CHAPTER 6

DISCUSSION

Adverse climatic conditions (heat stress) in terms of high ambient temperature and relative humidity are negatively correlated with production performance of the birds (Wiernusz, 1998). The term heat stress is often used to define response of birds to a hot and humid environment where an abnormal physiological response is observed. Although high temperature reduces feed intake as a natural physiological response of the birds, even then broilers have the ability to maintain growth rate during adverse environmental conditions (Jones, 1994) and there is a chance that the birds get overweight and make themselves more susceptible to heat stress. Leeson (1986) reported that thermic effect of feed should not coincide with the period of high ambient temperature, because body heat rises with increase in food intake and high environmental temperature (Zhou *et al.*, 1997) which may be further aggravated due to high humidity. Therefore, it may be envisaged that during summer when both temperature and relative humidity are high, continuous feeding may lead towards more heat production and can be a positive stressor. Hence, continuous feeding during high temperature has been taken as a control in this study, against intermittent feeding and feed withdrawal system. Similarly addition of fat is also a well-recognized factor to improve production performance under hot climatic conditions (Daghir, 1995) because of its specific dynamic effect (Fuller and Rendon, 1977; Fuller, 1978). Therefore, ration without fat supplementation was taken as another control against the ration supplemented with 3% fat.

High environmental temperature and relative humidity were predicted during the month of July and August based upon the last ten years of environmental data, obtained from the Department of Crop Physiology,

University of Agriculture Faisalabad, Pakistan. Environmental data of the year 2003 during which the experiment was conducted has also been presented in Fig.5.2, as a testimony that environmental temperature and relative humidity followed a similar pattern during the conduct of the study as it did in the last ten years (Fig. 5.1). The month of July and August were selected for the experiment because these months could be associated with great economic losses due to high mortality as a result of adverse climatic conditions.

6.1. Temperature and Relative Humidity

As expected, environmental temperature and relative humidity both remained high during the experimental period. Average temperature outside the shed was 1.5°C higher than that of inside temperature, whereas, average relative humidity during the experimental period was 8 % higher inside the shed. Comparatively low value of ambient temperature inside the shed than that of outside temperature may be due to the insulation effect of the building. Whereas, increase in relative humidity inside the shed, occurred due to liberation of moisture from the birds as a result of evaporative cooling and their droppings. Average temperature during the experimental period ranged between 28.80 to 34.99°C and the relative humidity ranged from 62.32 to 82.54 %. High temperature (35°C) and cyclic temperature (25-35°C) have also been reported to have adverse effects on the weight gain and feed efficiency of the birds (Acar *et al.*, 1995; Plavnik and Yahav, 1998). High ambient temperature (32 °C) caused a significant increase in rectal temperature. Gu *et al.* (1999) observed a significant increase in rectal temperature of the birds exposed to high ambient temperature when kept under 90% relative humidity than those maintained under 60% and 30% relative humidity. It can therefore, be concluded that the temperature and relative humidity during the experimental period was high enough for abnormal physiological behavior (heat stress) especially when accompanied by continuous feeding.

6.2. Rectal Temperature

Mean values of rectal temperature in all the treatment groups were higher at afternoon than those recorded at morning or evening times. It is quite obvious that high environmental temperature may have affected the thermoregulation process of the birds resulting into increased rectal temperature. Present results are in line with the findings of Ward and Peterson (1973), Ching and Ching (1992), Deyhim and Teeter (1994), Salvador *et al.* (1999) and Altan *et al.* (2000) who reported an increase in the rectal temperature of the birds exposed to high environmental temperature.

Feeding systems exhibited a significant effect on rectal temperature of the birds at afternoon and evening times. Both the methods of feed restriction (intermittent feeding and feed withdrawal) significantly reduced the rectal temperature of the birds recorded at afternoon. The lowest rectal temperature at afternoon was found in the birds kept under feed withdrawal system. As the birds kept under feed withdrawal system remained without feed (9 am. to 5 pm.) for longer time, resultantly the heat increment because of thermic effect of feed in the birds may have been less than those fed intermittently or continuously. The rectal temperature of the birds maintained on intermittent feeding was also less than those kept under continuous feeding system. However, it was higher than those kept under feed withdrawal system. This indicated that duration of feed restriction influenced the rectal temperature in the birds.

The results of the present study are in accordance with the findings of Smith and Teeter (1988), Francis *et al.* (1991), Smith (1992) Macleod *et al.* (1993), Teeter and Belay (1996) who observed that feed withdrawal during hot environment resulted in lower rectal temperature. However, rectal temperature of the birds at evening time maintained under different feeding methods presented a different scenario than those recorded at afternoon. The temperature of the birds kept under feed withdrawal system which was the lowest amongst the treatment groups at afternoon time exhibited higher values when recorded at evening time

than those maintained under intermittent feeding system. Probable reason of this fluctuation may be the re-feeding of the birds kept under feed withdrawal system, whereas, intermittently fed birds maintained their pace. Therefore, it may be inferred that rectal temperature of the birds had a significant bearing on the duration and method of feed restriction.

