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THE IMPACT OF AGE AND PHYSIOLOGICAL STAGE ON SOME BIOCHEMICAL PARAMETERS AND IMMUNE GLOBULIN IN LOCAL AND CROSSBREED HOLSTEIN DAIRY COWS

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Abstract:
This study was conducted in one of the animal fields in the Abi Al-Khassib area to investigate the impact of age and physiological stage on some biochemical parameters in the serum of local and crossbreed Holstein dairy cows. A total of 32 cows, including 16 local cows and 16 crossbreed Holstein cows, aged between 3 and 6 years were used during the period between 1st December 2019 to 31th May 2020, the total period of the experiment was 6 months, divided into two physiological stages: 3 months before parturition (gestation) and 3 months after parturition (lactation). The results showed a significant ($p<0.05$) increase in the concentrations of most biochemical parameters (cholesterol, glucose, triglycerides, urea, total protein, IgA, IgG, IgM, ALP) in older cows compared to younger cows, except for the ALT and AST enzyme concentrations. Pregnant cows had significantly($p<0.05$) higher levels of cholesterol, triglycerides and total protein compared to lactating cows, while lactating cows had significantly ($p<0.05$) higher levels of glucose, urea, enzymes (ALT, AST, ALP), and immunoglobulins (IgA, IgG, IgM). Additionally, the crossbred (H×L) cows had significantly ($p<0.05$) higher levels in cholesterol, glucose, triglycerides, urea total protein and ALP compared to the local breed cows. Whereas, the local breed cows recorded significantly ($p<0.05$) higher levels of ALT and AST enzymes and immunoglobulins (IgA, IgG, IgM) in the serum compared to the crossbred cows

Keywords: Age, Physiological stage, Biochemical parameters, Local breed, Holstein, Dairy cows.

INTRODUCTION

Cows are the first in produce milk when compared with other ruminant animals, and this production primarily depends on the content and components of milk, especially fat and protein (Parwin et al., 2005). Milk production is a quantitative trait influenced by various factors, including both genetic and non-genetic factors, genetic factors are associated with the breed and the genes carried by the animal, while non-genetic factors include the sex of the offspring, age of the animal, physiological status, health condition and external influences such as season and nutrition (Moretti et al., 2017; Dillane et al., 2018; Consolo et al., 2018).

Pregnancy and lactation stages in farm animals are considered a physiological condition that leads to dramatic changes, pregnant and lactated cows, in particular, often experience stress that results in significant alterations in blood parameters and metabolic components, there are many various changes occur in the female during, some of these changes involve fluctuations in nutrient metabolism, especially since the priority is to ensure fetal growth, which can lead to a decline in the body's blood parameters (Mir et al., 2008 ; Piccione et al., 2012).

Most of the problems faced by high-production cows occur immediately after calving and at the beginning of their production, this is due to negative energy imbalance, leading to increased lipolysis in the body, accumulation of fat in the liver, development of fatty liver deposition and increased concentration of enzymes (ALT, AST) (Bobe et al., 2004; Mohamed, 2014). Many studies have confirmed that breed, age and physiological status of cows have a significant effect on several biochemical parameters in their blood, including glucose, cholesterol, triglycerides, protein, albumin and globulin (Aarif and Aggarwal, 2015; Arfuso et al., 2016). When these parameters are within normal physiological limits, they reflect good health, with a positive impact on dairy production (Corian et al., 2017). Furthermore, the stability of immune globulin levels such as IgG, IgA, and IgM within the normal range reflects the health status of the animal and its resistance to stress, these immunoglobulins are influenced by various factors such as the age of the animal and its physiological status (Mehdi and Dufrasne, 2016; Butler,1969). So, the purpose of the study was to investigate the impact of age and physiological stage (pregnancy and lactation) on some biochemical parameters in the serum of local cows and crossbreed Holstein cows.

1. METHODOLOGY

The purpose of the study was to investigate the impact of age and physiological stage (pregnancy and lactation) on some biochemical parameters in the serum of local cows and crossbreed Holstein cows.

