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INDIRECT PASSIVE TRANSFER EVALUATION TECHNIQUES IN CALVES: A REVIEW

TEHNICI INDIRECTE DE EVALUARE A TRANSFERULUI PASIV DE ANTICORPI LA VIȚEI: RECENZIE

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ABSTRACT | REZUMAT

Judging by the histological type of placenta in ruminants, it has been determined that calves are naturally born with a low γ-globulin (IgG) level. Further deficient absorption of γ-globulins through colostrum intake is defined as failure of passive transfer (FPT). Current literature scrutinises the impact of this phenomenon on the overall economic setting of the farm. The unfulfillment of immunological transfer at this stage has been linked with higher mortality rates, higher infectious susceptibility, lower growth performance, and inferred financial losses. Several methods have been proposed for FTP evaluation in calves, but only a few of them are suitable for calf-side use. Brix refractometry (BR), Gamma glutamyl transferase (GGT) activity, and serum IgG levels measured with zinc sulphate or sodium sulphite turbidity tests (SSTT/ZSTT) are some of the reviewed techniques in this article. The aim of this paper is to gather data from the latest published research articles in order to determine which methods are relevant for in-farm applications.

Keywords: passive transfer, calves, serum immunoglobulins, serum total protein, serum IgG

Având în vedere tipul histo-morfologic al placentei rumegătoarelor, s-a stabilit că viței se nasc în mod natural cu un nivel scăzut de γ-globuline (IgG). Absorbția deficitară a anticorpilor prin colostru este definită ca deficit al transferului pasiv (DTP). Literatura actuală examinează impactul acestui fenomen asupra cadrului economic general al fermei. Neîndeplinirea transferului imunologic în această etapă a fost corelată cu rate mai mari ale mortalității, susceptibilitate mai mare la infecții și performanțe de creștere mai scăzute cu pierderi financiare implicite. Au fost descrise mai multe metode pentru evaluarea DTP la viței, dar doar câteva metode s-au dovedit a fi fezabile pentru a fi utilizate în teren. Refractometria Brix (RB), activitatea gama-glutamiltransferazei (GGT) precum și evaluarea nivelurilor serice ale IgG măsurate prin testul cu sulfat de zinc sau sulfit de sodiu TSZ/TSS, sunt parte din metodele discutate în acest articol. Scopul acestei lucrări este de a colecta date din cele mai recente publicații pentru a determina metodele relevante pentru a fi aplicate la nivelul fermei.

Cuvinte cheie: transfer pasiv de anticorpi, viței, imunoglobuline serice, proteină serică totală

Due to the synepitheliochorial placenta, the passage of serum proteins such as immunoglobulins is inconceivable, resulting in the calf having insufficient immunity at birth (6). Newborn calves rely strictly on colostrum antibodies. Therefore, failure of passive transfer (FPT) occurs when the neonatal calf fails to absorb adequate colostrum immunoglobulins within the first hours of life. FPT has been defined throughout the level of serum IgG; those lower than 10 g/L in dairy calves are considered to cause high mortality and morbidity rates (7). Subjects that did not exceed this limit have proven to be twice as likely to have diarrhoea and require antibiotic therapy before weaning (4, 7).

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FPT's prevalence has been intensively discussed; a survey conducted in Australia on 3,608 dairy calves considered a 36% rate (33), which is similar to previous findings of 38%–42% (1, 41). These results are in agreement with Elsohaby et al. (2019), who previously set the odds at 43.3% and 46.5% (16).

Consonant findings have been released in Italy by Lora et al. (2018), with 41% of the included calves being subjects to FPT, with IgG serum levels lower than 10 g/L (25).

Strikingly different outcomes have been reported by Aleri et al. (2021), who applied both refractometry for serum protein evaluation and radial immunodiffusion (RID) for IgG levels, setting the prevalence rate to 8.7% and 7.1%, respectively. In light of their results, the authors stated that the good colostrum management from the selected farms and the high quality of it determined such low prevalence rates, which revealed that calf-related factors such as age, sex, and

breed might not be associated with FPT (3). The latter conclusion opposed Vogels et al.'s (2013) allegations, who affirmed that bull calves and dairy-beef cross-breds' calves were more likely to develop FPT than female calves and Holstein-Friesians calves (41). Older papers have considered that sex is directly linked with high prevalence rates of FPT. Bragg et al. (2020) proclaimed that male calves were more prone to FPT (7). These observations have been scrutinised and generally speaking, male calves have a higher rate of dystocia (27). Moreover, through the same multivariable risk analysis, the authors conclude that other aspects are more important, such as colostrum feeding assistance, supplementation, and calving assistance (7).