Fat supplementation also influenced the rectal temperature of the birds recorded at morning and afternoon time. Rectal temperature of the birds using fat supplemented ration was found to be significantly lower than those fed ration without supplementation of fat. Similar findings have been reported by Fuller and Rendon (1977) and Fuller (1978) who observed reduced rectal temperature in birds due to the specific dynamic effect of fat supplemented diet. Lower heat increment of fat than carbohydrates and protein was also reported by Blaxter (1989). Therefore, lower rectal temperature of the birds fed fat supplemented ration than those fed ration without fat supplementation may be attributed to the lower heat increment of fat. Both, feed restriction systems and supplementation of fat significantly reduced rectal temperature of the birds hence, it can be inferred that a combined effect of both treatments at a time may be a useful tool to reduce body temperature when a heat wave is expected.

6.3. Respiration Rate

As expected, the mean values of respiration rate of broilers maintained under different feeding management systems were higher at afternoon time than those recorded at morning or evening time. The reason for higher respiration rate may be the high ambient temperature at afternoon time as compared to morning or evening times. The birds might have started panting because at high ambient temperature evaporative cooling through panting is the most effective method to get rid of increased body temperature (Wiernusz and Teeter, 1993; Daghir, 1995; Wirnusz, 1998). Similar findings were reported by Arad *et al.* (1975) and Anjum (2000) who found increased respiration rate in fowls due to increase in ambient temperature.

The respiration rate recorded at afternoon and evening time was significantly affected due to the feeding methods used. Intermittent feeding and feed withdrawal system influenced the respiration rate of the broilers and kept it significantly lower than those kept under continuous feeding system. Probable explanation of lower respiration rate of the birds may be less heat increment in the body of the birds as a result of lower feed intake. This may have resulted into decreased body heat load and less panting of the birds. However, Garcia *et al.* (1992) did not find any noticeable change in the respiration rate of the birds due to feed deprivation. Probable explanation of this contradictory finding may be the difference in the duration of feed restriction and/or environmental conditions.

The above discussion provides a strong indication that there may be a positive correlation between environmental temperature and respiration rate, and continuous feeding at high ambient temperature may increase respiration rate of the birds. Hence feed restriction could be an effective practice to reduce thermal load of the birds to keep them within comfortable physiological conditions.

6.4. Weight Gain

The birds maintained under intermittent feeding or feed withdrawal system, in general, gained significantly less body weight as compared to the birds using continuous feed during the trial. Lower body weight gain in the feed restricted birds may be due to less feed intake than those fed *ad libitum*. Findings of Fontana *et al.* (1992), Deaton (1995), Renden *et al.* (1996), Gonzales *et al.* (1998b) and Su *et al.* (1999) are in line with the results of present study that feed restriction resulted into decreased weight gain. Whereas, Smith (1992), Spinu and Degen (1993), Wong *et al.* (1993), Smith (1994), Zakia *et al.* (1995) and Zhong *et al.* (1995) found that feed restriction did not affect the weight gain adversely. The difference in the findings of present study may possibly be due to the difference in feed withdrawal duration in the studies mentioned above.

Fat supplementation in the ration of broilers kept under various feeding methods significantly reduced the body weight gain than those fed ration without

supplementation of fat. The reduction in weight gain may be due to the lower feed intake of the birds using ration supplemented with fat (Crespo and Esteve-Garcia, 2001). However, the findings of Korver *et al.* (1998), Olomu and Baracos (1991) and Peebles *et al.* (1997) are quite contradictory to the results of the present study who observed no effect on weight gain or even improved body weight of broilers due to supplementation of fat (Rotter *et al.*, 1987). Boosting the energy content of diet (Junqueira *et al.*, 1999 and Oliveira *et al.*, 2000) was also found to be beneficial in improving the weight gain. The difference in the results may be due to the difference in the level of fat used or the environmental conditions in which fat supplemented rations was fed.

As feed intake is energy dependent therefore, decreased energy requirement during hot weather resulted into lower feed intake and consequently lower body weight gain when birds were fed fat supplemented ration. However, in the present study reduced weight gain was required for better survival of the birds. Therefore, addition of fat may be a good feeding practice during summer working in two ways; first it reduces weight gain in hot climate, second it reduces the thermic effect of feed in the body of the birds, both the factors are vital for the survival of the birds during hot and humid conditions.

6.5. Feed Consumption

Feed consumption of the birds kept under various feeding methods was found to be significantly different. Birds kept under intermittent feeding and feed withdrawal system consumed 11% and 6% less feed, respectively, than those fed *ad libitum*. The results of present study are in line with the findings of Smith and Teeter (1988), Wiernusz and Teeter (1993), Suprunov *et al.* (1995), Buyse *et al.* (1996), Bhat and Banday (2000) who reported a reduced feed intake in fowls when different methods of feed restriction were applied. However, findings of Smith (1994) and Gonzalez *et al.* (2000a) are not in accordance with the present results, where a non-significant effect of feed restriction was observed on the

feed intake of birds. The difference in the findings may be either due to the difference in the methods of feed restriction or the duration of feed restriction.

The lower feed intake of birds kept under intermittent feeding or feed withdrawal system may be due to less time allowed to the birds for feed intake than those fed continuously. Birds kept under intermittent feeding system in which they had 6 hours access to the feed, consumed significantly lower feed when compared with those kept under feed withdrawal system, which allowed the birds 16 hours access to feed during 24 hours. This indicates that feed consumption decreased with decrease in the time of access of the birds to the feed. The addition of fat significantly reduced (7.22%) the feed consumption of the birds than those fed ration without fat supplementation. Similar findings have been observed by (Jaffar *et al.,* 1996) who observed a decrease in feed consumption due to increase in the energy density of the ration at high ambient temperature.