2. MATERIALS AND METHODS

This study was conducted in one of the animal fields in the Abi Al-Khassib area of southern Iraq (27 km from the centre of Basra governorate. A total of two- thirty dairy cows, including 16 local cows and 16 crossbreed Holstein cows (local × Holstein), aged between 3 and 6 years were used during the period between 1st December 2019 to 31th May 2020, the total period of the experiment was 6 months, divided into two physiological stages: 3 months before parturition (gestation) and 3 months after parturition (lactation). The cows were fed on bran (twice a day) and straw (ad libitum), and received continuous clean water inside the pins. They were also vaccinated against triple fever, malignant charcoal and hemorrhagic septicaemia before the start of the experiment.

Blood samples were taken from the jugular vein with a sterile medical syringe, with a volume of 10 ml for each cow. Samples were transferred to the laboratory using clot-free test tubes and centrifuged at 5000 rpm for 15 minutes to separate the serum, then, the serum was withdrawn and placed in clean, sterile test tubes and stored at -20C° until laboratory testing. Cholesterol, glucose, triglyceride, urea, total protein (Alanine amino transferase (ALT) and Aspartate aminotransferase (AST) concentrations were determined using the kit manual provided by Biolabo - France Company. To measure the concentration of immune globulins, the Enzyme Linked Immune Sorbent Assay (ELISA) method using a kit provided by (Lipoteichoic Acid) an Italian Company, following the manual of the kit.

Experimental data were analyzed statistically using (SSPS, 2019) as a factorial experiment with three factors. First factor: the age of cows, second factor: physiological status (lactating and pregnant cows) and the third factor: cows breed (local and crossbred Holstein) on the studied traits. To test the significance of the differences between the studied means, the Least Significant Difference (LSD) test was used at a significant level ($p<0.05$). The analysis was performed using the following mathematical model:

$Yijrk=M +Ai +Bj+Cr +TBCijr + eijr$

Where: Yijrk = value of the study traits, μ = the overall mean of the trait, Ai = effect of age cows (i=2), Bj = effect of the physiological status (j=2), Cr= effect of cows breed (r=2), TBCijr= the interaction between age, physiological status and breed, eijrk= The experimental error is randomly and normally distributed with a mean of zero and a variance of σ^2e.

3. RESULTS AND DISCUSSION

Tables 1, 2 and 3 indicate a significant effect ($P<0.05$) of age on the biochemical parameters (cholesterol, glucose, triglycerides). The age group 5-6 years was higher in serum concentrations of these parameters in local and crossbred cattle than the 3-4 year group. This can be attributed to the increased energy requirements as animals age, leading to larger rumen size, increased feed intake, elevated metabolic processes and energy production represented by these three parameters (Nath, 2006; Coroian et al., 2017).

In addition, the tables indicate a significant effect ($P<0.05$) of the physiological status of all three biochemical parameters. Pregnant cows exhibited significantly higher concentrations of cholesterol and triglycerides compared to lactating cows, while lactating cows showed significantly higher levels of glucose in the blood serum, may due to that the energy parameters, which include cholesterol and triglycerides, are linked to a change in the physiological state of the animal, especially at the stage of pregnancy, additionally, the decrease in cholesterol and triglyceride levels during lactation stage may result from increased lipase activity to provide higher energy levels and initiate milk synthesis in the mammary gland (Agarwal et al., 2008; El-Tarabany et al., 2016). It is also that the elevation of cholesterol levels during pregnancy is due to its role in synthesizing steroid hormones such as estrogen and progesterone (Prava and Dixtt, 2006; Arfuso et al., 2016). As for glucose levels, the high levels observed in lactating cows compared to pregnant cows in both local and crossbred cows can be attributed to increased feed consumption during milk production and the subsequent energy metabolism required for milk synthesis (Abd-ElNaser et al., 2014; Abdul Kareem, 2013). Azza et al., (2010) reported that elevated glucose levels may be attributed to the need for meeting the requirements of lactose synthesis, particularly in high-production cows.

