

Study of the Metabolic Profile in Buffaloes with Prolonged Anestrus

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Abstract

The metabolic profile test in animals is used to assess their nutritional status, to diagnose disease and assess fertility. The aim of this paper was to investigate the metabolic profile in buffalo cows. Forty buffaloes from the Research and Development Station for Buffaloes Secaia - Romania, were divided into two groups (20 heads/group). Estrus induction protocols were applied to the first group (G1), while the second group consisted of buffalo cows that express natural oestrus (G2). When the artificial insemination was performed, blood samples were taken and the following parameters were determined: hemoglobin, total serum protein, phosphorus, calcium, magnesium, alkaline phosphatase, cholesterol, glucose, lipase, urea. G2 buffalo cows had significantly higher concentrations of hemoglobin, serum protein, glucose and inorganic phosphorus, while the serum calcium levels were lower in the group. The concentration of magnesium, alkaline phosphatase, cholesterol, lipase and urea did not differ significantly between the buffalo groups ($p>0.05$). The Ca:P ratio for G2 buffaloes was lower (1.94:1) compared to G1 buffaloes (2.89:1). It can be concluded that the level of hemoglobin, serum protein, glucose, calcium and inorganic phosphorus plays a major role in the reproduction of buffaloes.

Keywords: anestrus, buffaloes, estrus, metabolic profile

1. Introduction

The analysis for metabolic profile in dairy animals is used to assess nutritional health status, to prevent the metabolic diseases, to diagnose the diseases and to assess the animal fertility status [1]. Among the factors influencing the metabolic profile in buffalo were highlighted: diet, reproduction, age, sex and climatic factors [2, 3]. Nutritional deficiencies cause metabolic, endocrine and nervous disorders, disrupting the activity of the hypothalamus-pituitary-ovarian circulation, with negative effects on the reproductive process, oogenesis and follicles-genesis, extending the postpartum anestrus period and decreasing fertility rates of cows [4-6]. Results regarding analyzes of the metabolic

profile in the Romanian Buffalo breed are limited, moreover, there are limited references available to the physiological parameters for blood metabolites [5]. In this regard, collection data on the metabolic profile from a large number of animals on sex, age, physiological status and health, are required to improve the current knowledge and implications [7, 8]. The aim of this study was to investigate the metabolic profile in buffaloes with reproductive cycle disorders and induced estrus through therapeutic protocols, compared to buffaloes that showed physiological estrus.

2. Materials and methods

Animal management

The study was carried out at the Research and Development Station for Buffaloes Șercaia, during 2020, on 40 adult buffalo cows, aged between 7 and 13 years, with good body condition and in different physiological conditions, divided into

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two groups (20 buffaloes/group). The first experimental group consisted of buffaloes with reproductive cycle disorders, with more than 60 days after calving (active anestrus caused by the presence of the *corpus luteum* and passive anestrus without a *corpus luteum* on the ovary) to which specific induction treatments were applied and estrus synchronization. The second experimental group consisted of buffalo cows that normally showed estrus after calving. The buffalo cows from both experimental groups were raised under identical conditions, in free housing and under tied stanchion barn conditions, being fed identically with green fodder, hill pasture hay, corn silage and concentrates.

Collection of samples and studied parameters

After artificial insemination was performed, blood samples were taken (10 ml) from the jugular vein. The blood samples were transported frozen to the laboratory, where the serum was extracted and stored at -20°C , until further analysis. Serum samples were analyzed using spectrophotometric and colorimetric-enzyme methods, for the following parameters: hemoglobin (g/dL), total serum protein (g/dL), phosphorus (mg/dL),

calcium (mg/dL), alkaline phosphatase (IU/L), magnesium (mg/dL), cholesterol (mg/dL), glucose (mg/dL), lipase (IU/L) and urea (mg/dL), using commercial kits (Span Diagnostics) according to the protocol the manufacturer.

Statistical analysis

Data from biochemical estimates were expressed as a mean \pm standard error (X \pm SD) and variation coefficient (CV) for each group of buffalo cows. The data were statistically analyzed by applying the Student's T test to obtain the significance of the difference of the mean values of the two groups (considered statistically significant at $p<0.05$).

All experimental procedures were performed in accordance with the Romanian Legislation no. 43/2014 and the European Union's Directive for animal experimentation (Directive 2010/63/UE).

3. Results and discussion

The results concerning laboratory determinations in buffaloes in the two experimental groups are summarized in Table 1.

