Journal of Cell and Tissue Research Vol. 15(3) 5221-5225 (2015)
(Available online at www.tcrjournals.com)
ISSN: 0973-0028; E-ISSN: 0974-0910

Original Article

COMPARATIVE EVALUATION OF DIFFERENT THERAPEUTIC REGIMES IN PRIMARY KETOTIC BUFFALOES

KUMAR, A.?, KUMAR, T., KUMAR, P., SINDHU, N., CHARAYA, G., GOEL, P., SRIDHAR, AND SURBHI

Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana - 125 004.
E. mail: tarunvet@gmail.com, Cell: 08901304347

Received: September 5, 2015: Accepted: September, 25, 2015

Abstract: The investigation was conducted to evaluate the therapeutic efficacy of different drugs in twenty four (n=24) buffaloes suffering from primary ketosis (screened out of 145 buffaloes). Primary ketotic animals were diagnosed on the basis of clinical signs (selective anorexia, drastic reduction in milk yield and absence of any other concurrent diseases) and two positive urine tests (Rothera's test and Keto-Diastix strip test). These twenty four animals were divided into three groups. Group A buffaloes (n = 8) were treated with propylene glycol 220 g b.i.d. orally on 1st day followed by 100g once a day along with dexamethasone (7 mg/100 kg BW I/M.). Group B (n = 8) were treated with dextrose 20 percent (1 litre) intravenously along with triamcinolone acetonide @ 0.1 mg/kg BW I/M once daily. Group C (n = 8) were treated with dextrose 20 percent (1 litre) intravenously along with isoflupredone acetate @ 1.0 mg/30 kg BW I/M once daily. Hemato-biochemical study revealed significant alterations in the values of various parameters before and after the treatment. The recovery time in group A animals was 4.88 ± 0.29 days, in group B animals was 4.12 ± 0.22 days and in group C animals was 3.00 ± 0.26 days. So comparative evaluation of three drugs combinations revealed better results for the therapeutic regimen dextrose (20 percent) along with isofluperadone acetate then combination of dextrose 20 percent along with triamcinolone acetonide and treatment of propylene glycol with dexamethasone

Key words: Primary ketosis, Buffaloes

INTRODUCTION

Ketosis is one of the most important production disorder characterized by increased level of ketone bodies (beta-hydroxybutyrate (BHBA), acetoacetate (AcAc), and acetone (Ac) at 70, 28 and 2%, respectively) in the blood, urine and milk [1,2] which is also used as indicators of physiological imbalance and clinical ketosis [3].

Ketosis is frequently observed in high yielding dairy animals mainly during transition period due to negative energy balance (NEB). As the negative effects of this disease may include displaced abomasum, increased culling risk, lower milk production and impaired reproductive performance [4-7], it will directly or indirectly affect the economics of dairy farm and ultimately dairy farmers suffer from huge financial losses. So this metabolic disease is more important in country like India which comprise approximately 58.6 percent i.e. 108.7 million of the total world buffalo population. Biochemical changes including hyperketonemia, hypoglycemia, hypoinsulinemia, increased hepatic triglycerides and non esterified fatty acids (NEFA) and increased blood activities of liver specific enzymes such as aspartate

aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) has been reported in ketotic animals [8-10].

Several therapeutic measures have been attempted for clinical ketosis with parenteral glucose, parenteral glucocorticoids and oral administration of some glucogenic compounds like glycerol and propylene glycol [11]. There is paucity of literature on therapy of clinical primary ketosis in buffaloes particularly from India and therefore, the present investigation was designed to evaluate comparative therapeutic efficacy of three drugs combinations in buffaloes suffering from clinical primary ketosis.

MATERIALS AND METHODS

Study area, design and animals: A total of 145 buffaloes were initially screened for ketosis brought to Teaching Veterinary Clinical Complex, Hisar from adjoining villages of the District Hisar. Twenty four buffaloes found positive for ketosis was selected for therapeutic trials whereas eight buffaloes found negative were considered as healthy control group. Diagnosis was made on the basis of clinical signs (selective anorexia, drastic reduction in milk yield and absence of any other concurrent diseases) and two positive urine tests (Rothera's test and Keto-Diastix strip test).

