*International Journal of Food, Agriculture and Veterinary Sciences (Online)*
*An Online International Journal Available at http://www.cibtech.org/jfav.htm*
*2012 Vol. 2 (1) January-April, pp. 47-53/Al-Seaf and Al-Harbi*
***Research Article***

# VARIABILITY OF DISEASE RESISTANCE, HEMATOLOGICAL PARAMETERS AND LYMPHOCYTE PROLIFERATION IN TWO GOAT BREEDS AND THEIR F1 AND F2 CROSSES

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**ABSTRACT**
This experiment has been carried out to study the comparative disease resistance of two breeds of goats and their F1 and F2 crosses. The breeds were a local Ardi goats (n=55), imported Syrian (Mediterranean) goats (n=51) and their F1 (n=30) and F2 (n=44) crosses. The goats were ear-tagged and kept in four different pens under one shade. Their health was observed for ten months by two qualified veterinarians. Disease resistance was measured by morbidity rate, mortality rate, incidence of respiratory diseases and incidence of gastro-intestinal diseases in each breed for the ten month period. The total erythrocytes (RBCs), Total leucocytes (TLC), packed cell volume (PCV), N (Neutrophils), L (Lymphocyte), N/L ratio and blood indices (CI, MCV, MCH and MCHC) beside *in vitro* lymphocyte glucose consumption were determined at the end of experimental period. Results showed that goats that fell ill during the experimental period were 25.5% in the Ardi breed, 28.8% in the Syrian breed, 26.6% in the F1 generation and 38.8% in the F2 generation. The percentages of goats that died were 9.1% in Ardi breed, 15.4% in the Syrian breed, 9.1% in F1 generation and 13.3% in F2 generation. Respiratory infections rates were 14.4% for the Ardi, 15.2% for the Syrian, 8% for the F1 generation and 17.2% for the F2 generation. The rates of enteric disease infections were 28.2% in Ardi goats, 30.2% in Syrian, 26.6% in F1 cross and 38.6 % for F2 generation. The Ardi breed of goats had obtained the highest values of RBC, L and TLC. This breed with its F1 generation suffered the least rate of mortality during the study period with overall the best performance of good health. The new cross-breed F2 generation showed the worst health performance of all groups with lowest N and highest glucose consumption by Phytohaemagglutinin-P (PHA) that probably form the physiological basis for the future adaptation of the species to the Saudi desert environment.
This study has indicated that hematological and glucose consumption values could serve as a baseline information for comparison in conditions of physiological and health status of goats kept under Saudi desert environment.

***Keywords:*** *Goats, Crossbreeding, Resistance, Glucose Consumption.*

## INTRODUCTION

Crossing local breeds of goats with foreign ones is a common practice in may countries with the aim of increasing production and disease resistance.
Breeding for disease resistance has been a target by many research workers because the conventional approaches to disease control (vaccination, medication, isolation of animals from pathogens, improved sanitation and eradication. The great challenges facing disease control in the veterinary profession such as the effectiveness of some vaccines and development of resistance of pathogens to drugs and chemicals are becoming increasingly common (Gruner 1991). Organic farming is gaining momentum and it necessitates the search for naturally resistant animals.
Small ruminant systems of production are based on pasture feeding and therefore parasitic diseases caused by helminthes are of primary importance to producers. The need to reduce the use of drugs has also involved interest in alternative and complementary methods of control such as increasing genetic resistance to nematode parasites (Cabaret and Gruner 1986; Gruner and Cabaret 1988; Stear and Murray, 1994).