6.6. Feed Conversion Ratio (FCR)

The feed conversion ratios have shown different responses to the feeding methods. The best feed to gain ratio was observed in the birds maintained under intermittent feeding system. Probably intermittent feeding (feed restriction) decreased the rate of passage of feed from the gastrointestinal tract (Sibbald, 1979) thus reducing the pumping up of more feed into the tract and might have resulted in increased feed utilization of the birds. Another probable explanation of improved feed efficiency of intermittently fed birds may be the reduced amount of energy required for the process of thermoregulation and or lower maintenance requirement due to relatively reduced body weight of the birds (Dickerson, 1978; Jones and Farrell, 1992). Moreover, associated digestive adaptation of restricted- refed (intermittently fed) broilers seems to be a contributing factor to improved feed efficiency (Zubair and Leeson, 1994).

Efficient feed utilization due to feed restriction has also been well documented (Fontana *et al.,* 1992; Deaton, 1995; Zhong *et al.,* 1995; Buyse *et*

al., 1996; Zulkifli and Fauzi, 1996), whereas, Sheila (1993) did not find any effect of feed restriction on the feed conversation ratio of the birds. In the present study two different methods of feed restriction were used and both the methods of feed restriction showed different trend for feed utilization. Therefore, it may be concluded that all the methods of feed restriction do not improve feed utilization in a similar fashion hence, selection for a right method for a specific climatic condition is important for economical production of broilers.

Feed conversion ratio was not affected by the addition of fat in the ration. Findings of the present study are in line with the findings of Olomu and Baracos (1991) who reported a non-significant effect of fat supplementation on feed utilization in broilers.

6.7. Water Intake

The effect of intermittent feeding on water intake of the birds was more pronounced than feed withdrawal and continuous feeding system. Broilers kept under intermittent feeding consumed less water than those kept under feed withdrawal and continuous feeding system. A probable reason of lower water intake of intermittently fed birds may be the reduced feed intake of the birds. Moreover, decrease in feed intake reduced the quantity of metabolic wastes as well as reduced body heat increment. Resultantly less water was required for thermoregulation and for the removal of metabolic wastes (Daghir, 1995).

Water intake of the birds increased at high temperature (Khadi *et al.*, 1988; Deyhim and Teeter, 1991; Kutlu and Forbes, 1993) because water works as heat sink and helps in process of evaporative cooling (Wiernusz and Teeter, 1993). Whereas, the birds kept under feed restriction methods showed a significant reduction in water consumption at the same ambient temperature as compared to continuous fed birds. This indicated that feed restriction (intermittent feeding) resulted in decreased thermal load.

Addition of fat caused reduction in water intake of the experimental birds. Increased energy contents of ration due to the addition of fat may have resulted

into reduced feed consumption and on the other hand specific dynamic effect of the fat resulted into lower body temperature thus reducing the requirement of water for thermoregulation and excretion.

The interaction between feeding system and fat supplementation in respect of water consumption was found to be highly significant. Broiler provided fat supplemented ration showed significant decrease in water consumption when they were kept under continuous feeding and feed withdrawal system. Whereas, the birds kept under intermittent feeding system did not follow this pattern. Birds kept under intermittent feeding system showed non significant difference in the values of water consumption when they were provided ration either supplemented with fat or without fat supplementation. Possible reason for this deviation may be the limited access of intermittently fed birds to feed (6 hours/24 hours). This resulted in reduced feed intake, reduced body temperature and respiration rate. Hence, it can be inferred that birds followed a normal pattern of water consumption under intermittent feeding due to a reduced stress on thermoregulation and excretion process inside the body of the birds. Therefore, it may be stated that intermittent feeding negated the effect of fat supplementation by lowering down the body temperature to its optimum levels.

6.8. Crude Protein and Metabolizable Energy Intake

The birds maintained under feed restriction systems (intermittent feeding and feed withdrawal) exhibited reduced intake of crude protein and metabolizable energy than those fed *ad libitum* probably due to reduced feed intake of the birds kept under these systems. Protein and energy intake of birds fed intermittently was lower than those kept under feed with drawl system. Therefore, it may be concluded that system of feed restriction may alter the intake of crude protein and metabolizable energy depending upon duration of feed restriction.

Supplementation of fat in the ration showed significant decrease of crude protein intake, but the results in case of metabolizable energy intake remained

unaffected, indicating that protein intake was dependent upon energy contents of the diet (Daghir, 1995). Fat supplementation also reduced the protein percentage in the ration to some extent and the other hand bird mostly ate for their energy requirements. Therefore, when energy contents of diet were increased due to addition of fat, it limited the protein intake (McNaughton and Reece. 1984).

6.9. Mortality

Feeding systems exhibited a marked effect on mortality of the birds. Mortality rate was higher (8.3%) in the birds kept under continuous feeding as compared to those maintained on feed withdrawal (1.66%) and intermittent feeding system (0%). These birds died during the last week (6^{th} week of age) of experiment at afternoon time and were over sized. Postmortem findings indicated that these birds died as a result of heat prostration. The death of heavy birds (above average) is an indicator that heavy birds are more prone to heat prostration (Ononiwu *et al.*, 1979). The situation may further aggravate if combined with *ad libitum* feeding during hot and humid climatic conditions (Daghir, 1995; Wiernusz, 1998). No doubt the mean body weight was lower in feed restricted birds but the restricted feeding did help in controlling the mortality of the birds. The results of present study are in accordance with the findings of Buckland *et al.* (1973), Smith (1992), Zakia *et al.* (1995) and Basilio *et al.* (2001) who observed a decreased mortality due to feed restriction in the birds kept under high environmental temperature. No mortality in the birds kept under intermittent feeding system suggested that the system helped to alleviate the effect of high ambient temperature during hot and humid environmental conditions.