Table 1. Blood biochemical results in buffalo cows from the two experimental groups

Blood parameters	Experimental group 1 N=20		Experimental group 2 N=20		t-test
	X \pm SD	CV %	X \pm SD	CV %	
Hemoglobin (g/dL)	9.73 \pm 1.02	14.37	12.68 \pm 0.73	20.12	$p<0.05$
Total serum protein (g/dL)	6.12 \pm 0.36	22.16	8.40 \pm 0.56	17.46	$p<0.05$
Calcium (mg/dL)	10.52 \pm 0.35	24.35	8.28 \pm 0.37	18.27	$p<0.05$
Phosphorus (mg/dL)	3.64 \pm 0.48	18.42	4.27 \pm 0.77	11.31	$p<0.05$
Magnesium (mg/dL)	3.06 \pm 1.22	6.83	3.63 \pm 1.32	8.46	ns
Alkaline phosphatase (UI/L)	181.95 \pm 53.17	23.17	175.19 \pm 48.29	27.36	ns
Cholesterol (mg/dL)	98.52 \pm 12.63	14.72	93.40 \pm 10.84	18.57	ns
Glucose (mg/dL)	42.18 \pm 2.62	34.58	54.18 \pm 4.95	26.12	$p<0.05$
Lipase (UI/L)	7.76 \pm 2.45	14.38	7.12 \pm 2.90	16.74	ns
Urea (mg/dL)	40.80 \pm 5.77	46.17	38.20 \pm 4.49	35.92	ns

ns=not significant ($p>0.05$)

The hemoglobin level in the experimental group 1 and 2 were 9.73 \pm 1.02 g/dL and 12.68 \pm 0.73 g/dL, respectively. The results highlighted a significant variation ($p<0.05$) of hemoglobin level in buffalo cows that showed normal estrus compared to buffaloes with prolonged anestrus. The higher hemoglobin concentration in buffaloes with a normal estrus expression can also be attributed to the fact that animals need more oxygen in any

stressful periods and, consequently, the hemoglobin concentration can increase [9]. Although the level of hemoglobin does not directly affect reproductive disorders, its low value could indirectly affect the functioning of the reproductive organs. A low level of hemoglobin in the organism influences the oxygenation of the tissues of the reproductive organs, which in turn could affect the reproductive cyclicity [7].

Total serum protein had values of 6.12 ± 0.36 g/dL in group 1 and 8.40 ± 0.56 g/dL in group 2. There was a significant difference ($p < 0.05$), higher in buffalo cows from group 2. Protein deficiency causes uterine involution which leads to the appearance of extended anestrus after calving. High level of protein increases the incidence of foetal attachment retention, genital infections and low fertility.

Serum calcium was on average with 10.52 ± 0.35 mg/dL in group 1 and 8.28 ± 0.37 mg/dL in group 2. The results of our study about blood serum calcium were within the limits described in the literature by other authors (8-10.8 mg/dl) [5, 6]. There was a significantly larger difference in calcium concentration ($p < 0.05$) in group 1 buffalo cows, compared to group 2.

Serum phosphorus levels in anestrus buffaloes (3.64 ± 0.48 mg/dl) were significantly lower ($p < 0.05$) compared to the physiological estrus cycle buffaloes (4.27 ± 0.77 mg/dl). Lower calcium levels in buffaloes from experimental group 2 could be attributed to the influence of the lactation existence [6].

The ratio of Ca:P for physiological estrus cycle buffaloes was lower (1.94:1) compared to anoestrus buffaloes (2.89:1). The ratio of Ca:P should be 2:1 for healthier reproduction performance [7].

Serum magnesium (G1- 3.06 ± 1.22 mg/dl; G2- 3.63 ± 1.32 mg/dl) was within the physiological parameters described in the buffalo literature (1.8-3.8 mg/dl) [5-8]. Magnesium has a vital role in the metabolism of carbohydrates, lipids, nucleic acids and proteins.

Alkaline phosphatase (ALP) (G1- 181.95 ± 53.17 IU/L; G2- 175.19 ± 48.29 IU/L), had values within the physiological parameters in buffaloes [5].

The individual values of cholesterol in buffaloes in both groups did not have significant differences (G1- 98.52 ± 12.63 mg/dL; G2- 93.40 ± 10.84 mg/dL), the reference range in the literature being 73-280 mg/dL [5, 6, 8].

In the present study, physiological cycle buffaloes (54.18 ± 4.95 mg/dL) had significantly higher glucose concentration ($p < 0.05$), compared to anoestrus buffalo cows (42.18 ± 2.62 mg/dL). Several authors have reported that glucose concentration reflects the energy status and reproductive activity of animals [7]. Hypoglycaemia influences the ovarian activity in animals by reducing the release of gonadotrophins

from the hypothalamus [7]. Blood glucose variations in buffaloes are associated to the estrus cyclicity and fertility of the animals [7].

The serum lipase level in buffaloes experimental group 1 and group 2 was 7.76 ± 2.45 IU/L and 7.12 ± 2.90 IU/L, respectively. No supporting previously published data for comparison was available, concerning the serum lipase. Lipase is a key enzyme in triglyceride metabolism. Urea recorded in both buffalo's experimental group's has values considered in the physiological thresholds (21-53 mg/dl).

4. Conclusions

We found that the levels of hemoglobin, serum proteins, glucose, calcium, inorganic phosphorus play an important role in buffaloes' reproduction. The ratio of Ca:P should also be around 2:1 for an adequate reproduction efficiency. In buffaloes, subclinical nutrient deficiencies could be the cause of clinical anestrus, more extensive studies are needed to identify the relationships between reproduction and the metabolic profile of buffalo cows.

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