Serum biochemistry: For evaluation of therapeutic efficacy serum analysis was done for calculating glucose, total cholesterol, triglycerides, calcium, total protein, inorganic phosphorous and alkaline phosphatase. Fully automated Random Access Clinical Chemistry Analyzer (EM 200™ Erba Mannheim – Germany) was employed for estimation of biochemical parameters using kits procured from Transasia Biomedical Limited. As per manufacturer's instructions, glucose was estimated by Trinder's method [12]; total cholesterol was measured by CHOD-PAP method [13]; triglycerides were measured by GPO-Trinder method [14]; calcium was measured by Arsenazo method [15]; inorganic phosphorus was estimated by UV phosphomolybdate method [16]; total proteins were estimated by Biuret method [17] and alkaline phosphatase was estimated by IFCC with AMP buffer method [18].

Therapeutic studies: To evaluate the therapeutic efficacy of different drugs, animals were categorized in three groups of eight animal each as per the protocol given below:-

Group A: were treated with propylene glycol 220 g bid orally on 1st day followed by 100 g once a day along with dexamethasone @7 mg/100 kg BW I/M.

Group B: were treated with dextrose 20% (1 litre) intravenously along with triamcinolone acetonide @ 0.1 mg/kg BW I/M once daily.

Group C: were treated with dextrose 20% (1 litre) intravenously along with Isoflupredone acetate @ 1.0 mg/30 kg BW I/M once daily.

Hematological parameters: Blood samples were collected aseptically using EDTA coated sterile vials from jugular vein of the affected animals (before and after treatment) as well as healthy control group animals by standard procedures **(**hemoglobin (Hb) was measured using Sahli's haemoglobinometer method, packed cell volume (PCV) was determined by Wintrobe method total erythrocyte count (TEC) was estimated by Haemocytometer method, total leucocyte count (TLC) was carried out by the Haemocytometer method, and differential leucocyte count (DLC) was estimated by counting the cells after staining with Giemsa stain) as prescribed by Weiss and Wardrop [19].

Statistical analysis: Data was expressed as mean (±standard error of the mean) and analyzed by applying One way ANOVA test using SPSS statistical software.

RESULTS

In group A animals (propylene glycol plus dexamethasone), the mean serum value of glucose, total cholesterol, triglycerides, inorganic phosphorus and alkaline phosphatase ($p<0.05$) differs significantly whereas values of calcium and total protein were unaffected before and after the treatment as evident in table 1. The mean blood values of eosinophil, TEC and MCV ($p<0.05$) differs significantly whereas the remaining blood parameters values were same before and after the treatment (Table 2). In all these clinical cases there was 100% recovery within a range of 4-6 days (Table 3). Further, the urine was negative to Rothera's test and Keto-Diastix test. Time required to restore milk production ranged between 5-7 days (Table 3).

Table 1: Biochemical parameters before and after the treatment:

