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Gagandeep Singh
Regional Research and Training Centre of Guru Angad Dev Veterinary and Animal Sciences University, Talwara, Hoshiarpur, Punjab, India

Randhir Singh
Department of Teaching Veterinary Clinical Complex, GADVASU, Ludhiana, Punjab, India

SNS Randhawa
Department of Veterinary Microbiology, Khalsa College of Veterinary and Animal Sciences, Amritsar, Punjab, India

Corresponding Author:
Gagandeep Singh
Regional Research and Training Centre of Guru Angad Dev Veterinary and Animal Sciences University, Talwara, Hoshiarpur, Punjab, India

Metabolic profiling of dairy cattle during transition period: A review

Gagandeep Singh, Randhir Singh and SNS Randhawa

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Abstract
The periparturient period in dairy cows is generally defined as the period from last three weeks of gestation to first three weeks after parturition and is considered as most important period for the subsequent lactation performances, due to the fact that as during this period, dairy cattle are at a high risk for most of the metabolic diseases, which is characterized by changes in their endocrine status and a reduction in feed intake when nutrient demand for the developing fetus and for the initiation of lactogenesis is increasing. Metabolic profiling is defined as the series of specific analytic tests run in combination and used as a herd based rather than individual based. Using metabolic profiling references ranges can be established for various parameters (Hb, PCV, TEC, TLC, MCV, MCH, MCHC, protein, albumin, BUN, creatinine, glucose, Ca, Mg, Pi, Na, K, Cu, Fe and Zn) which can act as a guide in the future for the advance diagnosis, prediction and management of various metabolic diseases.

Keywords: Dairy cows, metabolic, various parameters

Introduction
The concept of production diseases was elaborated by J. M. Payne (Payne, 1972) [1] and defined as group of conditions which were previously termed as metabolic diseases, some of which were very well known and extensively studied but others not so, with majority being due to deficit in input and output, or, in other words, to inadequate nutritional intake necessary for production. In broader terms, production diseases can be termed as man-made problem resulting from breakdown of the various metabolic systems of the body under the combined strain of high production and modern intensive husbandry.

As of now, production diseases include various infectious and non-infectious diseases thus bringing it under a broad classification. Roots of production diseases lie in the interaction between the animal's physiology, nutrition, production and the environment in which it lives. In addition to the traditional metabolic diseases in dairy cows like Parturient paresis and ketosis, production diseases now include subclinical acidosis, mastitis, endometritis, retained placenta and other post parturient reproductive disorders, and locomotive abnormalities inducing lameness, such as laminitis and digital dermatitis.

Transition Period and negative energy balance (NEB)
Negative energy balance (NEB) is a normal physiological phenomenon in dairy cattle during the physiological transition from late gestation to early lactation. This transition period is often considered to occur from three weeks' pre-partum to three weeks' post-partum (Drackley, 1999) [2], as during this period major homeorhetic regulation of various metabolic functions is necessary in order to accommodate additional demands of parturition and lactogenesis (Bauman and Currie, 1980) [3]. Recent, studies have demonstrated that adequate nutritional management during early dry period is pivotal for maintaining health and productivity of transition cows. (Dann *et al.* 2003) [4]. The major factor for NEB during a time of increased demand is the restricted availability of energy sources due to decreased dry matter intake (DMI) (decreases upto 30%) in the last 3 weeks of gestation. Maintaining health and productivity in dairy cows during the transition period is most important task for dairy farmers. Approximately 75 per cent of the diseases in dairy cows typically occur in the first month after calving (Leblanc *et al.,* 2006) [5]. During this period, dairy cattle are at a high risk for most of the metabolic diseases which is characterized by marked changes in their endocrine status that are much more dramatic than at any other time in the lactation–gestation cycle,

and a reduction in feed intake when nutrient demand for the developing conceptus and the impending lactogenesis are increasing (Grummer, 1995) [6]. Most commonly acetonemia, fat cow syndrome, sub-clinical hypocalcemia, peri-parturienthaemoglobinuria and hypophosphatemia are the major disease states during this transition period. Apart from the direct effect of these, there are also indirect effects which make an animal more prone to infections of udder and uterus, reduce fore stomach motility, and decrease appetite leading to decreased milk production.

