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Effect of *Haemonchus contortus* Infection and Nutrition on Glucose and Trace Elements

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ABSTRACT

The study was designed to know the effect of *Haemonchus contortus* infection and nutrition on plasma glucose and trace elements, i.e. cobalt, copper, zinc and selenium. Four groups of six animals of the Merino adult rams were made. Two groups, one fed a basal diet and the other a high protein diet, were drenched with 15000 L3 *H. contortus* larvae. The two other groups also fed a basal or high protein diet were used as controls. The rams infected with *H. contortus* were treated with Ivermectin, 10 weeks after infection. Plasma glucose concentrations from all four groups were measured once a week for a period of 11 weeks, during the first week before infection then for seven weeks during infection and for the three weeks following treatment with Ivermectin. The results showed neither parasites nor protein supplementation affected the mean values of plasma glucose. Plasma trace elements were analyzed on three occasions, one week before infection, during the 6th week of infection and during the 4th week after treatment with Ivermectin. The presence of parasites and protein had no significant effect on the mean values for cobalt, copper, zinc and selenium. However, copper values were lower in the infected rams fed the basal diet during the second collection (589.67±43.18 µg/L) compared to infected rams fed the high protein diet (705.67±178.46 µg/L).

Key Words: Haemonchosis; Ram; Trace elements; Glucose

INTRODUCTION

Glucose is an important carbohydrate present in the blood and extracellular fluids and it is an essential metabolite for several tissues, in particular the red blood cells and the brain. In the ruminants, there are two main sources of glucose, dietary and endogenous glucose. Ruminant absorbs little glucose from their small intestine, unless high levels of grain are fed, but they have an absolute requirement for glucose, as do non–ruminants (McDowel, 1983). Bergman (1973) reported that the contribution of renal gluconeogenesis in sheep contributed 8–15% to whole body glucose. Helminths use glucose as a source of energy. *H. contortus* uses the glucose in the blood that is sucked from the wall of the abomasum of sheep, the rate of absorption depending on the worm's need for nutrients (Thivend 1974; Ward 1974). Dick (1953) reported that copper deficiency is not a major nutritional factor influencing the susceptibility of calves to gastric infestation. Hucker and Yong (1986) found that hypocupraemic sheep had higher worm egg counts and worm burdens than sheep with normal copper levels, but the differences were not significant. Selenium deficiency suppresses many aspects of immunity in various animals' species (Blood & Radostits, 1989). However, Jelinek *et al.* (1988) reported that a low selenium status in Merino sheep alone does not subsequently compromise immunity when compared to a high selenium status.

Keeping in view of the above factors an experiment was designed to detect the effects of supplementary protein and *H. contortus* infection on blood glucose and plasma mineral concentrations.

MATERIALS AND METHODS

The experiment was carried out at The University of Queensland, School of Veterinary Science farm, Pinjarra Hills, Brisbane Australia (between 1996 & 1998). Four groups of six animals of adult Merino rams were made. Two groups, one fed a basal diet and the other a high protein diet were drenched with 15000 L3 *H. contortus* larvae. The two other groups also fed a basal or high protein diet were used as controls. The rams infected with *H. contortus* were treated with Ivermectin, 10 weeks after infection. The basal diet contained an essential 10 MJ of ME/kg DM and 145 g CP/kg DM. The high protein diet contained 20% crude protein. 1.5 kg of either feed was allowed to each ram daily of four respective groups.

Blood was collected in the 2.5 mL vial containing 2.5 mg of potassium fluoride–oxalate, for the seven consecutive weeks during which the rams were infected with *H. contortus* and for the three weeks following treatment with ivermectin.

For the trace element analysis for cobalt, copper, zinc and selenium plasma was collected in 5 mL vial containing 50 iu of lithium heparin. The samples taken were, one

before infection with *H. contortus*, one during the period when the blood values for Hb, PCV, TPP and RCC were significantly lowered (6th week after the infection) and one sample four weeks after treatment with Ivermectin.