Biochemical parameters	Animals Grouping								
	Group A			Group B			Group C		
	BT	AT	C	BT	AT	C	BT	AT	C
Glucose (mg/dL)	44.08 ± 3.87^{a}	59.12 ± 0.83^{b}	70.91 ±3.05^{c}	45.68 ± 3.13^{a}	60.5 ± 1.13^{b}	70.91 ± 3.05^{c}	49.38 ± 3.56^{a}	62.28 ± 0.67^{b}	70.91 ± 3.05^{c}
Total Cholesterol (mg/dL)	151 ± 6.42^{b}	105.93 ± 2.22^{a}	98.25 ± 1.82^{a}	157.38 ± 6.00^{b}	103.9 ± 1.60^{a}	98.25 ± 1.82^{a}	157.75 ± 7.50^{b}	100.85 ± 1.39^{a}	98.25 ± 1.82^{a}
Triglycerides (mg/dL)	77.62 ± 3.76^{c}	51.72 ± 1.19^{b}	38.50± 2.73^{a}	79.25 ± 5.11^{b}	47.38 ± 1.06^{a}	38.50 ± 2.73^{a}	76.62 ± 3.80^{b}	44.37 ± 0.90^{a}	38.50 ± 2.73^{a}
Calcium (mg/dL)	6.69 ± 0.47^{a}	7.00 ± 0.20^{a}	8.29 ± 0.36^{b}	7.15 ± 0.21^{a}	7.47 ± 0.14^{a}	8.29 ± 0.36^{b}	6.10 ± 0.30^{a}	7.62 ± 0.14^{b}	8.29± 0.36^{b}
Inorganic Phosphorus(mg/dL)	3.75 ± 0.29^{a}	5.40 ± 0.49^{b}	5.62 ± 0.60^{b}	5.02 ± 0.59^{a}	4.74 ± 0.15^{a}	5.62 ± 0.60^{a}	4.98 ± 0.51^{a}	5.75 ± 0.39^{a}	5.62 ± 0.60^{a}
Total Protein (g/dL)	7.42 ± 0.37^{a}	7.65 ± 0.34^{a}	8.12 ± 0.36^{a}	7.23 ± 0.57^{a}	7.54 ± 0.45^{a}	8.12 ± 0.36^{a}	6.84 ± 0.38^{a}	7.88 ± 0.12^{b}	8.12 ± 0.36^{b}
Alkaline Phosphatase (IU/L)	323.75 ±31.36^{b}	224.75 ± 7.50^{a}	182.5 ± 13.13^{a}	274.88 ± 19.53^{b}	217.2 ± 7.58^{a}	182.5 ± 13.13^{a}	267.75 ± 33.06^{b}	202.12 ± 5.35^{a}	182.5 ±13.13^{a}

(BT = Before Treatment; AT = After Treatment; C = Control Animals)
Note: means bearing different superscripts in a row differ significantly ($p< 0.05$)

Table 2: Hematological parameters before and after the treatment:

Hematological parameters		Animals Grouping								
		Group A			Group B			Group C		
		BT	AT	C	BT	AT	C	BT	AT	C
Hemoglobin (g/dL)		11.08 ± 0.43^{a}	11.45 ± 0.40^{a}	12.13 ±0.27^{a}	10.62 ± 0.49^{a}	11.27 ± 0.30^{ab}	12.13 ± 0.27^{b}	10.17 ±0.20^{a}	11.5 ±0.2^{b}	12.13 ±0.27^{b}
TLC (10^{3}/mm^{3})		9.08 ± 0.63^{a}	10.38 ± 0.40^{ab}	11.65 ± 0.30^{b}	9.7 ± 0.36^{a}	11.09 ± 0.28^{b}	11.65 ± 0.30^{b}	9.81 ± 0.52^{a}	11.10 ± 0.27^{b}	11.65 ± 0.30^{b}
DLC (%)	N	35.75 ± 1.65^{a}	38.88 ± 1.23^{a}	37.5 ± 0.90^{a}	35.88 ± 1.50^{a}	38.25 ± 1.66^{a}	37.5 ± 0.90^{a}	32.88 ± 2.20^{a}	39.38 ± 1.42^{b}	37.5 ± 0.90^{ab}
	L	58.88 ± 1.18^{a}	58.5 ± 1.22^{a}	60 ± 0.90^{a}	55.25 ± 1.97^{a}	58.88 ± 1.34^{ab}	60 ± 0.90^{b}	55.75 ± 3.64^{a}	58.38 ± 1.23^{a}	60 ± 0.90^{a}
	E	4.50 ± 0.75^{b}	2.38 ± 0.42^{a}	1.75 ± 0.52^{a}	5.62 ± 0.88^{b}	2.62 ± 0.59^{a}	1.75 ± 0.52^{a}	7.38 ± 1.59^{b}	2.12 ± 0.51^{a}	1.75 ± 0.52^{a}
	B	0 ± 0	0 ± 0	0 ± 0	0.25 ±0.16^{a}	0 ± 0^{a}	0 ± 0^{a}	0.12 ± 0.12^{a}	0 ± 0^{a}	0 ± 0^{a}
	M	0.88 ± 0.35^{a}	0.25 ± 0.16^{a}	0.75 ± 0.31^{a}	3.00 ± 0.32^{b}	0.25 ± 0.16^{a}	0.75 ± 0.31^{a}	3.88 ± 1.06^{b}	0.12 ± 0.12^{a}	0.75 ± 0.31^{a}
TEC (10^{6}/mm^{3})		4.95 ± 0.11^{a}	5.53 ± 0.12^{b}	5.87 ± 0.10^{b}	4.85 ± 0.17^{a}	5.62 ± 0.13^{b}	5.87 ± 0.10^{b}	4.93 ± 0.08^{a}	5.91 ± 0.13^{b}	5.87 ± 0.10^{b}
PCV (%)		31.88 ± 1.09^{a}	33.5 ± 0.82^{ab}	35 ± 0.70^{b}	31 ± 1.38^{a}	33.88 ± 0.83^{ab}	35 ± 0.70^{b}	29.88 ± 0.71^{a}	34.12 ± .71^{b}	35 ± 0.70^{b}
MCH (pg)		21.48 ± 0.41^{ab}	20.65 ± 0.43^{a}	22.52 ± 0.83^{b}	21.93 ± 0.91^{ab}	19.96 ± 0.28^{a}	22.52 ± 0.83^{b}	20.64 ± 0.53^{a}	19.49 ± 0.49^{a}	22.52 ± 0.83^{b}
MCHC (%)		34.71 ± 0.43^{a}	34.13 ± 0.53^{a}	34.63 ± 0.40^{a}	34.21 ± 0.26^{b}	33.27 ± 0.23^{a}	34.63 ± 0.40^{b}	34.15 ± 0.87^{a}	33.75 ± 0.65^{a}	34.63 ± 0.40^{a}
MCV (fl)		64.29 ±1.13^{b}	60.55 ± 1.17^{a}	65.80 ±1.42^{b}	64.05 ± 2.45^{ab}	60.00 ± .70^{a}	65.80 ±1.42^{b}	60.5 ± 1.02^{a}	57.77 ± 0.93^{a}	65.80 ±1.42^{b}

(N = Neutrophils; L = Lymphocytes; E = Eosinophils; B = Basophils; M = Monocytes)
Note: means bearing different superscripts in a row differ significantly ($p< 0.05$)

Table 3: Comparative therapeutic efficacy of different therapeutic protocol in primary ketotic buffaloes

Group	No. of cases treated	Therapy given	Dose	Route	Recovery (%)	Mean Recover-y time (days)	Time required to restore milk production (days)
A	8	Propylene glycol + Dexamethasone	220g bid on first day followed by 100g od following days 7 mg/100 kg BW od	Orally IM	100	4.88 ± 0.29	5.88 ± 0.22
B	8	Dextrose 20% + Triamcinolone acetonide	I Litre od 0.1 mg/kg BW od	IV IM	100	4.12 ± 0.22	5.38 ± 0.18
C	8	Dextrose 20% + Isoflupredone acetate	I Litre od 1.0 mg/30 kg BW od	IV IM	100	3.00 ± 0.26	4.25 ± 0.25

In group B (dextrose (20%) plus triamcinolone), the mean serum value of glucose, total cholesterol, triglycerides and alkaline phosphatase ($p<0.05$) differs significantly in normal and treated animals (Table 1), while other parameters were unaffected. Likewise, the mean blood values of TLC, eosinophil, monocyte, TEC, MCV and MCHC ($p<0.05$) were significantly different in treated animals as compared to control (Table 2), while rest showed insignificant changes. Recovery percentage in all these clinical cases was 100% within 3-5 days. The urine was negative to Rothera's and Keto-Diastix tests. Time required to restore milk production ranged between 5-6 days (Table 3).