In addition, the tables show a significant effect ($P<0.05$) of the breed on the concentrations of the three parameters. The crossbred cattle (H*L) showed higher concentrations of these parameters compared to the local breed, may be due to the larger rumen capacity and size of crossbred cattle, allowing for higher feed intake and increased metabolic rates which led to more production of these parameters (Ingvarsten et al., 2003; Roche et al., 2009).

Table (1) effect of age and physiological stage on cholesterol concentration (g/L) in the serum of local and crossbreed (H×L) (mean ±SD)

Breed	Age group	Cholesterol		
		Physiological stage		Breed average
		Dairy	Pregnant	159.87±4.28 B
Local	(3-4) Years	136.40±3.11	143.27±3.86	
	(5-6) Years	176.45±5.13	183.37±4.11	
Crossbreed(H×L)	(3-4) Years	152.82±3.81	168.71±4.09	180.39±5.16 A
	(5-6) Years	193.11±5.82	206.93±4.27	
Average physiological stage		164.70±3.73 B	175.57±4.06 A	
Average age effect	(3-4) Years	150.30±3.14 B		
	(5-6) Years	189.97±4.73 A		

^1 (H×L) : Holstein × local breed.^2 The means with different letters are differ significantly at level (P<0.05).

Table (2) effect of age and physiological stage on glucose concentration (g/L) in the serum of local and crossbreed (H×L) (mean ±SD)

Breed	Age group	Glucose		
		Physiological stage		Breed average
		Dairy	Pregnant	429.92±5.03 B
Local	(3-4) Years	387.45±6.16	143.27±3.86	
	(5-6) Years	562.14±7.86	183.37±4.11	
Crossbreed(H×L)	(3-4) Years	522.17±6.80	168.71±4.09	507.40±7.13 A
	(5-6) Years	613.93±8.27	206.93±4.27	
Average physiological stage		521.42±7.09 A	390.89±5.86 B	
Average age effect	(3-4) Years	403.43±6.14 B		
	(5-6) Years	533.88±7.25 A		

^1 (H×L) : Holstein × local breed.^2 The means with different letters are differ significantly at level (P<0.05).

Tables 4 and 5 show a significant age effect (P < 0.05) on urea and total protein levels in cows' serum. Cows aged 5-6 years exhibited higher concentrations of these parameters compared to cows aged 3-4 years in both local and crossbred breeds, may be attributed to the fact that older animals have a larger digestive capacity, allowing them to consume larger quantities of feed and digest more protein content present in the diet, resulting in higher ammonia production to the fact that as animals age, their digestive system and rumen capacity increase, allowing them to consume larger quantities of feed and produce higher amounts of ammonia which led to (Grasso et al., 2004; Harris and Bachman, 2008; Bertoni and Tervisi, 2013). This leads to an increase in urea and total protein concentrations in the blood. In their studies, Samanta and Dass (2007) and Hech et al., (2009) suggested that the elevation of protein levels in animal serum depends on several factors, including the age of the animal, hormonal balance and the nutritional status of the animal. They further noted that this increase in protein levels is indicative of anabolic processes and protein deposition in the body.

Table (3) effect of age and physiological stage on triglycerides concentration (g/L) in the serum of local and crossbreed (H×L) (mean ±SD)

Breed	Age group	Triglycerides		
		Physiological stage		Breed average
		Dairy	Pregnant	130.56±3.91 B
Local	(3-4) Years	109.11±3.14	117.81±3.85	
	(5-6) Years	133.21±3.17	162.11±4.11	
Crossbreed(H×L)	(3-4) Years	128.42±4.09	138.21±4.09	145.48±4.47 A
	(5-6) Years	142.13±4.75	173.17±4.27	
Average physiological stage		128.22±4.12 B	147.83±5.73 A	
Average age effect	(3-4) Years	123.39±4.86 B		
	(5-6) Years	152.66±5.96 A		

^1 (H×L) : Holstein × local breed.^2 The means with different letters are differ significantly at level (P<0.05).