Glucose estimation. Plasma glucose was estimated by using Roche Cobas Mira machine using Roche Unimate 5 Glucose Hk kit (Roche Diagnostic System, Basal, Swiss).

Trace elements estimation. Determination of cobalt, copper zinc and selenium were done by inductively coupled plasma–mass spectrometry (ICP–MS).

Statistical analysis. Repeated measure of analysis of variance (ANOVA) procedures used to estimate the effects of protein, parasite, parasite by protein, time, parasite by time, protein by time and parasite by protein by time interactions for each of the trace elements and plasma glucose studied using SAS (6.03 Version 1988).

RESULTS

The mean values for plasma glucose concentration before, during and after treatment with Ivermectin more or less remained unchanged. However, during the 2nd week of infection the mean values for infected rams fed the high protein diet were significantly lower (2.77±0.23 mmol/L) than the infected rams fed the basal diet (93.17±0.31 mmol/L) but were not significantly different from the non–infected rams fed the basal diet (2.93±0.19 mmol/L) or the non–infected rams fed the high protein diet (2.88±0.29 mmol/L). It is noted that during the period following treatment with Ivermectin that the values for infected rams fed the high protein diet were lower (2.57±0.33 mmol/L) than for the rams on the other three groups.

Presence of parasites or protein supplementation had no significant effect on mean plasma glucose concentration (Table I). However, time had a significant effect. There were significant parasite by time and parasite by protein by time interaction effects on mean protein interactions (Table I).

Trace Elements

Cobalt. There were no significant differences between any of these means. Presence of parasite or protein had no significant effect on plasma cobalt concentrations (Table II). However, time did have a significant effect. There were significant parasite by protein and parasite by time interactions but no interactions for protein by time and parasite by protein by time (Table II).

Copper. Plasma copper concentrations were lower but not significantly in infected rams fed the basal diet during the period of parasite infection (Period 2) 589.67±43.18 µg/L) than infected rams fed the high protein diet (705.67±178.46 µg/L) and the non–infected rams fed both the basal (755.0±88.85 µg/L) and the high protein diets (689.50±104.67 µg/L). The values for the non–infected rams were not different from each other during period 2. During the recovery period (period 3) the values for non–infected rams fed the basal diet were higher (883.98±112.51 µg/L). The former values were also higher than for the infected rams fed the high protein (784.17±150.37 µg/L) and basal diet (771.17±70.82 µg/L). The values for both non–infected groups during 3rd week were also different from each other Table III. Presence of parasite or protein had no significant effects on mean copper concentrations but time had a significant effect. There was an overall significant protein by time interaction on mean plasma copper concentrations. There was no significant parasite by time, parasite by protein by time or parasite by protein interactions on mean plasma copper concentrations (Table III).

Zinc. The plasma zinc concentrations in the infected rams fed the high protein diet during period 3 were (541.17±98.28 µg/L) significantly lower than in the infected rams fed the basal diet (570.33±91.57 µg/L). The former values were also significantly lower than for the non–infected rams fed the basal diet (546.37±35.28 µg/L) and the non–infected rams fed the high protein diet (633.67±73.40 µg/L). The values for the two non–infected groups of rams were significantly different each other (Table IV). Presence of Parasite or protein level had no significant effect on mean plasma zinc concentrations. There were significant time and parasite by protein by time interaction effects on mean values for plasma zinc. However, parasite by time, parasite by protein and protein by time had no significant interactions effects Table IV.

Selenium. Presence of parasites had a significant effect on mean selenium concentrations but protein and time had no significant effects. There were no significant parasites by time, protein by time, parasite by protein by time or parasite by protein interactions (Table V).

DISCUSSION

The normal range for plasma glucose concentrations in sheep given by (Boyd, 1984) is 2.4–4.5 mmol/L. All plasma glucose values recorded in the present study were normal.