In order to monitor, detect and predict such diseases, Metabolic Profiling was first developed by Payne *et al* (1970) [7]. This metabolic profiling is defined as series of specific analytic tests run in combination and used as a herd based rather than individual based diagnostic aid (Ingraham and Kappel, 1988) [8]. The original intent of metabolic profiling was to monitor the success of current management, early detection of problems or deviation from the management program, to identify the cows at high risk for disease and to develop intervention for preventing the clinical disease. Another driving force for renewed metabolic profiling has been increased awareness of the critical role of peri-parturient disease in dairy farm profitability. Peri-parturient disease-associated culling and reproductive infertility, coupled with the obvious health and production concerns, are driving interest in predicting potential risk for disease. However, most work in this area has focused on specific disease entities (i.e., milk fever, ketosis, left displaced abomasum); but it is well documented that peri-parturient diseases are interconnected and no single entities. As an example, dairy cattle that develop clinical hypocalcaemia (milk fever) are eight times more likely to develop mastitis (Curtis *et al*., 1983) [9]. Similarly, cows with sub-clinical ketosis are eight times more likely to develop left displaced abomasum (Le Blanc *et al*, 2005) [10].

This metabolic profiling is done by comparing the average concentrations of blood constituents viz. haemoglobin (Hb), packed cell volume (PCV), Total erythrocyte count (TEC), total leucocyte count (TLC), Differential leucocyte count (DLC), glucose, blood urea nitrogen (BUN), total plasma proteins (TPP), albumin, cholesterol, alkaline phosphatase (ALKP), calcium (Ca), inorganic phosphorus (Pi), magnesium (Mg), potassium (K), sodium (Na), copper (Cu), zinc (Zn) and iron (Fe), of a group of cows to the defined mean concentration values for the creation of reference values which will help in future for the advance diagnosis of various diseases and for studying the herd metabolic status. Now a days' a more holistic approach has gained attention which includes evaluation of body condition score (BCS), ultrasound guided back fat thickness measurement (BFT) along with metabolic profiling in order to gain a better insight into fat mobilization due to NEB.

Body condition score (BCS) and back fat thickness (BFT) in relation to metabolic profiling

The body condition score (BCS) is a subjective technique for assessing the condition of livestock at regular intervals. It is typically helpful in evaluating the body fat reserves of dairy animals by visual and manual inspection of the thickness of fat cover and prominence of the bone at particular points (Figure 1). Being non-invasive, quick and inexpensive the BCS system is accepted universally to estimate the degree of fatness (Bittante *et al*., 2004) [11]. It is particularly useful as an aid to dry cow and pre calving management with the main aim that the cows calve down safely and enter the lactation stage uneventfully. It is a well-known fact that the dairy cows utilize body energy reserves in the early lactation to cope up with NEB and thus BCS along with measurement of BFT by using real time ultrasound are more promising approaches to ensure an uneventful transition of dairy cows. Various studies on the precision of BCS system including the ultrasonographic assessment of subcutaneous back fat (Figure 2) indicated that BCS values were closely related to the actual measurement of subcutaneous fat (Lubis and Fletcher, 1985; Zulu *et al*., 2001) [12, 13]. As a thumb rule cows should calve with body score between 3.0 and 3.25 (5-point; equivalent to 5.0 to 5.25 on an 8-point scale and 5.0 to 5.5 on a 10-point scale). Cows having very low (<2.5) or very high BCS (>3.5) are prone to various production diseases compared to good (3±0.5) BCS cows. An increased risk of subclinical ketosis was observed when back fat thickness (BFT) was greater than 12 mm. For practical purposes if the average BFT in a herd is 12 to 14 mm 2 to 3 weeks before calving, cows should be evaluated for feed intake and diet should be evaluated for energy density (Scroder and Staufenbeil, 2006) [14].