A significant effect (P<0.05) of physiological state on urea and total protein levels was observed in bovine serum. Urea levels were higher for lactating cows than for pregnant cows. Nutrition is considered a major factor contributing to the variation in urea levels in the blood serum, and increasing energy levels, especially in the diets of lactating cows, leads to higher milk production rates, this increase is associated with an elevation in protein levels in the feed, resulting in higher ammonia production in the rumen and consequently higher urea levels in the blood serum (Harris and Bachman, 2008; Wathes et al., 2009). In addition, the crossbred cows had higher concentrations of these parameters compared with the local breed. This difference can be explained by the fact that crossbred cows have a higher body weight and larger digestive capacity, resulting in increased rates of metabolic digestion and its products, including urea and total protein in the blood, this finding agreed with the results of Wattiaux et al., (2005) and Abd-El Naser et al., (2014), which found that there are several factors influencing the concentrations of urea and total protein in animal blood, including nutrition, environmental conditions, physiological stage and breed.

Table (4) effect of age and physiological stage on urea concentration (mmol/L) in the serum of local and crossbreed (H×L) (mean ±SD)

Breed	Age group	Urea		
		Physiological stage		Breed average
		Dairy	Pregnant	23.52±2.27 B
Local	(3-4) Years	20.73±1.47	16.26±1.98	
	(5-6) Years	32.16±1.72	24.93±2.03	
Crossbreed(H×L)	(3-4) Years	22.93±1.51	18.47±2.11	26.87±1.95 A
	(5-6) Years	37.98±1.96	28.11±1.17	
Average physiological stage		28.45±2.01 A	21.94±1.93 B	
Average age effect	(3-4) Years	19.60±1.08 B		
	(5-6) Years	30.80±2.42 A		

^[1] (H×L) : Holstein × local breed.^[2] The means with different letters are differ significantly at level (P<0.05).

Table (5) effect of age and physiological stage on total protein concentration (g/L) in the serum of local and crossbreed (H×L) (mean ±SD)

Breed	Age group	Total protein		
		Physiological stage		Breed average
		Dairy	Pregnant	35.88±3.71 B
Local	(3-4) Years	21.98±3.18	32.14±3.27	
	(5-6) Years	36.11±3.21	53.28±5.13	
Crossbreed(H×L)	(3-4) Years	27.18±2.18	42.98±3.97	45.39±4.11 A
	(5-6) Years	48.27±3.20	63.11±4.11	
Average physiological stage		33.39±3.14 B	47.89±3.37 A	
Average age effect	(3-4) Years	31.07±3.30 B		
	(5-6) Years	50.19±4.28 A		

^1 (H×L) : Holstein × local breed.^2 The means with different letters are differ significantly at level (P<0.05).

The concentrations of AST and ALT enzymes increased significantly (P<0.05) in cows aged 3-4 years compared to cows aged 5-6 years (Tables 6 and 7). These enzymes are present in various tissues, including the heart, liver, skeletal muscles and blood, their presence in the blood serum indicates that the animals are exposed to stress or are under negative environmental and nutritional conditions, younger cows are generally less resistant and tolerant to stress compared to older cows, which explains the higher concentration of these enzymes in the first group (Milinkovic-Tur et al., 2005; Dokovic et al., 2017). In addition, lactating cows showed a significant increase in the concentration of these enzymes in comparison with pregnant cows. This means that the lactation stage is considered more stressful in animals than in pregnancy. Our result concurs with the results of Dokovic et al., (2017) and Fiore et al., (2017), which reported an increase in the concentrations of ALT and AST enzymes during the milk production stage in cows and buffaloes.

A significant (P<0.05) increase in enzyme concentrations was observed in local cows compared to the crossbred breed. This suggests that the crossbreed cows are more tolerant to stress conditions (pregnancy and lactation) compared to the local one, due to the process of selective breeding, which has improved many numerous in dairy cows. Numerous studies, including Yaylak et al. (2009), Mahima et al., (2013) and Das et al., (2019) found that hybridization enhances several characteristics, including production and stress resilience in local and crossbreed cattle.