Overall parasites infection had no significant effect on plasma glucose concentration. This finding is in agreement with that of Roseby (1973) who reported that plasma glucose concentration was not affected by experimental Trichostrongylus columbriformis infection in sheep. However, the findings are not in agreement with those of Kouider and Kolb (1994) who found significantly lower blood glucose concentrations in sheep infected with gastrointestinal nematodes. In the present study, diet had no significant effect on plasma glucose concentrations. These findings are in agreement with those of Adam *et al.* (1997) who reported that plasma glucose concentrations were not affected by a high energy high protein diet, a low energy low protein diet or a low energy high protein diet fed to castrated male sheep. In the present study it appears that the diet Fe provided sufficient glucose or glucose precursors to supply the rams and the parasites requirements.

Table I. Plasma glucose concentrations (mmol/L) (mean±SD) of rams fed on different diets

Time (week)	Basal non–infected	High protein non–infected	Basal infected	High protein infected
1 BI	3.45±0.27a	3.38±0.24a	3.46±0.24a	3.48±0.20a
2 PI	3.37±0.22a	3.28±0.25a	3.47±0.26a	3.25±0.19a
3 PI	2.93±0.19a	2.88±0.29a	3.17±0.31a	2.77±0.23ba
4 PI	3.53±0.36a	3.25±0.42a	3.6±0.35a	3.67±0.22bc
5 PI	3.22±0.12a	3.55±0.22a	3.57±0.23a	3.65±0.23bc
6 PI	3.20±0.10a	3.32±0.26a	3.43.±0.34a	3.±0.32a
7 PI	3.32 0.28a	3.37±0.15a	3.68±0.37a	3.54±0.30a
8 PI	3.28±0.19a	3.17±0.30a	3.55±0.33a	3.38±0.22a
9 AT	3.18±0.16a	3.32±0.18a	3.43±0.38a	3.37±0.21a
10 AT	3.08±0.32a	3.13±0.18a	3.22±0.37a	3.32±0.25a
11 AT	3.08±0.20a	3.6±0.76a	2.97±0.58ba	2.57±0.33ab

Values in the same row or column with different superscripts are significantly different (P <0.05); BI= Before infection; PI= Post-infection; AT= After treatment

Table II. Plasma cobalt concentrations (mmol/L) (mean±SD) of rams fed on different diets

Time	Basal non–infected	High protein non–infected	Basal infected	High protein infected
BI	3.17±0.45	3.33±0.52	3.67±1.21	2.83±0.41
6th week PI	2.17±0.45	2.50±0.55	3.33±0.52	2.67±0.82
4th week AT	2.67±0.58	2.50±0.55	2.83±0.98	2.0±0.0

BI= Before infection; PI= Post-infection; AT= After treatment

Table III. Plasma copper concentrations (mmol/L) (mean±SD) of rams fed on different diets

Time	Basal non–infected	High protein non–infected	Basal infected	High protein infected
BI	762.17±58.73	786.17±47.91	746.0±116.84	890.0±263.96
6th week PI	755.0±88.85	698.50±104.67	589.67 43.18	705.67 178.46
4th week AT	883.98±112.51	721.33±62.33	771.0±70.82	784.17 150.37

BI= Before infection; PI= Post-infection; AT= After treatment

Table IV. Plasma zinc concentrations (mmol/L) (mean±SD) of rams fed on different diets

Time	Basal non–infected	High protein non–infected	Basal infected	High protein infected
BI	644.83±62.81	637.0 86.87	542.33±59.05	570.17±79.68
6th week PI	553.50±56.97	567.50±86.64	552.0±114.25	579.67±128.92
4th week AT	546.37±35.28	633.67±73.40	570.33±91.58	541.17±98.28

BI= Before infection; PI= Post-infection; AT= After treatment

Table V. Plasma selenium concentrations (mmol/L) (mean±SD) of rams fed on different diets