Benefits of Metabolic Profiling

I. **Assessing nutritional status of feeding group or herd:** Using specific parameters known to be responsive to dietary intake, metabolic profiling, can be used for the dietary evaluation of current feeding program or a response to a feeding program change.
II. **Identifying disease conditions at an early stage:** Using metabolic profiling various subclinical metabolic disease conditions (ketosis, hypocalcemia, hypomagnesemia, subacute ruminal acidosis [SARA], respectively) can be identified at an early stage by using defined analytes (β-hydroxybutyrate [BHB], calcium [Ca], magnesium [Mg], rumen pH) in the absence of obvious clinical disease problems.
III. **Identifying individual animals who are at an increased risk of developing disease:** Using metabolic profiling, blood analytes that are either high or low relative to defined reference or cut point values before calving or immediately postpartum can be helpful in predicting potential for increased risk of experiencing specific or collective peri-parturient disease events.
IV. **Identifying herd based problems:** Metabolic profiling can be used as a screening tool for the studying the herd metabolic status and for identifying herd related problems.

Sampling Strategies

For the investigation of transition cow problems, blood samples collected from the cows just before and after calving are the most diagnostic. As a result of tremendous individual variation cows should not be sampled within 3 days before or following calving. As per the experience of authors it is recommended to first carry out BCS and BFT measurements of each animal to be sampled and sampling should be carried out as per stage of transition period i.e.,

Far off Dry (FOD): > 10 days following dry off and not less than 30 days prior to calving.

Close–up Dry (CUD): Between 3 and 21 days prior to calving.

Fresh: 3- 30 days in milk.

If the animals are at higher risk of developing fatty liver/ hepatic lipidosis (HL) concurrent sonography of liver is also indicated for early diagnosis and treatment strategies.

Individual Sampling: Samples from individual animal during the transition period is collected thrice during different periods (FOD, CUD and Fresh) for the estimation of various parameters of metabolic profiling and is compared with the standard reference ranges. With the help of this animals whose, blood parameters are showing abnormal values can be diagnosed for the subclinical metabolic diseases and can be treated at proper time, whereas, all other animals which were normal during the transition period and did not develop any abnormality during the later lactation period and their blood metabolites did not showed any deviation from the normal ranges, data from such animals is used for the establishment of normal reference values for the future.

Pooled Sampling: Instead of doing analysis from individual samples, pooled samples can be used to reduce the cost and provide some valid method of herd assessment. Equal amount of samples should be included from each individual in the pooled sampling procedure. Depending upon the total numbers of animals included, normally 100-500 ul of sample (plasma/serum) should be taken from individual animal and mixed into a new clean glass test tube (Van Saun, 2005) [15].
Sampling time should be selected depending upon the parameters of interest. If NEFA is of primary interest than the sampling should be done before the first feeding, where as if BHBA and BUN are of our interest than sampling should be done 3-5 hours after the first primary feeding bout (Hoff and Duffield, 2009) [16].

Analyte selection: The parameters to be determined can be selected as:

Energy Balance
Energy balance is one of the most important nutritional factors effecting animal health, lactation and reproductive performance. Non esterified fatty acid (NEFA) is generally regarded as the most important parameter in the estimation of energy balance. Concentration of NEFA directly reflects the amount of adipose tissue breakdown. Excessive NEFA concentration during the pre or post-partum period are predictive for increased risk of ketosis, left displacement of abomasum and other peri-parturient diseases (Cameron *et al*., 1988; Dyk *et al*., 1995; Holtenius and Horst, 1990) [17, 18, 19]. High NEFA concentration > 0.4 mmol/L in the 2 weeks before calving is associated with 2 to 4 times increased risk of LDA (Leblanc *et al*., 2005) [10], 1.8 times increased risk of retained placenta (RP) (Leblanc *et al.*, 2005) [10], two times increased of culling before 60 days in milk (DIM) and 1.5 times increased risk of culling over the whole lactation (Duffield *et al.*, 2009) [20] and 1.2 kg/day milk production loss for the 1st 120 days of lactation (Carson, 2008) [21].
β-hydroxybutyrate (BHBA), one of the ketone bodies is another important parameter useful in assessing energy status. Before calving BHBA is not of diagnostic value, however after calving excessive BHBA values are predictive for periparturient disease problems (Vansaun, 2006) [22]. Subclinical ketosis (BHBA > 1.2-1.4 mmol/L) in early lacatation is associated with 3 to 8 times increased risk of LDA (Geishauser *et al.*, 2000; Leblanc *et al*., 2005; Duffield *et al*., 2009) [10, 20, 23], decreased probability of pregnancy at first AI (Walsh *et al.*, 2007) [24], decreased milk production (Duffield *et al.*, 2009) [20], increased duration and severity of mastitis (Suriyasathaporn, 2000) [25]. Similarly, blood glucose concentration independently is not of any diagnostic value, however blood glucose concentration measured in conjunction with other parameters may provide some further insight into the underlying mechanisms of disease.