Addition of fat also exhibited reduced mortality in the broilers than those fed ration with out supplementation of fat. The mortality in birds maintained on fat supplemented ration was reduced 50% of the mortality, which occurred in their counterparts. Reduction in mortality may be due to reduced feed intake,

body weight and or lower heat increment of fat supplemented ration (Fuller and Rendon, 1977; Fuller, 1978).

6.10. Economics

Broilers kept under intermittent feeding system gained more profit per kilogram body weight followed by those kept under feed withdrawal system and continuous feeding system. Increased profit margin in the birds kept under intermittent feeding system and feed withdrawal system than those kept under continuous feeding may be attributed to lower mortality and improved feed utilization of birds kept under feed restriction systems. Whereas, addition of fat exhibited a negative effect on the profit margin of the broilers kept under various systems of feeding.

Highest profit margin was found in the birds kept under intermittent feeding system and provided ration without fat supplementation. Although birds kept under intermittent feeding and fed fat supplemented ration showed minimum feed consumption per kilogram weight gain, but they showed less profit margin than those provided ration without fat supplementation. This may be due to extra expenditure incurred on addition of fat in the ration. Hence, an inference can be drawn that improved feed utilization alone cannot be the only trait for a profitable poultry production but it must be accompanied by a cost effective balanced ration and lowest possible mortality as well. Therefore, during summer, more emphasis should be placed on to reduce mortality and improve feed utilization rather than to gain more weight of broilers, to fetch more profit from a poultry business.

6.11. Leg Abnormalities

The gait scoring and foot burn of both right and left sides did not show any difference due to the treatments, however, hock burn of both the sides showed higher values for abnormality in the birds maintained on continuous feeding followed by feed withdrawal and intermittent feeding system. Similar findings were observed by Buckland *et al.* (1976) who reported that birds kept

under continuous lighting (continuous feeding) had more leg abnormalities than those kept under intermittent lighting. Considering that intermittent lighting is associated with intermittent feeding, Edward and Sorensen (1987) found reduced growth rate as well as reduced leg abnormalities in broilers kept under intermittent lighting programs. A probable explanation of reduced leg abnormalities due to feed restriction may be the reduction in weight gain of the feed restricted birds. Reduced leg abnormalities due to lower growth/ body weight have also been observed by Buckland *et al.* (1973, 1974), Buckland (1975), Simons (1982, 1986), Wilson *et al.* (1984), Simons and Haye (1985), Duff and Thorp (1985), Ketelaars *et al.* (1986), Classen and Riddell (1989, 1990), Robinson *et al.* (1992) and Renden *et al.* (1991, 1996). These findings support the results of the present study that the birds kept on intermittent feeding and feed withdrawal system showed less leg abnormalities under the effect of fasting and reduced growth than those kept on continuous feeding system.

Non-significant differences regarding hock burn of the birds kept under feed withdrawal and intermittent feeding system are also supported by the findings of Su *et al.* (1999) who observed less hock burn in the birds kept under meal feeding than those kept under *ad libitum* feeding. Addition of fat did not show any difference in case of all the leg abnormalities mentioned above. Similar findings have been reported by Hulan and Proudfoot (1987) who observed that dietary energy had no significant effect on leg abnormalities except for curled toes, the incidence of which decreased with increasing dietary energy.

Keeping in view the discussion above, it may be concluded that intermittent feeding of broilers may help reduce the leg abnormalities and may be equally useful in this respect without addition of fat in the rations during hot and humid environmental conditions.

6.12. Slaughter Characteristics

Dressing percentage, intestine length and relative intestine weight of the birds were not affected due to the feeding methods used. This indicated that feed restriction methods did not influence these characteristics of the broilers when

compared with those fed *ad libitum* during summer. The results of the present study are compatible with those reported by Mizubuti *et al.* (2000) who observed non-significant effect of feed restriction on the carcass characteristics of broilers. Similarly fat supplementation did not affect dressing percentage of broilers. Whereas, Oliveira *et al.* (2000) reported that carcass yield of broilers maintained under thermo-neutral environment (23.2±0.74°C) linearly reduced according to levels of metabolizable energy in the rations. These contradictory findings may be due either to difference in climatic conditions or level of metabolizable energy in the ration used for the study or both.

Abdominal fat calculated on the basis of relative body weight was markedly affected in the birds kept under feed withdrawal system. These birds showed higher value of abdominal fat than those fed continuously. More fat deposition in the birds kept under feed restricted system (feed withdrawal) may be probably due to less energy requirements of the birds for the process of thermoregulation where more energy remained spared and was utilized for the synthesis of fat. Whereas, birds kept under intermittent feeding system did not show any difference in this regard when compared to the birds kept under continuous feeding or feed withdrawal system. The results of the present study are partially in line with the findings of Sheila *et al.* (1993) and Deaton (1995) who reported a non-significant effect of feed restriction of the birds on the abdominal fat as compared to those fed *ad libitum*. Whereas, Zhong *et al.* (1995), Santoso (1995) and Gonzalez *et al.* (2000a) reported a significant decrease in the abdominal fat due to feed restriction. Probable explanation of this contradiction in case of feed withdrawal system may be the difference in the intensity of feed restriction used in these studies as well as the environmental conditions under which the trials were conducted.