Time	Basal non–infected	High protein non–infected	Basal infected	High protein infected
BI	10.0±0	15.33±8.45	40.33±12.13	39.67±13.57
6th week PI	11.50±4.02	24.50±19.01	33.33±12.83	36.0±12.33
4th AT	19.55 7.79	23.0±15.80	33.50±17.77	42.0±22.10

BI= Before infection; PI= Post-infection; AT= After treatment

In the present study, neither parasite interaction nor diet had significant on plasma cobalt concentrations. There was no significant difference in the cobalt concentrations of four groups of rams at the second collection. However, there was a significant parasite by protein interactions effect on mean plasma cobalt concentrations. Cobalt concentrations in the plasma of a normal sheep are 1–3 µg/dL (Blood & Radostits, 1989). Cobalt adequacy can also be examined by determining vitamin B12 concentrations in plasma or serum or in liver, which are believed to reflect dietary cobalt intake and maintenance of vitamin B12 reserve (Langlands, 1987). Further the number of samples used in the present study may have also been inadequate.

In the present study, plasma copper concentrations were not significantly affected by parasite infection or protein content of the diet. But during the second collection the values for the infected rams fed the basal diet were lower than the values for the rams in the other three groups. Normal blood plasma copper levels in sheep are in the range 700–1300 µg/L (Blood & Radostits, 1989). These findings agree with those of Hucker and Yong (1986) who reported significant lower liver and plasma copper levels in sheep infested with nematodes than in non–infected sheep. Ortolani *et al.* (1993) also reported that plasma copper was decreased upto 50% in *H. contortus* infected lambs compared to non–infected lambs.

It should be pointed out, however, that in the present study copper levels were estimated by measuring plasma concentrations by spectrometry, as this was the only test available. More accurate estimation of copper is by determining liver concentrations using biopsy or sacrificing the animal (Langlands, 1987; Suttle, 1987; Grace & Lee, 1990). The results may also be limited by the small number of samples used.

In the present study, zinc concentrations were significantly affected by parasite infection or by diet. Normal serum levels of Zn reported by Blood and Radostits (1989) in sheep are above 78 µg/dL and values below 39 µg/dL are considered as deficient. None of the rams in the present study had plasma zinc concentrations that would be considered deficient. The significant difference in plasma zinc concentrations noted in the results suggested to be of no biological importance. The concentrations in the non–infected rams fed the high protein diet were elevated at the third sampling but the significance difference is not evident.

Zinc concentrations are usually monitored in plasma (Langlands, 1987) as was the case of the presence study. However, Mills *et al.* (1967) (cited by Langlands 1987) suggested that resampling on two occasions at least a week to give repeatable values is recommended to diagnose zinc deficiency.

In the present study, plasma selenium concentrations were significantly affected by parasite infection but diet had no significant affect. Examination of the values in the table shows that the significant parasite effect was due to the low concentrations in the un–infected rams fed the basal diet. There is no apparent reason for these values. Normal serum selenium levels in sheep given by Blood and Radostits (1989) is 80–500 µg/L. They also reported that marginal serum selenium levels are 30–50 µg/L and the deficient range is 6–30 µg/L. Therefore, the selenium status of all of the rams in the present study would be regarded as marginal or deficient.

Selenium status can be assessed from selenium content of whole blood, plasma or erythrocytes or glutathione peroxidase–px (GSH–px) activity in whole blood or erythrocytes (Langlands, 1987; Suttle 1987). In the present study the method used to estimate selenium plasma concentrations may have given less adequate results compared to the above methods. Also the number of samples tested may have been insufficient.

In Summary, the overall effects of the parasitic infection and diet were not significant for plasma cobalt, copper, zinc and selenium concentrations. However, due to the limitation of the tests available for use in this study, these results may not be a true reflection of the actual nutritional status of the rams.

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