Investigations on the genetic resistance of sheep and goats to intracellular bacteria such as Salmonella have been initiated in France, with the objective of finding a major gene for resistance. An additional aim is to increase our knowledge of the immune mechanisms involved in resistance to intracellular bacteria in ruminants and to demonstrate the feasibility of genetic improvement of innate resistance to such pathogens of worldwide importance. (Gruner et al 1988). Emerging viral diseases in sheep and goat are Maedi Visna and Caprine Arthritis Encephalitis caused by lentiviruses. Studies have be en undertaken in diagnosis research, epidemiology and vaccination .Scrapie is a widespread disease with genetically determined susceptibility. It has been studied since the sixties in the United Kingdom and more recently in France. A gene for susceptibility has been determined in mice and sheep (Hunter 1992). The mechanism of transmission of the disease and the nature of the infectious agent are largely unknown. Because of the spread of this kind of disease in Europe (and the Middle East) and the value of the work done by different teams in the United Kingdom, it seemed important to us that a short description of the current investigations should be included here.

Comparison of the susceptibility of various animal populations (inbred or outbreed lines, selected lines, flocks, breeds) is a powerful means of identification of mechanisms of resistance to natural and, more efficiently, experimental infectious or parasitic diseases.

Most of the available literature on genetic variation in disease resistance in Indian goats has originated at the Central Institute for Research on Goats (CIRG) (Acharya 1982), Makhdoom, near Agra in the state of Uttar Pradesh and at CIRG's Western Regional Research Centre (WRRC) located at Avikanagar in the state of Rajasthan. The breeds on which at least some information is available are the ]amunapari, Barbari, Black Bengal, Sirohi, ]hakrana, Beetal, Marwari and Kutchi. Mortality due to parasitic infestation in goats of the Barbari, Jamunapari and Jhakrana breeds was found to be 10% over the years 1985 to 1990 (Sharma e t al., 1992). These deaths occurred despite a regular deworming regime. Paramphistomiasis is reported to have caused 44 to 69% morbidity and 45 to 88% mortality in goats (Chattopadhyay e t al., 1992).

Colibacillosis in kids Diarrhoea is one of the primary causes of kid mortality in goats. Colibacillosis is highly prevalent in kids throughout the Indian country (Chattopadhyay et al., 1992). At the livestock farm of CIRG, 180 clinical cases of colibacillosis in Barbari and Jamunapari kids were observed during March-April 1985 (Vihan et al., 1990). The incidence of E. coli infection was greater in the age group 0-10 days in Barbari and 11-20 days in Jamunapari kids. The mortality was higher in Barbari kids (46%) than in Jamunapari kids (22%). The mortality due to coli bacillosis was also found to be higher in Barbari than in Jamunapari kids by Vihan (1991) after a study of the kid mortality at CIRG from 1985 to 1989. A similar study of the prevalence of E. coli infection among Barbari and Jamunapari kids born at CIRG during 1985-1988 indicated that mortality was higher in the Barbari breed in 1987 and it was higher in the Jamunapari breed in 1985 and 1988 (Vihan et al., 1990). But this difference in mortality between years and breeds was not statistically significant. Singh et al. (1992b) also found the mortality due to E. coli infection among neonates of Barbari to be significantly higher (12%) as compared to that in Jamunapari kids (6%). While evaluating these results, it must be borne in mind that the Jamunapari and Barbari breeds are kept at separate locations on the CIRG campus.

The immune system is crucial for the defense against organisms and toxic products that cause infection. A functional immune response requires rapid and extensive cell growth and proliferation. Glucose consumption test was employed to evaluate the transformation of lymphocyte in vitro. The test was comparable to the 3H-thymidine uptake assay (radioisotope-based methods of Simakura et al. (1985). This test evaluated the mitogenic response of lymphocytes that reflect the humoral and cell mediated immune response in vitro. Reference values for hematologic parameters are necessary for assessment of the physiologic status of animals, as well as for assessment of their health and nutritional status (Lo´pez-Olvera et al., 2006). The goal of the present study is to study the comparative disease resistance of two breeds of goats and their F1 and F2 crosses with a special reference to glucose consumption test and some hematological parameters.

## MATERIALS AND METHODS

### Experimental animal and protocol

This experiment was conducted on 4 flocks of goats, a local Ardi breed (n=55), A Seryian breed (n=52) and their F1 (n=30) and F2 crosses (n=44). The goat flocks were kept in four different pens under one shade. They were ear-tagged and fed on alfalfa (*Medicago sativa*) hey, a concentrate diet made of barley and wheat bran, and had free access to drinking water.