Supplementation of fat in the ration of broilers kept under different feeding methods did not influence their abdominal fat deposition. The results of present study are in accordance with the findings of Oliveira *et al.* (2000) who observed that dietary ME levels (3000, 3075, 3150, 3225 and 3300 kcal ME/kg

diet) did not affect abdominal fat of broilers. Contradictory to the findings of present study Yalcin *et al.* (1998) and Sanz *et al.* (2000) reported a significant increase in the abdominal fat due to increase in the energy level of diet or increasing the duration of feeding high energy ration. The difference in the results of present study with regards to fat deposition may be due to levels of fat or energy used in the rations as well as feeding methods applied in these studies.

The relative weight and length of the elementary tract did not show any difference due to the feeding systems used. Similarly the relative weight of elementary tract of the birds fed fat supplemented ration compared with those fed ration without fat supplementation remained unaffected. The results of the study are in partial agreement to the findings of Oliveira *et al.* (2000) who did not observe any effect due to dietary metabolizeable energy level on absolute and relative weight of intestine. However, length of elementary tract was significantly reduced in the experimental birds fed fat supplemented ration. This may be an adjustment of body as a result of addition of fat in ration which may have reduced the rate of food passage in the alimentary tract (Mateos *et al.*, 1982). Therefore, birds fed without fat supplemented ration extended their elementary tracts to enhance passage time in order to increase the feed uptake from the gut.

Neither feeding methods nor fat supplementation in the ration showed any influence on the relative weight of liver, gizzard, heart, lungs and kidney of the broilers. These results are in line with the findings of Oliveira *et al.* (2000) who observed no effect of dietary energy on the organ weight of broilers. Contradictory to the results of present study, Latour *et al.* (1994) reported that liver weight was suppressed by the inclusion of lard in the diet. Probable explanation of the difference in the results of the study may be the type of fat or level of fat used in the experiment.

Relative weight of pituitary, thymus, adrenal, bursa and spleen were also not affected due to the feeding methods used in the study. However, relative pancreas weight of the birds was significantly influenced due to the feeding

methods used. The birds kept under feed withdrawal system exhibited higher relative pancreas weight than those of the comparative groups. These results are in accordance with the findings of Mahmood *et al.* (1998) who observed pancreatic hypertrophy in birds due to decreased feed intake resulting into higher relative pancreas weight. Under the present study the birds kept on intermittent feeding system did not differ regarding relative pancreas weight than those kept under continuous feeding system. This indicated that all the feed restriction methods did not result in same physiological changes. Therefore, it is possible that change in the pancreatic weight may be associated with long term feed restriction as observed in feed withdrawal system.

6.13. Breast Meat Analysis

Dry matter, crude protein and fat percentage of breast meat were not affected by the methods of feeding used. However, the ash percentage was significantly higher in the birds kept under intermittent feeding than those maintained on *ad libitum* feeding or feed withdrawal system. Higher contents of ash in the breast meat of the birds fed intermittently may be due to lower water losses under the effect of thermoregulation than those kept under continuous feeding or feed withdrawal system and resulting in more retention of minerals in the meat. On the other hand birds kept under continuous feeding in hot climatic condition not only increased water consumption but also had increased water loss from the body and consequently mineral retention in their body.

Supplementation of fat did not influence the parameter discussed above, indicting that energy level had no effect on the composition of broiler meat. Similar findings were reported by Yalcin *et al.* (1998) who did not observe any effect of dietary energy levels on the nutrient composition of breast meat.

6.14. Leg Meat Analysis

Dry matter contents in the leg meat of the birds were significantly infuelenced by the feeding methods used, whereas, crude protein, ash and fat contents remained unaffected. The birds kept under intermittent feeding and feed

withdrawal system exhibited higher dry matter contents/lower moisture level in the leg muscles of broilers than those kept under continuous feeding system. Lower moisture contents in the feed restricted birds may be atrributed to decreased body temperature and water retension in musle of the birds.

Fat and protein percentage in the leg and breast meat of the birds was not affected by the methods of feeding. Addition of fat also failed to produce any significant effect on these parameters. Results of the present study are not in line with the findings of Latour (1994) who observed an increase in body fat and protein contents of chickens fed ration with 7% added lard. A probable reason of this contradiction may be the intensity of the feed restriction as well as the level of fat supplementation in the ration of the experimental birds.

6.15. Blood Parameters

The values for blood pH, haemoglobin contents, erythrocytes sedimentation rate and erythrocyte count were not affected in the birds kept under various feeding systems. These findings are in line with those observed by Deyhim and Teeter (1991) who reported no effect of high ambient temperature on the blood pH of broilers when they were subjected to either a thermoneutral (TN; 24°C) or cycling temperature (24 to 35°C) at 5 weeks post hatch. However, these results are not in keeping with the findings of Hocking *et al.* (1994) because they found that *ad libitum* feeding in heat stressed birds resulted in increased pH whereas, feed restriction significantly decreased the blood pH in those birds. Although the difference in present study was non-significant, but slightly lower blood pH was observed in the birds kept under feed restriction systems than those kept under continuous feeding system. Slight decrease in the pH of fed birds indicates that feed restriction may have the ability to reduce blood pH in the birds exposed to elevated temperature.

