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Research Article
Blood Biochemical Profile in Fertile and Repeat Breeder Ongole Cross Breed Cows

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Abstract

Background and Objective: Repeat breeding is a major problem in beef cows. The objective of the present study was to determine the blood biochemical levels in repeat breeder and fertile Ongole cross breed cows. **Materials and Methods:** This study compared the blood biochemical profiles from 30 repeat breeders and 30 fertile Ongole cross breed cows. Plasma glucose, total cholesterol, total protein and urea nitrogen concentrations were examined in repeat breeder cows and compared with those of fertile cows. Blood samples were collected from the caudal vein into anticoagulant-coated tubes. Cells were removed from plasma by centrifugation and blood glucose, total cholesterol and urea nitrogen concentrations were measured using commercially available kits; a non-commercial kit was used to determine total protein content. **Results:** Significantly different blood profiles between repeat breeder and fertile cows were reported. Specifically, repeat breeder cows had lower levels of glucose, total cholesterol and total protein, but higher levels of urea nitrogen than fertile cows. **Conclusion:** Finally, a low level of total protein, total cholesterol and glucose, accompanied by a high level of urea nitrogen, may cause reproductive problems in repeat breeders. Additionally, present findings indicated that biochemical blood profiling could be used as a diagnostic tool for repeat breeding.

Key words: Blood biochemical profile, ongole cross breed cows, reproductive problem, fertile cows and repeat breeder cows

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cows that fail to conceive after three or more inseminations and in the absence of detectable abnormalities, are described as repeat breeders[1]. Repeat breeding has adverse effects on fertility, leading to substantial economic losses in the cattle industry, a reduction in milk production, an increase in calving intervals and an increase in culling rates[2]. Fertility is related to nutritional adequacy and energy reserves[3]. Furthermore, nutrient imbalances, starvation and wasting can all reduce fertility. Nutritional status is reflected in the blood biochemical profile, in which normal levels of various biochemical components are crucial for proper function of all physiological processes, including reproduction. Changes in some biochemical components can have adverse effects on reproductive performance. Previous studies showed that blood glucose levels, plasma cholesterol and plasma total protein were lower in repeat breeders than in fertile cows and buffaloes[3-5], whereas plasma urea appeared to be higher[4,6]. Low levels of serum glucose may inhibit the synthesis or release of gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone and luteinizing hormone (LH), thus resulting in inhibition of follicle and ovum development. In addition, nutritional deficiency affects mortality rates due to insufficient ovarian steroid hormone. Cholesterol is a compound that functions in the formation of body cell walls, precursors of steroid hormone synthesis, vitamin D and bile salts[7]. Proteins are a source of amino acids required for the biosynthesis of gonadotropins and gonadal hormones. Higher urea nitrogen levels in the body will negatively affect the quality of uterine fluid due to an increased concentration of ammonia and the direct toxic effect of ammonia and urea on endometrium, leading to conception failure[8].

The present study aims to be an important reference for the maintenance, optimum production, propagation and lowering of reproductive failure in Ongole cross breed cows. The main objective of the present study was to measure serum glucose, total cholesterol, total protein and urea nitrogen concentrations in repeat breeder cows, compare them with those in fertile cows and determine their possible involvement and usefulness as a tool in the clinical diagnosis of repeat breeding.

MATERIALS AND METHODS

The study design was approved by the Animal Ethics Committee of Universitas Gadjah Mada. The research subjects were 60 Ongole cross breed cows with body condition scores of 2.0-3.0[9]; they were divided equally into repeat breeder and fertile groups. A cow that fails to conceive after three or more successive inseminations, in spite of having normal oestrus and lacking detectable clinical abnormalities, is described as a repeat breeder. Blood samples and reproductive data of the subjects were obtained from smallholder farmers in several areas of Sleman District, Yogyakarta, Indonesia. Blood biochemical analyses were carried out at the Integrated Research Laboratory, Universitas Gadjah Mada and the entire study took place between February 1 and October 31 2017. The cows were fed concentrates (wheat pollard and rice bran) and *ad libitum* forage (parra grass, native field grass and rice straw) and access to drinking water. Forage was provided to the animals in the morning and evening, whereas the concentrate was provided in the afternoon. Dietary nutrients are listed in Table 1.