Defining NEFA and BHBA cut points
In recent years, there has been a tremendous research in transition cow biology and it had subsequently lead to various studies determining the effective cut points of NEFA and BHBA for predicting various production/ metabolic diseases particularly SCK. Multiple studies have documented that the pre-partum NEFA cut-points with the highest sensitivity and specificity for the prediction of post-partum health problems ranged from 0.3 mEq/L to 0.5 mEq/L (LeBlanc *et al*., 2005; Ospina *et al*., 2010, Chapinal *et al*., 2011; Roberts *et al*., 2012) [26, 27, 28, 29]. Likewise, post-partum NEFA cut-points for the prediction of post-partum health problems ranged from 0.70 to 1.0 mEq/L (LeBlanc *et al*., 2005; Ospina *et al*., 2010; Chapinal *et al*., 2011; Roberts *et al*., 2012) [26, 27, 28, 29]. The single most pre-partum BHBA cut-point reported was by Chapinal *et al*., (2011) [27], who reported that pre-partum mean BHBA concentration of 0.8 mmol/L was highly associated with post-partum problems (McArt *et al*., 2013) [30]. Postpartum BHBA concentration cut-points that maximize accuracy of disease prediction and production measures range from 0.9 mmol/L to 1.6 mmol/L with the majority between 1.2 and 1.4 mmol/L (LeBlanc *et al*., 2005; Walsh *et al*., 2007; Duffield *et al*., 2009; Ospina *et al*., 2010; Chapinal *et al*., 2011; Roberts *et al*., 2012) [10, 20, 26, 27, 28, 31],. BHBA concentration of ≥ 1.2 mmol/L is used as standard for diagnosing SCK. Similarly in our study Singh *et al*. (2018) [32], a significant decrease was redcorded in milk yield in subclinical ketotic cows having blood BHBA levela >1.2 1.2 mmol/L as compared to healthy cows (<1.2 1.2 mmol/L) during the fresh period in these cows.

Protein Evaluation
Evaluating protein status in dairy animals is not as easy as that of energy balance because there are number of parameters such as BUN, creatinine, total protein, albumin, and creatinine kinase which needs to be measured for accessing the protein status, and also there are number of interrelated factors which influences the concentration of a parameter in blood for example BUN concentrations are influenced by a wide variety of interrelated parameters such as: dietary protein intake, rumen degradability, dietary amino acid composition, protein intake relative to requirement, liver and kidney function, muscle tissue breakdown, dietary carbohydrate amount and rumen degradability, similarly total protein and albumin concentration reflects protein availability and their concentration decline in the face of protein deficiency. Creatinine is used to evaluate renal function and its impact on BUN values. Creatine Kinase is released from muscle when it is catabolized or injured. Fresh cows that could maintain serum albumin concentaions ≥ 3.5 g/dl were less likely to have postpartum disease. Similarly, serum albumin concentration ≤3.25 g/dl in close up dry cows resulted in a threefold greater risk for post-partum diseases (Van saun, 2005) [15]. Close up dry cows will generally have low to moderate BUN, lower albumin and elevated CK values. Fresh cows generally have low BUN and low albumin (< 3.0 g/dl) (Van saun, 2005) [15]. (Table 1).