Haemoglobin (Hb) values did not differ due to the feeding methods used in this study. However, a slight increase of Hb in feed restricted birds indicates that feed restriction has a tendency to increase Hb level during summer. The

results of present study are not in line with the findings of Garcia *et al.* (1992) who found that food depressed males expose to 40°C for four hours had a marked increase in haemoglobin concentration. The difference in the results may be due to the different in ambient temperature during the study.

Hot environmental conditions are known to adversely affect the erythrocyte count in the blood (Huston, 1960; Washburn and Huston, 1968; Moye *et al.*, 1969; Soliman and Huston, 1974). Decrease in red blood cell number due to acute heat stress has been observed by Furlan *et al.* (1999). A slight increase in erythrocyte count of feed restricted birds than those fed continuously indicates that feed restriction may have a tendency to overcome the adverse effects caused by hot climatic conditions.

A significant reduction in packed cell volume (PCV) has been reported (Huston, 1960; Huston, 1965; Washburn and Huston, 1968; Moye *et al.*, 1969; Deaton *et al.* 1969a, 1969b; Kubena *et al.*, 1972b; Zimmerman *et al.*, 1973; Soliman and Huston, 1974; Vo and Boone, 1975; Doerr *et al.*, 1975; Zimmerman *et al.*, 1975; Andrade *et al.*, 1976; Deyhim and Teeter, 1991 and Sahota *et al.*, 1993) at high ambient temperature. Whereas, present results revealed a significant increase in PCV of feed restricted birds than those fed *ad libitum* under hot environmental condition. It is evident from the results of present study that with the help of feed restriction adverse effects of high environmental temperature and thermic effect due to continuous feeding may effectively be appeased.

The addition of fat did not influence the above mentioned blood parameters when compared with those fed ration without supplementation of fat. Contradictory findings were reported about addition of fat on the PCV by Peebles *et al.* (1997). They found that haematocrits increased across time in birds fed 3% fat in starter diets through 10 d of age. Whereas, in the present study only a slight increase was observed in the PCV values due to addition of fat. The difference in the results may be due to difference in environmental conditions in

which these experiments were conducted as well as duration of incorporation of fat in the diets.

Both total leukocyte count and differential leukocyte count (TLC, DLC) were neither influenced by feeding methods nor by the addition of fat in the diets of the experimental birds except that of monocytes count. Highest count of monocyte was observed in the birds kept under feed withdrawal system followed by intermittent feeding and continuous feeding respectively. However, no difference was observed in the birds kept under different methods of feed restriction. Results of present study are partially in line with the findings of Al-Rawashdeh *et al.* (2000). They reported that feed-restricted birds had slightly higher heterophils, lymphocytes and monocytes than birds fed *ad libitum* whereas, in present study monocytes are significantly higher in feed restricted birds. Hocking *et al.* (1994) found that feed restriction was related with lower numbers of heterophils and monocytes. The contradictory findings may be due to the difference in the intensity of the feed restriction.

6.16. Blood Biochemistry

6.16.1. Glucose

Lower concentration of blood glucose was recorded in the birds kept under intermittent feeding system than those maintained under feed withdrawal or continuous feeding system. Results of the present study are in line with those of Suprunov *et al.* (1995) who observed that level of blood sugar increased in the broilers due to high environmental temperature but it was significantly reduced due to the interrupted feeding because of decreased insulin production. However, Zulkifli *et al.* (2000) reported that serum glucose concentration was elevated by the heat challenge but was not affected by the feeding regimes.

Reduction in blood glucose level in the domestic fowls was also reported by Hazelwood and Lorzen (1959) as a result of prolonged fasting whereas, Pierce and Fanguy (1971) noted a reduction in the blood glucose during the first 72 hours under food deprivation stress but slightly elevated at 144 hours post

fasting. These findings indicated that the process of gluconeogenesis was involved in the increase of glucose after prolonged fasting as a result of feed deprivation stress. Whereas, in the present study prolonged feed restriction (stress) was not involved. Therefore, rise in glucose level of full fed and those who were fasted for 8 hours a day than those of intermittently fed birds may be due to either increased feed intake or may be due to the effect of chronic thermal stress. It can be envisaged from the above discussion that heat stress caused increase in the glucose level but the effect was appeased due to intermittent feeding.

6.16.2. Cholesterol

Feed restricted birds did not show any difference in blood cholesterol levels when compared to those fed *ad libitum*. These findings are not in accordance with Hevia and Vinsek (1979) who observed that starvation increased the blood cholesterol level, because fasting caused mobilization of fat through gluconeogenesis which ultimately increased blood cholesterol level. The difference in the results may be due to the reason that in the present study fasting was not so prolonged that it could cause fasting stress and mobilize fat.