Table 1: Nutrient content of Ongole cross breed cow diets

	Dry matter (%)		
Type of feed	Crude protein	Extract ether	Crude fiber
Rice bran	9.96	2.32	18.51
Pollard	16.41	4.01	5.86
Parra grass	1.31	43.41	12.08
Native field grass	8.59	6.93	36.38
Rice straw	4.04	0.53	31.62

Blood sampling: Ten millilitres of blood were collected from the caudal vein of repeat breeder and fertile cows into anticoagulant-coated tubes. Of these samples, 4 mL were collected in sodium fluoride-coated tubes for estimation of glucose and 6 mL in ethylenediaminetetraacetic acid (EDTA)-coated tubes for estimation of all other biochemical parameters. The tubes were transported in an ice container to the laboratory. Cells were removed from the plasma by centrifugation at 2000 rpm (670.8×g) for 20 min. Plasma was stored at -20°C for further analysis.

Chemical analysis: Blood glucose, total cholesterol, total protein and blood urea were measured using a Microlab 300 spectrophotometer (ELITech, Logan, UT, USA). Blood glucose, blood cholesterol and blood urea were analyzed using commercially available kits (DiaSys Diagnostic System, Holzheim, Germany). Specifically, blood glucose was estimated using Glucose Oxidase-Phenol 4-Aminoantipirin (GOD-PAP, Liquid kit), cholesterol with the cholesterol esterase method (CHOD-POD, Liquid kit) and blood urea with the urease method (Urease-GLDH, Kinetic UV kit). Plasma total protein was estimated using the Biuret method (Biuret, Colorimetric kit).

Statistical analysis: Data were analysed using one-way analysis of variance (ANOVA) to compare means between the two groups. Results are expressed as Mean±standard deviation (SD). All statistical analyses were performed using Statistical Program for Social Science (SPSS) version 16.0 (IBM, Chicago, IL, USA). Probability at $p \leq 0.01$ was considered statistically significant.

RESULTS

This study observed significant differences between the blood biochemical profiles of repeat breeder and fertile Ongole cross breed cows (Table 2). Compared with fertile cows, the concentration of protein was lower in repeat breeder cows ($p \leq 0.01$). Furthermore, significantly lower ($p \leq 0.01$) levels of total cholesterol and glucose were also found in repeat breeder cows than in fertile cows. In contrast, the levels of blood urea nitrogen were significantly higher in the former than in the latter.

Table 2: Blood biochemical profiles of repeat breeder and fertile Ongole cross breed cows

Blood biochemical profile	Repeat breeder (n = 30)	Fertile (n = 30)
Total protein (g dL^{-1})	5.67±0.86[a]	8.68±0.58[b]
Total cholesterol (mg dL^{-1})	124.65±12.84[a]	151.45±26.25[b]
Glucose (mg dL^{-1})	48.48±10.55[a]	69.28±4.03[b]
Urea nitrogen (mg dL^{-1})	27.60±4.25[a]	16.97±2.55[b]

[a,b]On the same row indicate a significant difference ($p \leq 0.01$)

DISCUSSION

Repeat breeding is a reproductive disorder that hinders the productivity of livestock and results in economic losses. The present study sought to determine if there were any biochemical differences in the blood of repeat breeders and fertile Ongole cows and if such biochemical "signatures" could be used for diagnostic purposes. In the present study, total protein content was found to be significantly lower in repeat breeder cows than in fertile cows. Low serum protein contributes to a deficiency in amino acids. It is well known that amino acids are the main precursors to the synthesis of hormones that are essential for regulating metabolic pathways for survival, growth, development and reproduction[10]. Furthermore, some amino acids, such as glutamate and aspartate, are important components of the neuroendocrine pathways that regulate the secretion of LH and FSH hormones[11].

