MERINO EWES DIVERGENTLY SELECTED FOR CALM TEMPERAMENT HAVE A GREATER CONCENTRATION OF IMMUNOGLOBULIN G IN THEIR COLOSTRUM THAN NERVOUS EWES

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SUMMARY

At birth, the concentration of serum immunoglobulins (Ig) in the plasma of lambs is close to nil. A lamb must absorb Ig from its mother's colostrum to acquire immunity. There is considerable variation in the concentration of Ig in the colostrum of different ewes and the serum concentration of a lamb is influenced by the concentration in its mother's colostrum and the quantity of colostrum available. While Ig concentration in colostrum is influenced by environmental factors there is also evidence to show there is some genetic control. Temperament of the ewe has been shown to influence other aspects of colostrum quality. We hypothesised that temperament would also influence the concentration of Immunoglobulin-G (IgG), the most abundant Ig in ewe colostrum. Ewes from selection lines for calm and nervous temperament from the University of Western Australia's Merino flock were in individual pens and fed at maintenance for a single bearing late pregnant ewe. A 50 ml sample of colostrum was collected from each ewe shortly after she gave birth. IgG concentration was analysed using single radial immunodiffusion (IDRing® SHEEP IgG Test Plates: IDBiotech). The mean IgG concentration in the colostrum of ewes in the calm line (35.69 ± 2.47 mg/ml) was greater than ewes in the nervous line (30.26 ± 1.45 mg/ml; $P < 0.05$). Lambs of calm ewes can acquire increased immunity compared to lambs of nervous ewes.

INTRODUCTION

When a lamb is born its serum Ig concentration is close to nil (Parker and Nicol 1990). Neonatal lambs passively acquire immune competency by absorbing Ig that are present in colostrum (Halliday 1974). The total quantity of Ig produced by the ewe is correlated to the volume of colostrum produced (Shubber *et. al*. 1979a, 1979b). The Ig concentration in colostrum is greatest during the lambs first feed and it rapidly declines in subsequent meals (Shubber *et al*. 1979b) with the concentration by the fifth feed only being about 6% of the level available in the first meal. It declines even further over subsequent meals. This means there is a strong correlation between the volume of colostrum consumed by the lamb at its first meal and its serum Ig concentration 30 hours after that meal (Shubber *et al*. 1979b).

As this rapid decline in colostral Ig concentration is occurring there is a simultaneous rapid decline in the ability of the lamb to absorb Ig (Dominguez *et al.* 2001). As soon as 12 hours after it is born a lamb's ability to absorb Ig is only 40% as efficient as it is 30 minutes after birth.

The decline in both colostral Ig concentration and the lambs ability to absorb Ig means a peak in serum Ig concentration occurs about 24 hours after the first meal (Cabello and Levieux 1981; Parker and Nicol 1990). Once this peak is reached there is then a steady decline in the lambs serum Ig concentration until the lamb begins to develop the capacity to produce its own Ig. This process does not begin until the lamb is about four to six weeks of age (Ducker and Frazer 1976). It is crucial for a lamb to have access to a good quantity of colostrum with a high concentration of Ig.

Neonatal deaths are not due to low levels of colostral Ig. Lamb deaths related to inadequate Ig occur during the first 5 to 6 weeks of life. About 14% of lamb deaths occur between 2 - 10 days of age and a further 5% after day 10 (Khalaf *et al*. 1979). Serum IgG concentration at 24 hours of age

was much lower (< 18 mg/ml) in lambs that died compared to lambs that survived beyond 10 days of age (> 20 mg/ml; Khalaf *et al.* 1979). In addition McGuire *et al*. (1983) found 45% of lambs died within three weeks of birth where serum IgG concentration was low compared to only 5% where the serum IgG levels were adequate. In fact, serum IgG concentrations are lower in lambs whose mothers had less than 30 mg/ml of IgG in their colostrum compared to lambs with mothers that had more than 110 mg/ml (McGuire et al. 1983). While the volume of colostrum produced is an important factor for lambs to acquire adequate serum Ig, colostral Ig concentration also has an important role. There are also important environmental factors that influence colostral Ig concentration. These include nutrition of the ewe (Hall *et al*. 1992), age of the ewe (Halliday 1976) and litter size (Hall *et al.* 1992). There is also evidence of genetic control of Ig concentration in both colostrum and lamb serum. The performance of ewes in the transfer of Ig to their lambs over different years has a moderate repeatability (0.3; P< 0.001; Halliday 1974).

Recently we have shown ewe temperament is associated with differences in some components of colostrum, such as lactose, fat and viscosity, that relate to its quality (Hart *et al*. 2006). In addition, previous observations over two or more lambing opportunities have shown a difference in lamb mortality from day 3 to weaning, when the mortality rate of lambs born to calm ewes was lower (5.7%) than the lambs of nervous ewes (22.9%, P < 0.001; Murphy 1999). One of the factors underlying the difference in medium-term lamb mortality between calm and nervous ewes in Murphy's experiments may be differences in colostral Ig concentration. We hypothesised that ewes with calm temperament will have greater colostral IgG concentrations than nervous ewes.