Evaluation of hepatic function
Fatty liver is a production disease of dairy animals in

transition affecting almost half of the herd immediately after calving (Jorritsma *et al.*, 2000) [33]. It occurs most commonly within first 2 weeks after parturition, usually in association with other metabolic diseases. Occasionally it appears before calving. It is characterized by accumulation of fat (lipid) in the liver. The build up of fat in majority of affected cases is within 10 days of calving and ranges from 60 to 125 g per day (Ametaj *et al.*, 2002) [34]. Mild cases of fatty liver are associated with reduced fertility and severe cases with increased culling, disease and death. Liver function can be accessed through a variety of enzymes (gamma-glutamyltransferase [GGT], aspartate aminotransferase [AST] and sorbitol dehydrogenase [SDH], Lactate dehydrogenase (LDH), Glutamyl dehydrogenase (GDH), triglycerides (Tg) and total bilirubin concentrations in the blood. Bilirubin values are most specific to bile flow problems than overt liver cell damage. These enzyme values need to be interpreted in conjunction with total cholesterol and NEFA results. Fatty liver/ hepatic lipidosis (HL) is usually diagnosed by taking a liver sample through biopsy and measuring the amount of total lipids in the liver. Biopsy being an invasive technique involving risk of bleeding and infection is now a day's widely replaced by use of ultrasound. Results of our recent study (Singh *et al.*, 2017) [35] in both cows and buffaloes during different transition stages in Punjab show that ultrasound can be used in both species to diagnose HL with promising results (Figure 3). The results of ultrasound correlated well with various metabolic parameters like NEFA, BHBA along with concentration of liver enzymes in serum like AST, ALP, GGT, LDH, GDH and triglycerides.

Macro mineral Evaluation

Macro minerals such as calcium, phosphorus, potassium, magnesium, sodium, chloride and sulfur are regularly assessed in transition cows due to their role in the causation of milk fever, alert downer cows, and weak cow syndrome. As, number of homeostatic mechanisms are controlling these minerals, so the blood concentrations of these macro minerals are not reflective of dietary status when the homeostatic system is functioning properly, whereas, phosphorus, potassium, magnesium and sulphur are macro minerals in which blood concentrations are somewhat sensitive to dietary intake (Herdt *et al*., 2000) [36]. Sodium and chloride concentrations are altered when renal or digestive function is compromised or in extreme dietary deficiency states. Evaluating Ca concentrations during the transition period is an important indicator of assessing that how well the Ca regulatory system is working and is also helpful in the advance diagnosis of clinical or subclinical hypocalcemia problems (Oetzel, 2004) [37]. Other than the 2 weeks prior to and following calving, blood Ca is not a very diagnostic value as a result of the intact regulatory system. (Ghergariu *et al*, 1984) [38]. Therefore, macro mineral blood concentrations will need to carefully interpret in light of whether or not the homeostatic system is in proper operation. Van saun (2005) [15] in his study reported that cows with serum calcium concentration < 8.0 mg/dl during the pre or post-partum period are 4 times more likely to have postpartum disease problems. Further studies are needed to identify the relationship between minerals concentrations and peri-parturient diseases (Table 2).

Micro mineral and Vitamin Evaluation

As the trace minerals and fat-soluble vitamins are not in a single large pool in the body, but are distributed into a number of different pools including storage, transport, and biochemical function pools (Suttle, 1986) [39]. As a result of this moderate dietary deficiencies or short-term severe deficiencies can be overcomed without any effect on the critical biochemical functions, however if the dietary insult is severe or prolonged enough to drain the storage pool, then some effects might be seen in the transport pool. Finally, when the transport pool has been compromised, the biochemical function pool will be compromised resulting in some dysfunction. It is only when the biochemical function pool reaches a critically low level that we see the overt clinical deficiency diseases (Van Saun, 2005) [15]. Before we reach the clinical disease stage, we will see problems associated with subclinical disease including increased disease susceptibility as a result of compromised immune function. Associations between trace mineral concentrations and risk of peri-parturient disease were minimal, though high pre-partum iron and low postpartum zinc concentrations tended to be associated with infectious disease problems (Van saun *et al*, 2006) [22]. Ratio of copper to zinc or iron, potentially reflecting changes indicative of an acute phase inflammatory response, tended to be associated with increased mastitis and metritis risk. There are number of (Enjalbert *et al.*, 2006); Zhang *et al.*, 2010) [40, 41] studies reporting the relationship between the micromineral status in the body and peri-parturient diseases