Table (6) effect of age and physiological stage on ALT enzyme concentration (IU/L) in the serum of local and crossbreed (H×L) (mean ±SD)

Breed	Age group	ALT		
		Physiological stage		Breed average
		Dairy	Pregnant	30.07±4.67 A
Local	(3-4) Years	37.92±3.11	32.11±4.56	
	(5-6) Years	28.51±3.30	21.73±3.12	
Crossbreed(H×L)	(3-4) Years	32.77±2.95	27.11±2.91	24.53±3.61 B
	(5-6) Years	21.11±2.15	17.14±3.43	

Average physiological stage		30.08±3.51 A	24.52±3.75 B
Average age effect	(3-4) Years	32.48±4.81 A	
	(5-6) Years	22.12±3.43 B	

^1 (H×L) : Holstein × local breed.^2 The means with different letters are differ significantly at level (P<0.05).

Table (7) effect of age and physiological stage on AST enzyme concentration (IU/L) in the serum of local and crossbreed (H×L) (mean ±SD)

Breed	Age group	AST		
		Physiological stage		Breed average
		Dairy	Pregnant	43.86±2.91 A
Local	(3-4) Years	52.13±3.14	46.73±2.14	
	(5-6) Years	40.74±2.17	36.21±2.97	
Crossbreed(H×L)	(3-4) Years	48.91±4.27	41.28±3.91	38.78±2.24 B
	(5-6) Years	36.27±2.91	28.64±4.08	
Average physiological stage		44.51±4.01 A	38.22±3.11 B	
Average age effect	(3-4) Years	47.28±4.85 A		
	(5-6) Years	35.47±3.28 B		

^1 (H×L) : Holstein × local breed.^2 The means with different letters are differ significantly at level (P<0.05).

The concentration of the ALP enzyme was significantly (P<0.05) higher in older cows compared to younger cows (Table 8). The levels of this enzyme are typically associated with milk production, and an increase in its concentration in the blood serum is correlated with an increase in the calcium content of milk, since older cows generally have higher milk production and higher calcium concentrations, so, it is normal that the enzyme concentration will arise in her blood serum (Mohamed, 2014). Lactating cows showed a significantly higher concentration of this enzyme than pregnant cows. This is because ALP plays a role in promoting and maintaining natural calcium levels, especially during milk production stages, while, the decrease in ALP concentration during pregnancy is a result of increased requests for calcium to completing the growth of the fetal skeleton (Carlos et al., 2013). Finally, the concentration of the ALP enzyme was significantly higher in crossbreed cows than local cows.

Table (8) effect of age and physiological stage on ALP enzyme concentration (IU/L) in the serum of local and crossbreed (H×L) (mean ±SD)

Breed	Age group	ALP		
		Physiological stage		Breed average
		Dairy	Pregnant	166.33 ±7.38 B
Local	(3-4) Years	168.27±6.92	130.13 ±6.52	
	(5-6) Years	203.12 ±9.78	163.81 ±7.54	
Crossbreed(H×L)	(3-4) Years	184.15 ±7.44	147.11 ±7.83	184.42 ±8.02 A
	(5-6) Years	224.28 ±10.07	182.18 ±10.13	
Average physiological stage		194.95 ±8.11 A	155.80 ±7.23 B	
Average age effect	(3-4) Years	157.42±8.26 B		
	(5-6) Years	193.35 ±9.02 A		

^1 (H×L) : Holstein × local breed.^2 The means with different letters are differ significantly at level (P<0.05).

Tables 9, 10 and 11 indicate that age has a significant effect (P<0.05) on levels IgA, IgG and IgM immunoglobulins. Cows in the older age group (5-6 years) showed a significantly increased in the concentration of these proteins compared to younger cows (3-4 years), may be due to the fact that these substances are glycoproteins, and their production primarily depend on the development of the immune system in the animal's body, especially the production of white blood cells, which increase in numbers with the advancement of the animal's age (Herr et al., 2011; Dugovich et al., 2017).