High serum cholesterol concentrations in the broilers in response to the heat treatment, 36±1°C has been reported by Zulkifli *et al.* (1999), probably due to increased energy requirements and mobilization of fat to cope with high ambient temperature. Although, in the present study the results were non significant but a mild increase in serum cholesterol was observed in the birds kept under continuous feeding system. It indicated that a slight mobilization of fat was present in these birds and they used their fat in order to maintain their excessive energy requirement in response to high environmental temperature and additional thermal effect of feed or their energy may have been already used in the process of thermoregulation instead of getting stored in the body. A strong evidence of this hypothesis is the lower abdominal fat in the birds kept under continuous feeding system than those kept under feed restriction systems, in the

present study. Zulkifli *et al.* (1999) also observed that glucose intake alleviated the influence of heat stress, therefore increased blood glucose level in the birds kept under continuous feeding system and feed withdrawal system than those of intermittently fed bird may be due to a natural response of birds to cope with heat stress caused by high ambient temperature and more feed intake.

6.16.3. Urea

Feeding methods significantly influenced the blood urea concentration in the experimental birds. The birds maintained under feed restriction systems exhibited lower concentration of blood urea than those fed *ad libitum*. The cause of reduction in blood urea concentration of the birds fed under restricted feeding program may be the lower intake of protein than those of continuously fed birds. Blood uric acid has been found to increase with high environmental temperature probably as a result of gluconeogenesis in heat distressed birds (Ward and Peterson, 1973). Where broilers exposed to a temperature of 33-35°C for 4 hours had higher plasma levels of uric acid (6.7 against 5.5 mg/dL) than the birds kept at 18-22°C. Similar findings were observed as regards to blood urea of the birds under the effect of fasting which averaged 5.08 in feed restricted birds as against 6.83 mg/dL in full fed birds at 25-35°C. This indicates that the use of feed restriction methods during summer may reduce the adverse effects expected due to increase in blood urea under the influence of high ambient temperature.

6.16.4. Albumin

The highest albumin concentration was obtained from the birds kept under feed withdrawal system followed by those kept under continuous feeding and intermittent feeding, respectively. A probable explanation of decrease in albumin concentration in the birds kept under intermittent feeding may be the reduced protein intake of the birds. Similarly lower protein intake observed in the birds provided fat supplemented ration also resulted in a noticeable decrease in the albumin concentration when compared with those fed ration without fat supplementation.

Although fat supplementation may have been involved in reducing the albumin concentration in the blood under the effect of reduced feed intake, both in the continuous and feed withdrawal systems yet, it presented an opposite trend in case of intermittent feeding where fat supplementation did not reduce the albumin concentration. This change in the trend also made the interactions between feeding methods and fat supplementation significant. The results in respect of provision of fat supplemented ration to the birds kept under intermittent feeding are in line with the findings of Ehinger (1977) who reported that diet did not affect plasma albumin when the broilers were fattened from 1 to 46 days on 1 of 4 diets containing metabolizable energy 2800 or 3250 Kcal/Kg with 25.0 or 20.0% protein. However, these findings are not in agreement with the present results regarding provision of different energy levels (fat supplementation) to the birds kept under continuous feeding and feed withdrawal systems. These results indicated that method of feed restriction might alter the blood albumin concentration in broilers.

6.17. Enzymes and Hormones

Neither feeding systems nor supplementation of fat influenced the glutamate pyruvate transaminase (GPT) and glutamate oxaloactate transaminase (GOT) values in the plasma of broilers used in this study indicating that feed restriction had no adverse effect on these liver enzymes. Similarly the plasma concentration of the hormones triiodothyronine (T_3), thyroxine (T_4) and cortisol remained unaffected. These results are in agreement with the findings of Renden *et al.* (1994) who observed that intermittent feeding, under the effect of intermittent lighting, was not associated with alterations in corticosterone or thyroid hormone concentrations.

Contradictory results regarding the effect of feeding method on plasma T_3 and T_4 concentration were observed by Gonzales *et al.* (1998a). They reported that during the period of feed restriction, plasma T_3 concentration decreased whereas, the value for plasma T_4 increased than those of the age-matched *ad*

libitum fed counterparts. The difference in the results may be due to the difference in the climatic conditions or the methods of feed restriction used.

An increased corticosteroid level, contradictory to the findings of present study, due to feed restriction was observed by Freeman *et al.* (1981), Mench (1991), and Latshaw (1991). A probable reason which may be advanced to the increased level of corticosteroid concentration in the blood of feed restricted birds may be the prolonged fasting stress.

The results regarding the supplementation of fat and plasma concentration of triiodothyronine and thyroxine are in line with the findings of Junqueira *et al.* (1999) where no correlation existed between plasma thyroid hormone (T_4 and T_3) concentration and energy intake (2600, 2900, and 3200 Kcal ME/Kg).

6.18. Micro Minerals

The concentration of iron (Fe) in the plasma of the birds maintained on intermittent feeding was higher than those kept under continuous feeding or feed withdrawal system. Plasma Fe concentration was found to be linked with high ambient temperature as reported by Jamadar and Jalnapurkar (1995) who observed that mean serum Fe concentration of chicks exposed to high ambient temperature (40 ± 1°C) for 12 h of a day (07.00 to 19.00 h) was lower than those maintained at the prevailing room temperature (22 to 31°C). However, in the present study temperature ranged from 28 to 34°C, and thus was not supposed to reduce the plasma Fe concentration of the bird. Most probably the additional thermic effect of feed at 28 to 34°C ambient temperature may have resulted in reduced Fe level in full fed birds along with those kept under feed withdrawal system.