All the gathered data proves that, no matter the predisposing factors, FPT remains a contemporaneous issue within both dairy and beef cattle farms, with good monitoring of passive transfer efficiency being compulsory at this stage. Therefore, indirect methods that can be easily applied on farms have been reviewed.

GAMMA GLUTAMYL TRANSFERASE (GGT)

GGT is better represented in colostrum in comparison with milk and maternal serum (5). Thus, it is considered a marker for colostrum intake; its levels in neonatal serum rise proportionally to the latter (22). In accordance with these statements, Ganz et al. (2021) used GGT evaluation in order to assess the influence of first colostrum pasteurisation on serum IgG (17). Thus, the stated outcomes contradicted the previous results of Weaver et al. (2000). According to the latter, calves that ingest heat-treated colostrum should not be subjected to GGT evaluation (42). However, Ganz et al. (2021) have proved that feeding pasteurised colostrum does not have an effect on the activity of GGT (17).

Older studies suggest that this test should be applied at certain times during the first 8 days of life in beef calves (43) and the first ten days of life in dairy calves (29). Generally speaking, with levels dropping after 24 hours of age, GGT values can vary significantly after this stage (26). Therefore, false positives for FPT diagnosis may appear secondary to inaccurate age reporting.

In terms of specificity, Hogan et al. (2015) stated that GGT, along with enzyme-linked immunosorbent assays (ELISA), returned rates higher than 98% at the recommended cut-off point (100 IU/L). The same study marked a 97.26% sensitivity when compared to RID results. Conversely, Güngör et al. (2004) admitted obvious differences between RID and GGT results, which may be linked to the quality level of the colostrum per se. Nearly the same considerations were pointed out by Cuttance et al. (2017), their work highlighting only a moderate correlation between IgG concentrations and GGT activity, in accordance with Šlosárková et al. (2014) (10, 35).

Thresholds used vary among different laboratories, according to Cuttance et al. (2017), who presented the current considerations in New Zealand (10). One establishment uses a threshold of 200 IU/L to identify FPT in calves up to three days of age, and 75 IU/L for 5–7-days-old calves. Another lab considers thresholds ranging from 600 to 50 IU/L over the same age range. Nevertheless, as other research has stated, commercial kits and automated solutions are available in order to reduce the time needed for GGT analysis, making it a reasonable, cheap, and flexible solution in terms of sample quantity (22, 44).

SODIUM SULPHITE TURBIDITY TEST (SSTT)

Throughout the first scientific works citing SSTT as an indirect method to assess FPT in calves, Pfeiffer and McGuire (1977) proposed the use of three different sodium sulphite concentrations, which rendered diverse proportions of correct estimations (from 90% to 96%) (30). This approach derives from the fundament of the technique. Thus, different sodium solutions are needed in order to selectively precipitate high-molecular-weight proteins, such as immunoglobulins (11, 31). Most applications employed the historical strategy of the three-step semiquantitative test using 14%, 16%, and 18% sodium sulphite test solutions (42). SSTT was previously applied to lambs (9), but also to Springbok calves (36). In bovines, Tyler et al. (1996b) classified mean serum IgG concentrations at different endpoints (Table 1). Consequently, an 18% test solution provided the best diagnostic utility (40). Although SSTT did not receive the same attention as the other indirect methods described by the literature, it was considered to be superior to similar methods such as ZSTT or glutaraldehyde coagulation (GC) (39), being one of the fastest and cheapest alternatives for FPT assessment (max. time 1 h and USD 0.57/unit) (11).

Table 1

IgG levels at different endpoints (40)

Endpoint	IgG (g/L)
1+	12.50
2+	21.16
3+	29.48

ZINC SULPHATE TURBIDITY TEST (ZSTT)

Another indirect method for IgG determination, ZSTT, shares similar principles as SSTT; total IgG concentration is being assessed thanks to the turbidity induced by cross-linking immunoglobulin molecules due

to the addition of zinc sulphate (44). ZSTT has been extensively used for FPT diagnosis in foals (32), lambs (9), and calves (14, 44). According to Zakian et al. (2018), ZSTT returned the lowest correlation between the evaluated methods (GGT, total serum protein, BR) when compared to ELISA IgG. Denholm et al. (2022) made the same observations as Hogan et al. (2015), thus lowering the test cut-off points should improve accuracy. Further, Hogan et al. (2016) compared results from ZSTT with RID, simultaneously examining cut-off points and parameters such as the wavelength of light used to read the turbidity reaction in order to optimise diagnosis accuracy. Therefore, the author claimed that ZST units and IgG concentration are linear, with a cut-off point of 12.5 ZST units being equivalent to 10 g/l of IgG. The latter observations permitted the optimisation of sensitivity rates with less possible effect on specificity. Moreover, various solution strengths between 250 and 400 mg/l have been suggested, but if a one-hour solution is used, then 350 mg/l zinc sulphate should return the highest specificity. In terms of reaction reading, the authors stated that a 680 nm light was not able to increase the specificity of the ZST test. However inexpensive and easy to perform, ZSTT is impacted by several aspects, such as sample haemolysis, reaction time, ambient temperature, and the CO_2 reacting with the $ZnSO_4$ solution (30, 42).