Table (9) effect of age and physiological stage on IgA concentration (mg/100ml) in the serum of local and crossbreed (H×L) (mean ±SD)

Breed	Age group	IgA		
		Physiological stage		Breed average
		Dairy	Pregnant	2.16 ±0.36 A
Local	(3-4) Years	1.26±0.11	0.70±0.13	
	(5-6) Years	4.28 ±0.13	2.41±0.14	
Crossbreed(H×L)	(3-4) Years	1.47±0.05	0.85±0.09	1.41.±0.27 B
	(5-6) Years	2.17±0.08	1.15 ±0.07	
Average physiological stage		2.30 ±0.51 A	1.28 ±0.42 B	
Average age effect	(3-4) Years	1.07 ±0.18 B		
	(5-6) Years	2.51 ±0.48 A		

^1 (H×L) : Holstein × local breed.^2 The means with different letters are differ significantly at level (P<0.05).

Similarly, lactating cows showed a significantly increased in the concentration of immune globulins compared to pregnant cows , perhaps the reason is due to that in the stage of pregnancy, the immune bodies decrease in the body, which causes a decrease in the concentration of immune proteins, as they (in the blood) are linked with a positive relationship (Gunti and Notkins, 2015). However, the local breed outperformed the crossbreed in concentrations of these parameters in all age groups. The local breed may be more resistant to nutritional and management stress as well as adverse weather conditions, resulting in better overall health and this may lead to increased immunity and reduced susceptibility to diseases due to the higher levels of immune bodies and elevated immune protein concentrations (Herr et al., 2011).

Table (10) effect of age and physiological stage on IgG concentration (mg/100ml) in the serum of local and Crossbreed (H×L) (mean ±SD)

Breed	Age group	IgG		
		Physiological stage		Breed average
		Dairy	Pregnant	
Local	(3-4) Years	28.16±0.91	21.95±1.17	32.76 ±2.01 A
	(5-6) Years	46.13 ±0.96	34.78 ±1.79	
Crossbreed(H×L)	(3-4) Years	23.29 ±1.02	18.14 ±1.02	26.72 ±2.47 B
	(5-6) Years	37.15 ±1.04	28.31 ±1.12	
Average physiological stage		33.68±1.54 A	25.80±1.93 B	
Average age effect	(3-4) Years	22.89 ±1.43 B		
	(5-6) Years	36.60 ±2.17 A		

^1 (H×L) : Holstein × local breed.^2 The means with different letters are differ significantly at level ($P<0.05$).

Table (11) effect of age and physiological stage on IgM concentration (mg/100ml) in the serum of local and Crossbreed (H×L) (mean ±SD)

Breed	Age group	IgM		
		Physiological stage		Breed average
		Dairy	Pregnant	
Local	(3-4) Years	1.84 ±0.07	1.07 ±0.08	2.04 ±0.35 A
	(5-6) Years	3.09 ±0.27	2.15±0.29	
Crossbreed(H×L)	(3-4) Years	0.95 ±0.04	0.64 ±0.07	1.19 ±0.18 B
	(5-6) Years	1.93 ±0.32	1.22 ±0.14	
Average physiological stage		1.95 ±0.81 A	1.27 ±0.57 B	
Average age effect	(3-4) Years	1.26 ±0.36 B		
	(5-6) Years	2.09 ±0.67 A		

^1 (H×L) : Holstein × local breed.^2 The means with different letters are differ significantly at level ($P<0.05$).

4. CONCLUSIONS

The current study concluded that biochemical parameters in dairy cows are influenced by a number of factors, including breed, age and physiological stage. These parameters can be used as indicators of the health status and the response of dairy cows to stressful conditions throughout their productive lives at different ages whether they are of local or crossbred breeds, especially, when raised in southern Iraq.

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