In group C (dextrose (20%) plus isoflupredone acetate), the changes in mean serum value of glucose, total cholesterol, triglycerides, calcium, total protein and alkaline phosphatase ($p<0.05$) were significantly different (Table 1). No significant change in inorganic phosphorus was observed in control and treated animals. Likewise the mean blood values of Hb, TLC, neutrophil, eosinophil, monocyte, TEC and PCV ($p<0.05$) differs significantly, while rest parameters were unchanged before and after the treatment (Table 2). Recovery percentage in all these clinical cases was 100% within a range of 2-4 days (Table 3). Like other groups urine was negative to Rothera's and Keto-Diastix tests. Time required to restore milk production ranged between 3-5 days (Table 3).

DISCUSSION

The present study revealed a better therapeutic efficacy of Isoflupredone acetate (glucocorticoid) along with dextrose 20% (Group C) in which the clinical recovery took a shorter time compared to triamcinolone acetonide along with dextrose 20% combination (Group B) and oral propylene glycol alongwith parenteral dexamethasone combination (Group A). As the biochemical parameters in all the three groups increased significantly but their recovery is more and quick in group C as it took shorter time to improve. Shpigel et al. [20] reported that treatment of ketosis in dairy cattle with glucose and corticosteroids was more efficacious than treatment with corticosteroids alone, the fact which was kept in mind while planning the present investigation in which corticosteroids were used in combination with dextrose in group B and C. The frequency of administration of therapeutic regimen in the present investigation was once a day and gave promising results (100% in Groups B and C) whereas in contrast to present study, Bihani and Gahlot [21] reported that the intravenous administration of glucose in ketotic cattle needs to be repeated every six hours for optimum results. In group A, in which oral glucogenic compound (propylene glycol) along with parenteral dexamethasone was given as therapy, the time taken in recovery was comparatively longer than other two groups (B and C). Tufani et al. [22] observed excellent recovery rate and less evidence of relapse in clinical cases of ketosis treated with dexamethasone in association with parenteral glucose which is in partial confirmation to the findings of therapeutic trial in present investigation in which dexamethasone was used in combination with oral propylene glycol. These findings have also been supported by several investigators [23,24]. Reddy et al. [25] also indicated good efficacy of parenteral dexamethasone, 25% dextrose and oral glycerol (another oral glucogenic compound) in ketotic cows.

Isoflupredone acetate is a comparatively recent introduction to the group of glucocorticoids and considered many times more potent compared to other members of this group. It helps in increasing protein mobilization which increases blood glucose level as the availability of gluconeogenic amino acids is also raised. With result of present study showing better cure rate with least recovery time which is also in accordance with the study of various authors [26,27].

To conclude, isoflupredone acetate @ 1.0 mg/30 kg b.wt. along with dextrose (20 percent) @ 1.0 litre once a day parenterally for 3 days was found most effective therapy of primary ketosis in buffaloes when compared with triamcinolone acetonide plus dextrose (20 percent) combination and oral propylene glycol plus parenteral dexamethasone combination.

REFERNCES

[1] Tehrani-Sharif, M., Hadadi, M., Noughabi, H.H., Mohammadi, A., Rostami, F. and Sharifi, H.: Comp. Clin. Path., 10: 1007-1008 (2011).

[2] Zhang, Z., Liu, G., Wang, H., Li, X. and Wang, Z.: Pak. Vet. J., 32(2): 156-160 (2012).