The finding that repeat breeders present low protein plasma levels is in agreement with previous reports linking low serum protein levels with the deficiency of certain amino acids essential for gonadotropin and gonadal hormone synthesis, which might consequently result in hormonal imbalance and disrupted ovarian function. Through hormonal disruption, low serum protein levels can have a severe impact on reproduction, resulting in weak lust, silent heat, anoestrus, repeat breeding, early embryonic death, absorption of the embryo by the uterine wall and the birth of a weak foetus[3,4,12-15].

Similarly, the total cholesterol level was also significantly lower in repeat breeding cows compared to fertile animals. This finding corroborates similar previously reported observations[3,5,16]. Low lipid levels contribute to a low-energy status and decrease the presence of precursors for all other steroids synthesized in the body, such as adrenal cortex hormones, sex hormones, vitamin D and bile acids[17]. Furthermore, cholesterol is crucial for the biosynthesis of androstenedione, progesterone and oestrogen by granulosa cells under the influence of LH[9]. Specifically, LH stimulates production of pregnenolone by granulosa cells; pregnenolone is then converted to androstenedione by theca cells. From there, most androstenedione returns to the granulosa cells, where it is converted to oestrone and oestradiol. Fat-deficient cattle might prolong the anoestrus period, resulting in repetitive mating and a decrease in the number of ovulated eggs[18].

Repeat breeder cows had significantly lower glucose levels than normal cyclic cows, as previously reported in studies that showed low levels of glucose had adverse effects on reproduction, such as repeat breeding[3,12,13]. Low levels of blood glucose are indicative of a scarce energy level and consequent infertility. Low energy availability in cows in a negative energy balance shift to a catabolic condition, can result in increased plasma growth hormone, non-esterified fatty acid levels and decreased insulin hormone and glucose levels[19,20]. Moreover, compromised metabolic status reduces the function of preovulatory follicles in oestradiol production[21], which in turn suppresses pulsatile LH secretion and reduces ovarian responsiveness to LH stimulation, resulting in ovulation interference. In addition, low glucose levels impair the pituitary-hypothalamus axis, thus inhibiting GnRH secretion[22]. GnRH regulates growth, follicular development and ovulation and maintains an appropriate uterine environment for embryonic development. Thus, inhibited GnRH secretion blocks follicular development and ovulation, which might result in anovulation and anoestrus[22], as well as the inhibition of oestrogen and progesterone secretion[18], which lead to repeat breeding.

Finally, the plasma level of urea nitrogen was significantly higher in repeat breeder cows than in fertile cows. Similar findings of higher blood urea or milk urea levels have been

reported in repeat breeder cows and does[23-25]. High blood urea nitrogen (BUN) levels (>19 mg dL^{-1}) reduce pregnancy rates[26]. A similar result in this study indicated that in repeat breeder cows, BUN was higher than the above-mentioned limit. Elevated plasma urea nitrogen has negative impacts on uterine fluid quality, uterine pH and has a toxic effect on the endometrium, which adversely affects the motility and viability of sperm, resulting in the production of abnormal oocytes and reduced conception rates[6,27,28]. It has also been reported that high urea levels interfere with the expression of the mRNA of endometrial fertility-related genes[8].

CONCLUSION

The present study demonstrated that repeat breeder Ongole cross breed cows exhibit low blood levels of total protein, total cholesterol and glucose, but high levels of urea nitrogen. Therefore, blood biochemical profiles could be a useful diagnostic tool for evaluating the fertility of cattle and other livestock.

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SIGNIFICANCE STATEMENT

This study aimed to investigate total protein, total cholesterol and glucose, urea nitrogen and the feasibility of using those parameters for evaluating the fertility of cattle and another livestock. We believe that our study makes a significant contribution to the literature because We discovered that significantly different blood profiles between repeat breeder and fertile cows. Specifically, repeat breeder cows had lower levels of glucose, total cholesterol and total protein, but higher levels of urea nitrogen than fertile cows. Our study demonstrates that the low level of total protein, total cholesterol and glucose, accompanied by a high level of urea nitrogen, might cause reproductive problems in repeat breeders. Additionally, our findings indicate that biochemical blood profiling could be used as a diagnostic tool for repeat breeding.

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