GLUTARALDEHYDE COAGULATION (GC)

A whole-blood GC test was first used to detect hypergammaglobulinemia in adult cattle (34). The test relies on the activity of uncharged amino groups, which will form cross-linkages with aldehyde groups, forming a visible clot due to globulin gelation (42). For FPT evaluation, a small sample size study assessed IgG levels via electrophoresis and compared the results to 10% glutaraldehyde, obtaining 100% agreement between the results (36). Another paper proposed GC for IgG-level evaluation, gathering samples from 242 calves (40). In this situation, the authors stated that sensitivity was highly variable depending on the chosen endpoint (0.00–0.41). Moreover, the relationship between serum IgG and GC results was proven, but it was considered of no biological significance (r^2=0.034). A recent study regarding FPT in Springbok calves stated that a positive GC result of ≥ 28 min had low sensitivity (57.1%) but 100% specificity (36).

PROTEIN REFRACTOMETRY (PR) AND BRIX REFRACTOMETRY (BR)

Refractometry is a field-friendly method extensively applied by practitioners in order to determine protein concentrations in plasma or other biological fluids (38). Refractometers typically measure how much light bends as it passes through the interface of two materials with different densities (19). Therefore, the refractive index is able to reflect the concentration of proteins in the sample (18). BR gauges the amount of sucrose present (21). It approximates the serum total solids percentage in non-sucrose-containing liquids, which could lead to an indirect measurement of serum IgG (21). A comparative study between PR and BR stated that the two methods can be used interchangeably for serum total protein and serum IgG evaluation (21). Moreover, in Holstein calves, BR has been shown to have a good correlation with serum IgG determined by RID (12). Buczinski et al. (2018) have considered, through their systematic review, that cut-off values dictate different specificity and sensitivity rates for PR when diagnosing FPT. Thus, PR turned out to be more specific but less sensitive at the 5.2 g/dL cut-off compared to 5.5 g/dL (8). Through the use of digital refractometers, Thornhill et al. (2015) proved that BR can be a useful tool for in-farm FPT assessment, with scores above 10% defining subjects with adequate immunity. However, Elsohaby et al. (2019) and Morrill et al. (2013) considered different FPT cut-off points of 7.8% and 8.3%, respectively. Variations between studies were justified for different reasons, such as age, breed, health status, or different refractometers used (16). The high applicability of PR and BR was ratified by even more recent incidence studies (33). PR and BR refractometers are convenient for daily use (8), the authors of the present paper, considering them still among the very feasible FPT assessment methods.

BIURET METHOD (BM) FOR TOTAL PROTEIN CONCENTRATION (TPC)

Serum biochemical profiling in ruminants most commonly involves total protein level evaluation (24). Besides the use of specific chemistry analysers, serum or plasma proteins can be assessed through the biuret method, which relies on copper chelate formation due to the enolized peptide bonds of proteins at alkaline pH (15). Alberghina et al. (2011) applied BM in order to investigate reference intervals for TPC along with different protein fractions and albumin/globulin ratios in dairy cows. In calves, Denholm et al. (2021) tested BM utility for FPT diagnosis, with results returning superior accuracy compared to refractometry (83.1% vs. 69.3%). Moreover, the same authors suggested that specificity rates for BM may reach 93.9%, making the latter preferable to refractometry for FPT detection (13). Zakian et al. (2018) considered the BM their reference method for TPC evaluation. The latter, used BM in 160 Holstein heifer calves, along with other direct or indirect methods (refractometry, ELISA, GGT,

and ZSTT) for FPT diagnosis. Among others, their work resulted in establishing strong correlations between TPC values obtained by ELISA and BM (44).

However satisfactory in terms of accuracy and specificity, as it was previously stated, BM remains a laboratory-based method that may be hardly applied in farm conditions but may serve for periodic assessment of the equipment being used on the farm (11).

CONCLUSION

FPT is a reality among both dairy and beef cattle farms; therefore, the application of diagnostic methods in a dedicated manner is particularly important. Among the described indirect methods, BM lacks in-field applicability, which is not the case for BR or PR. In terms of costs, more than one technique may be preferred, such as SSTT or ZSTT, but dealing with sensitivity rates, another approach is more the golden standard (RID). GGT remains a good method for colostrum intake evaluation and is thus variable secondary to signalling errors, such as age. Overall, all methods have different advantages and drawbacks; therefore, adapted decisions and method-dependent thresholds have to always be considered.

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