[3] Enjalbert, F., Nicot, M.C., Bayourthe, C. and Moncoulon, R..: J. Dairy Sci., 84(3): 583-589 (2001).
[4] Collard, B.L., Boettcher, P.J., Dekkers, J.C.M., Petitclerc, D. and Schaeffer, L.R.: J. Dairy Sci., 83: 2683-2690 (2000).
[5] LeBlanc, S.J., Leslie, K. and Duffield, T.F.: J. Dairy Sci., 88: 159-170 (2005).
[6] Duffield, T.F., Lissemore, K.D., McBride, B.W. and Leslie, K.E.: J. Dairy Sci., 92: 571-580 (2009).
[7] McArt, J. A., Nydam, D.V. and Oetzel, G.R.: J. Dairy Sci., 95: 5056-5066 (2012).
[8] Radostits, O.M., Gay, C.C., Blood, D.C. and Hinchcliff, K.W.: *Veterinary Medicine*, 9th Edn. ELBS, Bailliere Tindall, London, UK, pp 1452-1462 (2000).
[9] Dann, H.M., Morin, D.E., Bollero, G.A., Murphy, M.R. and Drackley, J.K.: J. Dairy Sci., 88(9): 3249-3264 (2005).
[10] Hayirli, A.: Vet. Res. Commun., 30: 749-774 (2006).
[11] Nielsen, N.I. and Ingvartsen, K.L.: Ani. Food Sci. Tech., 115: 191-213 (2004).
[12] Dingeon, B., Ferry, J.P. and Roullet, A.: Annales de biologie Clinique, 33(1): 3-13 (1974).
[13] Allain, C.C., Poon, L.S., Chan, C.S., Richmond, W.F.P.C. and Fu, P.C.: Clini. Chem., 20(4): 470-475 (1974).
[14] Klotzach, S.G., McNamara J.R.: Clin. Chem. 36(9): 1605-1613 (1990).
[15] Morgan, B.R., Artiss, J.D. and Zak, B.: Clini. Chem., 39(8): 1608-1612 (1993).
[16] Rubino, L., Catapano, V. and Guerra, G.: J. Analytical Methods Chem., 11(4): 164-167 (1989).
[17] Nowotny, A.: Basic Exercises in Immunochemistry., Springer Berlin Heidelberg, pp 168-169 (1979)
[18] Lamb, E.J., Browne, M., John, W.G. and Price, C.P.: Annals Clin. Biochem.: An Inter. J. Biochem. Med., 35(1): 120-127 (1998).
[19] Weiss, D.J. and Wardrop, K.J.: *Schalm's Veterinary Hematology*, 6th Edn. Wiley Blackwell, State Avenue, Ames, Iowa, USA (2010)
[20] Shpigel, N.Y., Chen, R., Avidar, Y. and Bogin, E.: J. Am. Vet. Med. Assoc., 208(10): 1702-1704 (1996).
[21] Bihani, D.K. and Gahlot, A.K.: Ind. J. Ani. Sci., 73(9): 1049-1050 (2003).
[22] Tufani, N.A., Hafiz, A., Muhee, A. and Makhdoomi, D.M.: Ind. J. Vet. Med., 31: 38-39 (2011).
[23] Asrat, M., Tadesse, G.H., Gounder, R.V. and Nagappan, R.: British J. Dairy Sci., 33: 26-30 (2013).
[24] Gordon, J.L.: *Risk Factors for and Treatment of Ketosis in Lactating Dairy Cattle*. Ph.D. Thesis. University of Guelph, Guelph, Ontario, Canada (2013).
[25] Reddy, B. S., Reddy, B.S.S., Reddy, Y.P. and Vennkatasivakumar, R.: Int. J. Bio. Res., 2(2): 143-144 (2014).
[26] Dar, K.H., Ansari, M.M., Tantary, H.A., Baba, M.A. and Ashraf, I.: Inter. J. Vet. Sci., 3(3): 147-150 (2014).
[27] Van der Drift, S. G., Houweling, M., Bouman, M., Koets, A.P., Tielens, A.G., Nielen, M. & Jorritsma, R.: The Vet., J.,204(2): 144-149 (2015).