Summary

With the increasing herd size and the increase in the number of high yielding cattle in India, regular assessment of dairy herds performance has become an important part of dairy farming. Metabolic Profiling has been developed as a new tool in the evaluation of dairy herd performance. This metabolic profiling along with the ultrasonography at individual cow level helps in identification of animals which are at increased risk of developing clinical and subclinical form of metabolic diseases and at herd level helps in to study the herd metabolic status and at to identify metabolically superior cows which can be further used in subsequent lactations.

References Values

Table 1: Normal range of various blood parameters over the peri-parturient period for healthy mature dairy cows (Van saun, 2009)

Analyte	Close up Dry	Fresh
Albumin (g/dl)	3.3-3.7	3.2-3.6
AST(IU/L)	46.5-82.6	61.1-103
BHBA (mg/dl)	1.25-4.2	1.7-8.9
Cholesterol (mg/dl)	65-114	63-253
Glucose (mg/dl)	51-74	42-68
NEFA (mEq/L)	0.03-0.46	0.01-0.52
Total protein (g/dl)	6.9-8.5	7.3-8.9
NEFA to Cholesterol ratio	0.03-0.2	0.03-0.4

Table 2: Fresh dairy animal mineral concentrations in healthy population and concentration that are of concern for potential disease risk (Van saun, 2009)

Analyte	Adequate range	Concern level
Calcium (mg/dl)	8.7-11	<8
Phosphorus (mg/dl)	4.5-8	<3.5
Magnesium (mg/dl)	2-3.5	<1.5
Sodium (mEq/L)	137-148	<137
Potassium (mEq/L)	3.8-5.2	<3 or >5.5
Copper (ug/ml)	0.6-1.5	<0.45
Zinc (ug/ml)	0.8-1.4	<0.5