The goat flocks were assessed for disease resistance for a period of ten months. The criteria of evaluating disease resistance was done by recording morbidity rates (total number of animals that fell sick), mortality rate (total number of animals that died), incidence of respiratory diseases and incidence of gastro-intestinal diseases. Diagnosis of respiratory and enteric infections was carried out by two qualified veterinarians who employed routine clinical veterinary diagnosis. Post mortem examination was performed and tissues were processed for histopathology for confirmation purposes.

### Lymphocyte transformation assay:

Assay of lymphocyte transformation was done using glucose consumption test as previously described by Ishikawa and Shirahata (1986) and Walters et al. (2003). Briefly, citrated blood from different flocks at the end of ten months was diluted 1:1 with phosphate –buffered saline (PBS, pH 7.2) and layered over Ficoll histopaque solution(Sigma® H8889 d = 1.077 g mL-1). The blood was centrifuged at 400 xg for 30 minutes after which the mononuclear cell layer was aspirated. The cells were washed three times with PBS and twice with the culture medium. The medium consisted of RPMI- 1640 (Gibco Laboratories, Paisley, UK) supplemented with 10% fetal calf serum (Gibco Laboratories, Paisley, UK), 2 mmol/l l-glutamine (Sigma Laboratories, St. Louis, Mo, USA), 100 IU/mL penicillin and 100 mg/mL streptomycin (Sigma Laboratories, St. Louis, Mo, USA). The cells were resuspended in the medium and were examined for lymphocyte purity and viability using trypan blue dye exclusion method where the viable (unstained) cells were counted in a hemocytometer under a light microscope (Huttunen et al., 2004). Phytohaemagglutinin-P (PHA, Sigma-Aldrich, USA) was used for T cell mitogen (Kosti et al. 2010 and Marko et al. 2010) and lipopolysaccharide (LPS, Sigma- USA) was used for B cell mitogen (Dardick et al., 1983). Each mitogen was reconstituted with sterile PBS and divided into small aliquots and stored at -30°C until used.

Lymphocytes were cultured in triplicate in the presence of either 5 $\mu$g/mL PHA or 10 ug/100ul LPS in 24-well plates (Corning Costar® No. 3526). Each well contained 200µl of culture suspension containing $2 \times 10^6$ cells . The cultures were kept at 37◦C in a humidified 5% $CO^2$ atmosphere for 72 hours. **(**Skendros et al.,2008). Glucose was estimated in the terminal incubation medium using assay kits by monitoring the change in optical density at 500 nm due to reduction of NAD through glucose consumption by hexokinase (Chang et al., 1992). The lymphocyte stimulation was estimated as the quantity of glucose (mg/dL) consumed minus the concentration of stimulated cell culture of control samples.

### Hematological analyses:

Portion of blood samples were put into vacutainers with K3-EDTA for total leukocyte count (TLC), red blood cell count (RBCs), neutrophil (N), lymphocyte (L) and N/L ratio and packed cell volume (PCV) within one hour after collection according to the methods of Schalm et al.(1975). Blood indices as Color index (CI), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC) were calculated.

### Statistical analysis:

Differences between means are analysed for significance by using a INSTAT 2 computer-adapted program.

## RESULTS

Total mortality, morbidity, respiratory and enteric diseases in the four flocks of goats during the 10 month period of observation is shown in table 1.The percentage of goats that fell ill during the experimental period was 25.5% in the Ardi breed, 28.8% in the Syrian breed, 26.6% in the F1 generation and 38.8% in the F2 generation. The total number of goats that died 9.1% in Ardi breed,

15.4% in the Syrian breed, 9.1% in F1 generation and 13.3% in F2 generation. Respiratory infections rates were 14.41% for the Ardi, 15.2% for the Syrian, 8% for the F1 generation and 17.2% for the F2 generation. The rates of enteric disease infections was as follows Ardi goats was 28.2%, Syrian was 30.2%, F1 cross 26.6 and 38.6 for F2 generation.