Jamadar and Jalnapurkar (1995) also reported that a possible release of corticosteroids during heat exposure might have caused low serum Fe concentration. Iron concentration in the plasma and leg muscle did not reduce in the plasma and meat samples of the birds kept under intermittent feeding system, indicating a less corticosteroids availability in the plasma of feed restricted birds

than those fed *ad libitum* and kept under feed withdrawal system. Whereas, low iron concentration in the plasma of the birds fed fat supplemented diet may be due to reduced availability of iron in the feed as a result of additional fat which consequently resulted in the reduction of iron percentage in the ration as well as reduction in the feed intake under the affect of fat supplementation.

Concentration of copper was found to be higher in the leg and breast meat of bird kept under continuous feeding and feed withdrawal system than those kept under intermittent feeding system. The exact reason why copper concentration reduced in the muscles of the birds kept under intermittent feeding is not known, however, it may probably be due to reduced feed intake of the birds in this group.

6.19. Macro Minerals

Electrolyte balance in the plasma, leg and breast meat of the birds kept under different feeding practices was not influenced which indicated that feed restriction did not has any adverse effect on the electrolyte balance in the birds. However, the birds kept under intermittent feeding showed more retention of phosphorous and potassium in the leg meat and sodium in the breast meat than those kept under continuous feeding system. Whereas, birds kept under feed withdrawal system showed less retention of potassium and phosphorous in leg meat, and exhibited a non significant difference in terms of sodium retention in the breast meat than those kept under intermittent feeding system. This showed that over all retention of macro minerals in the birds kept under intermittent feeding system was more than those kept under continuous feeding and feed withdrawal system regardless of the fact that feed intake was lower in the birds kept under intermittent feeding than those of the other two groups. These results indicate more efficient intake and retention of macro minerals in the intermittently fed birds. Whereas, less retention of sodium potassium and phosphorous in the birds kept under continuous feeding and feed withdrawal system may be due to their excessive feed intake in hot climate. Increased feed

intake resulted in increased heat production as well as increased water requirement for heat dissipation and removal of metabolic wastes which may be related to the loss of macro minerals. These results are in keeping with those observed by Belay *et al.* (1993). They found that heat stress increased urinary excretion for potassium and sodium whereas, mineral retention for magnesium and phosphorous was reduced by a combination of urinary and fecal excretion.

The birds kept under feed restriction methods showed less magnesium concentration in their plasma than those kept under continuous feeding system. Reduced magnesium concentration in feed restricted birds may be ascribed to their reduced feed intake. Whereas, intermittently fed birds showed more magnesium retention than those kept under feed withdrawal system. These results indicate that intermittent feeding system may be a better choice to be practiced in broiler rearing as compared to feed withdrawal system in this regards.

6.20. Immune Response

The highest values of cumulative mean titre (CMT) for Newcastle Disease (ND) were observed in the birds kept under intermittent feeding followed by those kept under feed withdrawal and continuous feeding system. High ambient temperature has been known to affect immune response of birds (Suba Rao and Glick, 1977). El-Gendy *et al.* (1995) and Zhang *et al.* (1998) reported that high ambient temperature resulted in reduced immunological response. It is therefore, possible that additional thermic effect of feed in hot climate may reduce antibody titre of the birds kept under continuous feeding system.

Results of present study indicated that both the methods of feed restriction resulted in higher antibody titre than continuous fed birds. Contrary to the findings of present study, Ben Nathen *et al.* (1976) found that feed withdrawal resulted in lower antibody titre against viral diseases. Difference in

the results may be due to the methods used for feed restriction, which may have caused fasting stress and increased corticosterone level (Latshaw, 1991), resulting into decreased antibody titre possibly through effects on cytokines.

In case of Infectious Bursal Disease (IBD) maximum value for CMT was found in the birds kept under intermittent feeding followed by those maintained on feed withdrawal and continuous feeding system. However, the difference between the birds kept under continuous feeding and feed withdrawal system was found to be non-significant. The results of present study are in line with the findings of Zulkifli *et al.* (1997) who observed that feed-restricted (9am to 5 pm) chicks had a higher antibody response to IBD vaccination than those fed *ad libitum* at 42 days of age.

Better immune response of the birds kept under intermittent feeding system may be attributed to their efficient use of energy and better retention of iron (Fe) in the plasma of intermittently fed birds. These results are in line with the findings of Berger (1996) who reported that increased plasma Fe was associated with enhanced immune response in broilers.

A transient decrease in GMT of the birds kept under all the treatments was observed at 28 days of age against both ND and IBD virus, which may have resulted due to the stress caused by revaccination of the broilers, as IBD booster vaccine was applied just a day before the scheduled collection of blood.

The addition of fat adversely affected the geometric mean titre (GMT) than those fed non-supplemented fat ration at 21 day of age against ND and IBD. However, in the later stage this condition got reversed; at 28, 35 and 42 days of age, which indicated that energy levels in the feed exhibited a significant effect on the immune status of the birds. Fat supplementation resulted in higher antibody titre when compared with those fed ration without supplementation of fat. Fat supplementation also resulted in higher CMT in the

broilers than those maintained on ration without fat supplementation, indicating that antibody titre was positively correlated with energy level of ration. These results are in agreement with the findings of Friedman and Sklan (1995) who observed that addition of fat resulted into higher antibody titre than those provided ration without fat supplementation.