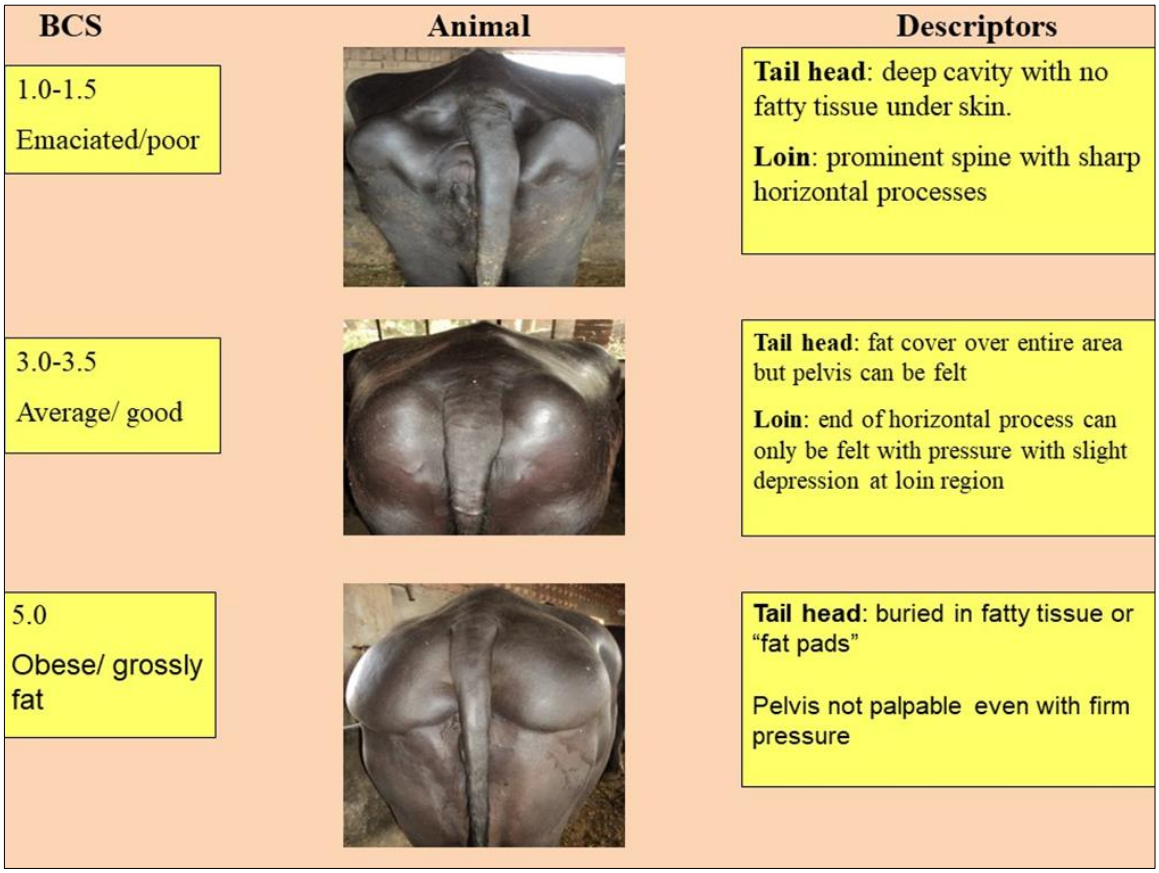

Fig 1: Body condition score along with principal descriptors in transition buffaloes (Singh *et al.*, 2017) [35]