In this experiment, the mean lymphocyte population was more than 90% pure and they were more than 95% viable by trypan blue exclusion.

**Table 1: Total morbidity, mortality, respiratory and enteric diseases in the four flocks of goats during 10 months period.**

| **Breed** | **Ardi (n=55)** | **Syrian (n=52)** | **F1 (n=30)** | **F2 (n=44)** |
|---|---|---|---|---|
| Morbidity rate | 25.5%[a] | 28.8%[a] | 26.6%[a] | 38.6%[b] |
| Mortality rate | 9.1%[a] | 15.4%[b] | 9.1%[a] | 13.3%[b] |
| Respiratory diseases | 14.4%[a] | 15.2%[a] | 8.0 %[b] | 17.2 %[a] |
| Enteric diseases | 28.2%[a] | 30.2%[a] | 26.6%[a] | 38.6 %[b] |

*number in the same raw with similar letters are statistically insignificantly different
*numbers in the same raw carry different letters are significantly different at $p< 0.05$

**Table 2: Hematological indices of the four flocks of goats after 10 months period.**

| **Breed** | **Ardi** | **Syrian** | **F1** | **F2** |
|---|---|---|---|---|
| RBCs $10^6$/µl | 10.74± 2.33 | 9.93±1.65 | 9.76±1.58 | 10.10±2.12 |
| Hb g% | 10.14±1.24 | 11.58±2.47 | 10.81±1.65 | 12.77±2.65 |
| TLC $10^3$ / µl | 8.80±1.09 | 8.88±1.72 | 9.70±1.26 | 8.86±0.95 |
| CI | 1.08±0.31 | 1.39±0.13 | 1.28±0.28 | 1.23±0.31 |
| MCV (fl) | 20.45±0.35 | 19.56±3.74 | 22.48±1.62 | 20.61±3.01 |
| MCH (pg) | 8.53±0.11 | 7.71±1.91 | 8.31±1.27 | 8.88±1.11 |
| MCHC (g/dl) | 42.94±0.30 | 42.83±3,86 | 45.81±4.44 | 46.60±5.32 |
| PCV% | 37.4±3.25 | 33.83±4.12 | 36.04±5.11 | 37.67±2.74 |
| N % | 29.01±2.12 | 29.01±3.17 | 29.2±2.63 | 24.02±3.64 |
| L % | 49.10±3.07 | 45.00±4.18 | 43.4±2.32 | 44.66±2.74 |
| N/L ratio | 0.57±0.03 | 0.65±0.03 | 0.64±0.01 | 0.56±0.03 |

**Table 3: Glucose consumption (mg\dl) of LPS - and - PHA stimulated lymphocytes of the four flocks of goats after 10 months period.**

| **Breed** | | Glucose concentration in the incubation medium (mg/dl) | | | |
|---|---|---|---|---|---|
| | | **Ardi** | **Syrian** | **F1** | **F2** |
| **LPS** | Without LPS | 33.88±4.02 | 34.49±4.28 | 35.31±2.11 | 33.50±5.21 |
| | With LPS | 18.14±3.57 | 15.20±2.26 | 21.25±2.25 | 16.63±1.73 |
| | Glucose consumption | 16.52 ±2.20 | 19.88±2.96 | 14.41±3.61 | 18.92±3.67 |
| **PHA** | Without PHA | 32.64±4.48 | 33.65±3.39 | 33.72±3.64 | 34.16±3.26 |
| | With PHA | 17.31±2.20 | 16.59±1.47 | 11.47±2.18 | 9.18±1.44 |
| | Glucose consumption | 15.25±2.65[a] | 18.13±2.34[a] | 21.61±3.50[a] | 24.29±2.71[b] |

**values in the same raw with similar letters are statistically insignificantly different*
**numbers in the same raw carry different letters are significantly different at p< 0.05.*

Results showed that stimulation of lymphocyte via PHA led to highly inducing glucose uptake for F1 and F2 crosses than related parents. The pattern of change in glucose consumed by LPS stimulated lymphocyte did not differ between the two breeds of goats and their F1 and F2 crosses. Statistically insignificant values of blood parameters measured here were obtained as illustrated in table 2.

