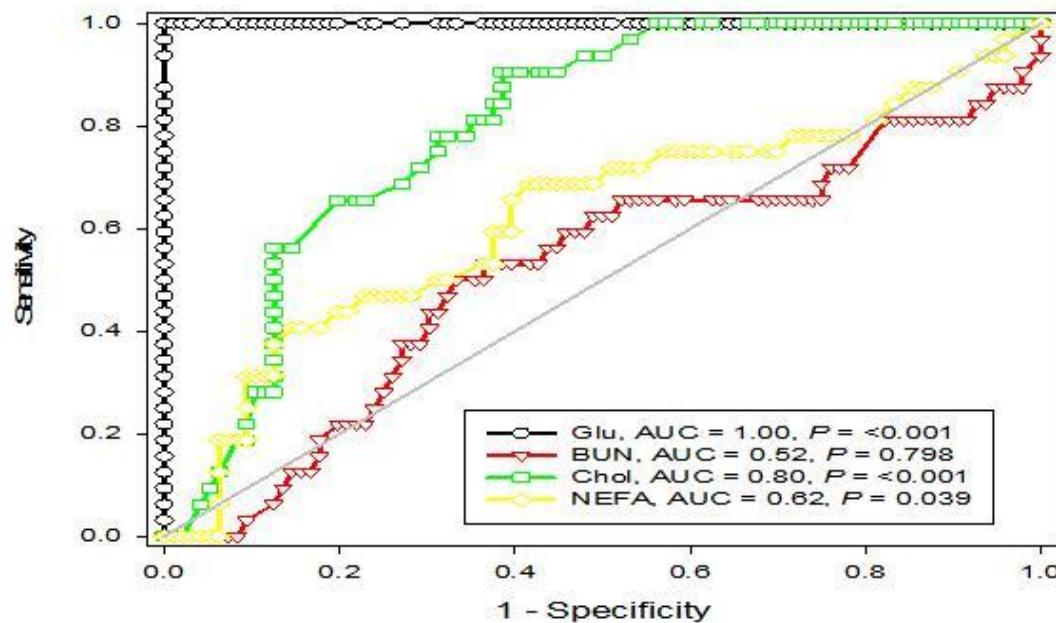


Figure.3 Area Under Curve (AUC) of plasma minerals during calf diarrhea

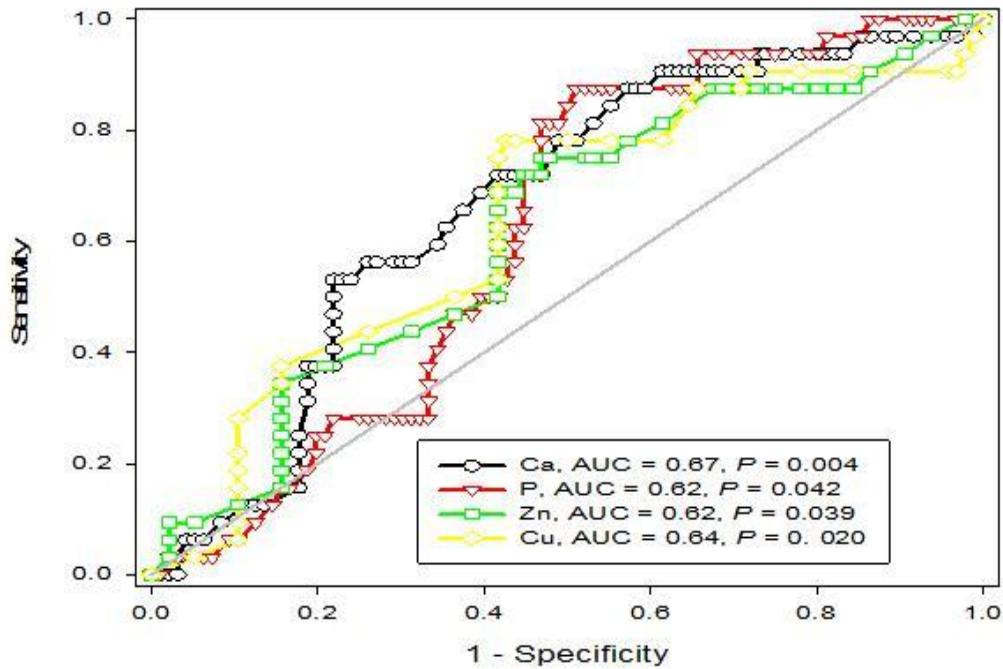
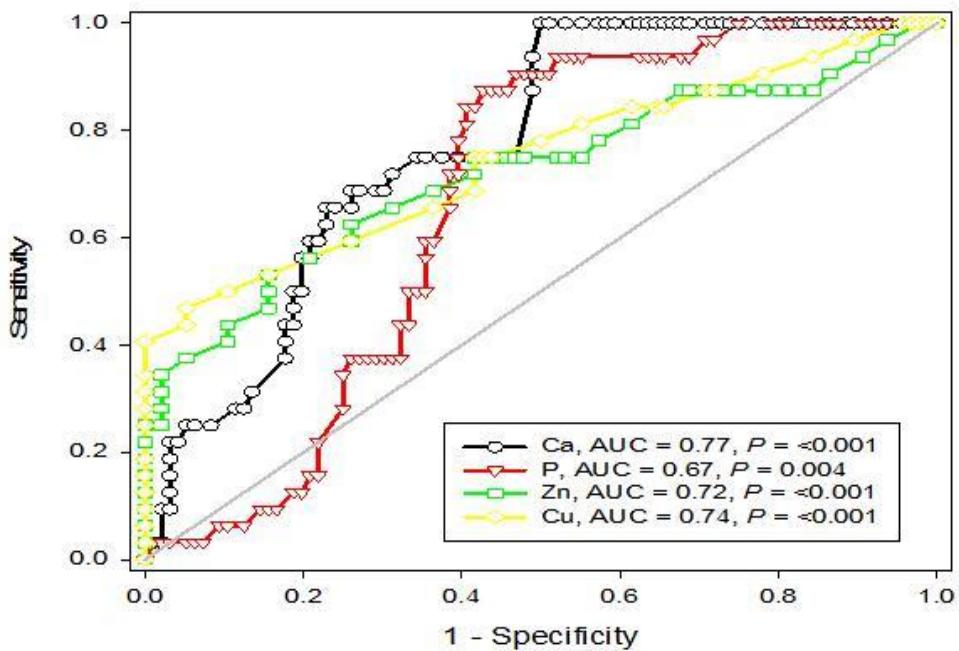


Figure.4 Area Under Curve (AUC) of plasma minerals during calf pneumonia



Accuracy of plasma metabolites and minerals

Area under the curve (AUC) is considered as good measure to assess accuracy of diagnostic markers to differentiate diseased and healthy animals as it does not depends on the prevalence of disease (Kumari *et al.*, 2016). Higher the AUC value of a diagnostic marker, then higher is the accuracy to differentiate diseased animals from their healthy counterparts (Swets, 1988; Patbandha *et al.*, 2013). The AUC of the plasma metabolites to discriminate diseased calves from healthy ones are presented in figure- I (diarrhoea condition) and figure- II (pneumonia condition). Further, AUC of the plasma minerals to discriminate diseased calves from healthy ones are depicted in figure- III (diarrhoea condition) and figure- IV (pneumonia condition). Among the different metabolites, plasma glucose differentiated diseased calves (both diarrhoea and pneumonia) from their healthy counterparts with 100% accuracy (AUC=1.0). In this study, 100% accuracy indicated that all calves affected with either diarrhoea or pneumonia had lower level of plasma glucose. On the other hand, none of the plasma minerals had higher accuracy to differentiate diseased and healthy calves (accuracy maximum 67% in calf diarrhoea i.e. AUC=0.67 and 77% in calf pneumonia i.e. AUC=0.77). The lower intake owing to anorexia condition may lead to hypoglycaemic condition in diseased calves. Hence, by proper supportive therapy could ameliorate hypoglycaemic condition in diseased calves.

Taken together it may be concluded that hypoglycaemia condition occurred in both calf diarrhoea and pneumonia condition. Plasma glucose was 100% accurate to discriminate diseased calves from their healthy counterparts.

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