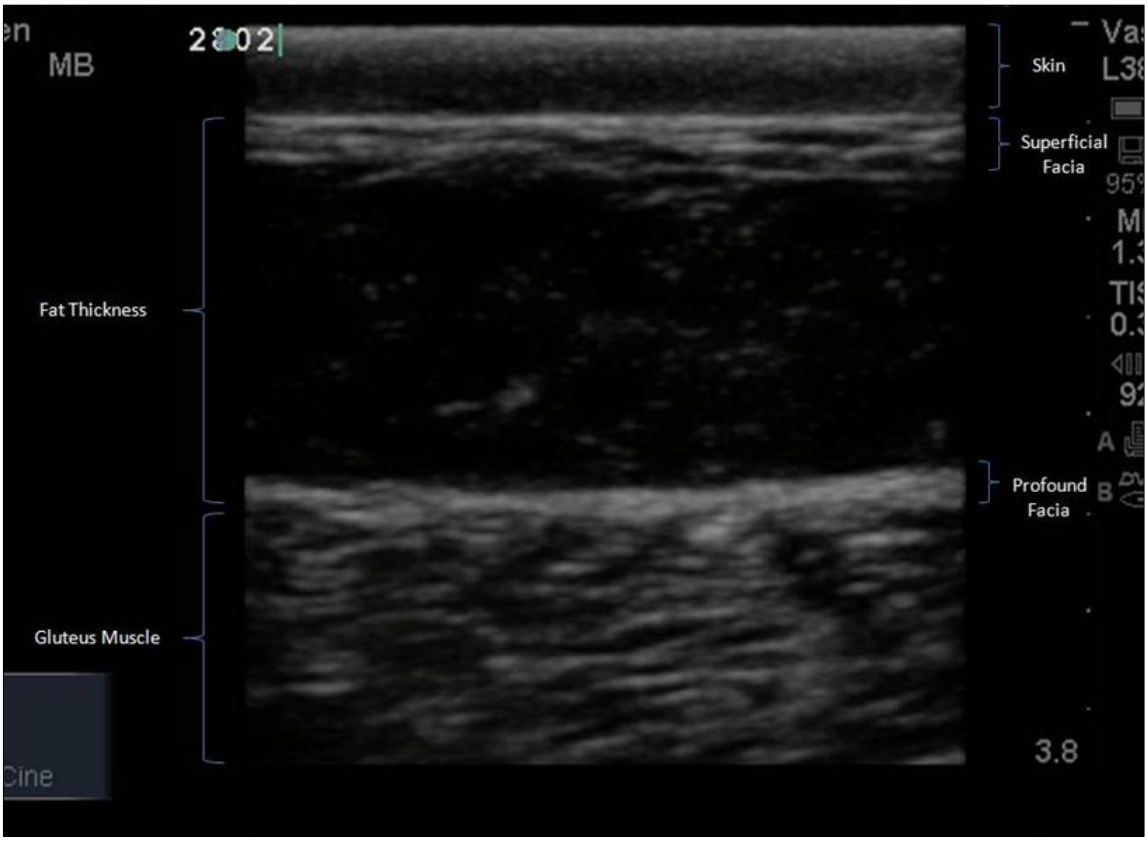

Fig 2: Measurement of back fat thickness (BFT) using real time ultrasound (Singh *et al.*, 2015)

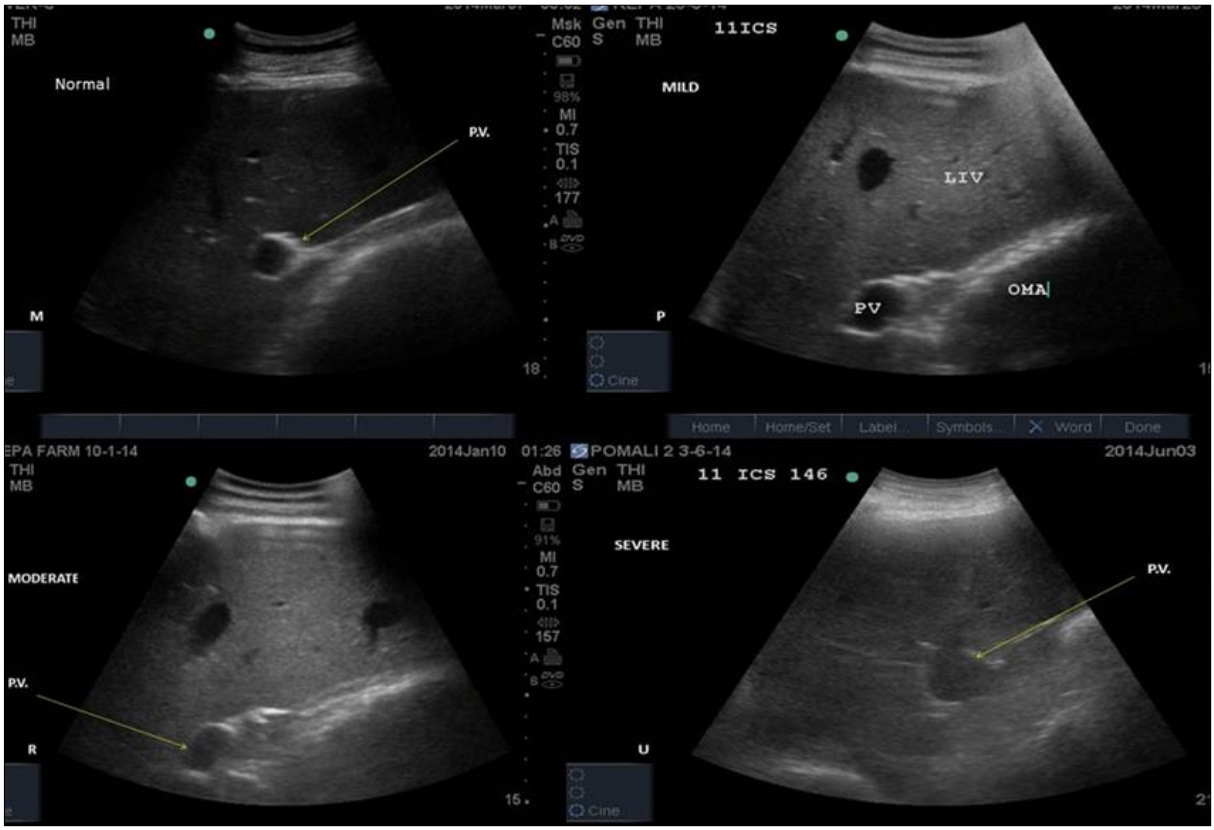

Fig 3: Diagnosis and grading of hepatic lipidosis in dairy cows using ultrasonography (Singh *et al.*, 2017) [35]

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