**DISCUSSION**

Previous studies on the effect of crossbreeding the local Saudi Ardi goats with Syrian Mediterranean goats on disease resistance are poorly documented in the literature.

Results reported in this paper have shown that the imported Syrian breed of goats had suffered the highest rate of mortality and this is expected as its not adapted to the Saudi desert environment.

Experiments have been conducted worldwide to transfer disease resistance between breeds. For instance, the Red Masai sheep has proven to be genetically resistant, or less prone, to infestation with intestinal worms (Geerlings et al., 2002). The Uda sheep of Northern Nigeria is much less susceptible to foot rot, while the Kuri cattle kept along the shores of Lake Tchad are very resistant to insect bites. N'dama and some other breeds of indigenous African cattle are resistant to infection with trypanosomes. Such disease resistance is compromised when animals are crossed only for high productivity. For example, the Orma Boran cattle kept by the Orma people in the Tana River District of Kenya are much more resistant to trypanosomes than their relative, the Improved Kenya Boran, which has been selected for meat gains over several generations. Thus in areas where tsetse pressure is high, the Orma Boran gains weight faster than the Improved Kenyan Boran. Similarly, local "backyard" chickens are likely to be more resistant to diseases and to certain parasites than are the "improved" breeds.

Imported Saanen goats and South African Indigenous goats were crossbred, and all three types were compared in terms of productivity, milk production and disease resistance between 1988 and 1994. The annual mortality rates of 10% for Saanens and 15% for Crossbreds were high, compared to that for the Indigenous goats of 4%. The most important causes of death were mastitis, ketosis and pneumonia. Few cases of dystocia were recorded, but some goats were lost as a result of uterine infections and peritonitis. Pregnancy toxaemia occurred with increased demand for energy late in gestation. Only two cases of heartwater were recorded (Donkin and Boyyazoglo 2004).

Scott et al (2007) reported resistance of cross-bred goats in relation to rinderpest infection. The epidemiological character of rinderpest is influenced by the innate resistances of the hosts at risk. The classic example being the difference in the responses of Zebu cattle breed in the foothills of the Himalayas and on the plains of India; most hill cattle died when infected with rinderpest whereas susceptible plains cattle often survived. Variations in disease resistances using purebred and crossbred Nigerian Dwarf goats that were shown to be due genetic factors (Scott 2007).

The evaluation of the haematological parameters is a good way of assessing the health status of animals as it plays a vital role in the physiological, nutritional and pathological status of animals. The values of PCV and RBCs reported here in agreed with the values reported by Lazzaro and Saanendoah (2005) and Belewu et al. (2006) for similar animal. The value of the white blood cell (WBC) obtained supported the reports of Daramola et al. (2005) that goats possesses a protective system providing a rapid and potent defense against infectious agent. The percentage of the Neutrophil and lymphocyte in all the animals conform to the reported values of Lazzaro and Saanendoah (2005) and Belewu et al. (2006). This probably shows that animals maintained an active immune system that defends the body against infection, allergic reactions, parasites and antigens**.**

In conclusion, results obtained in this paper have shown that the local Ardi breed of goats had obtained the highest values of RBC,L and TLC. This breed with its F1 generation suffered the least rate of mortality during the study period with overall the best performance of good health. The new cross-breed F2 showed the worst health performance of all groups with lowest N and highest glucose consumption by PHA that probably form the physiological basis for the future adaptation of the species to the Saudi desert environment.

**ACKNOWLEDGEMENTS**
The research team is thankful to the Deanship of Scientific Research, Qassim University for financing this project. Thanks are extended to staff of the Department of Animal Production at the University's Agricultural and Veterinary Research and Experimental Station, for care of experimental animals and help in sample collection. Last and by no means least, the research team is thankful to Dr. Ibrahim Mosa for caring for the health of experimental animals.

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