MATERIALS AND METHODS

This project was approved by the University of Western Australia's Animal Ethics Committee (Approval number: RA/3/100/466). Two groups of single bearing ewe's were established using the temperament selection lines from the University of Western Australia's Merino flock. There were 18 ewes in the calm group and 23 in the nervous group. Ewe age ranged from 3 to 7 years and was not different between groups (Table 1). All ewes had previously reared at least one lamb and the mean weight of ewes was no different between groups (Table 1). The ewes were synchronised for artificial insemination using intravaginal sponges containing 40 mg of Fluogestone Acetate (Chrono-gest® 40, Intervet, Australia) for 14 to 15 days. At sponge withdrawal the ewes were injected with 200 IU of eCG (Folligon®, Intervet, Australia). The ewes were inseminated by laparoscopic intrauterine insemination about 50 hours after sponge removal.

Two weeks prior to lambing the ewes were moved indoors into single pens. Ewes were fed one kilogram of pellets per day (SPECIALTY FEEDS; late pregnant ewe cubes), which contained 12% protein and 10.5 MJ/kg M.E. Oaten hay and water were provided *ad lib.*

The ewes were observed 24 hours per day during lambing. Within 5 minutes of giving birth a sample of approximately 50 ml of colostrum was collected from each ewe. Each sample had 50 µl of potassium dichromate added and was then frozen until required for analysis.

Prior to analysis the frozen samples of colostrum were thawed slowly. The concentration of IgG was determined using single radial immunodiffusion (IDRing® SHEEP IgG Test Plates: IDBiotech).

Statistical analysis. A comparison of the means for IgG concentration between the treatment groups was made using a one-tail heteroscedastic t test.

RESULTS

The mean colostral IgG concentration is greater for calm ewes than for nervous ewes (P < 0.05; Table 1). Both groups showed a large variation between individuals in the IgG concentration in colostrum that was available at birth. Calm ewes ranged from 20.05 to 60.04 mg/ml, while the

range among nervous ewes was from 20.93 to 44.69 mg/ml. Mean birth weight of the lambs are presented in Table 1 and were not different between the selection lines.

Table 1: Shows the means ewe body weight, ewe age, lamb birth weight and the IgG concentration of colostrum available at birth from Calm and Nervous ewes (± S.E.)

Treatment group	No.	Ewe WT	Ewe age	Lamb BWT	IgG mg/m
Calm	18	57.7 ± 1.28 kg	4.28 ± 0.32	5.04 + 0.20 kg	35.69 ± 2.47^{a}
Nervous	23	56.1 ± 1.31 kg	4.13 ± 0.29	5.33 + 0.24 kg	30.26 ± 1.45^{b}

Values in a column with different superscripts are significantly different ($P < 0.05$).

DISCUSSION

Our hypothesis that ewes with calm temperament will have a greater concentration of colostral IgG than nervous ewes was supported. Given the narrow window of opportunity for a lamb to acquire Ig it is imperative that the concentration in colostrum is sufficient to acquire an adequate serum concentration. These results suggest that up until lambs begin to synthesise Ig the lambs of calm ewes have an increased chance of survival compared to the lambs of nervous ewes .

Colostrum produced by calm ewes has a greater concentration of lactose, which leads to lower viscosity (Hart *et al.* 2006). These factors mean the colostrum of calm ewes provides distinct advantages to their lambs during the critical neonatal period. It has more energy available to meet the lambs' immediate metabolic requirements and uses less energy as it is easier to suck. Having a greater concentration of colostral Ig provides the lambs of calm ewes with a greater level of immune competency until the lamb is able to synthesise its own Ig. These factors improve the chances of survival for the lambs of calm ewes compared to those with nervous mothers.

While the colostral IgG concentration is different between the selection lines the results of both groups are toward the lower end of the acceptable levels (~30 mg/ml) indicated by McGuire *et al*. (1983). Several environmental factors have been identified as influencing colostral Ig concentration. These include litter size (Hall *et al*. 1992), ewe age (Halliday 1976) and nutrition (Hall *et al*. 1992). Litter size is excluded as a factor in this experiment as all ewes produced singles. The ewes in this experiment are between 3 to 7 years old. The mean age (± se) of the ewes was 4.2 ± 0.2 years and was not different between the groups. Ewe age is excluded as a factor as Halliday (1976) found that colostral IgG concentration was only reduced in ewes that were 8 to 9 years old. This leaves nutrition as a possible explanation for low colostral IgG concentration in these ewes. If the relatively low IgG concentrations in colostrum are due to a nutritional challenge it may be postulated that the result in this experiment is due to calm ewes being better able to cope with that challenge.

The volume of colostrum is increased where particular nutritional supplements are given to ewes late in pregnancy (Banchero *et al*. 2004). Just as other factors associated with colostrum production by Merino ewes are associated with temperament of the ewe it may be that the response to such supplements would also be influenced by temperament. This is an important question as Banchero *et al*. (2004) found the response to be greater in twin bearing ewes. If temperament is also related to differences in response to supplements this may provide a useful strategy to reduce the mortality of lambs born as multiples.

Shubber *et al*. (1979a) found that the greater the total volume of colostrum the greater the amount of Ig produced and this was irrespective of litter size. However the total yield of colostrum is influenced by litter size. While twin bearing ewes produce more colostrum there is less available for each lamb (Shubber *et al.* 1979a, 1979b; Banchero *et al*. 2004). This suggests that the concentration of Ig in colostrum becomes an important factor for the survival of lambs born as multiples. There is a degree of compensation for multiples, as lambs that are less mature at birth

have an increased window of opportunity to absorb Ig (Cabello and Levieux 1981). While this provides some degree of compensation to multiple lambs it is confounded by lamb vigour. Shubber *et al*. (1979a, 1979b) found wide variation in the volume of colostrum produced by triplet bearing ewes. Ewes whose lambs were heavier at birth and had a greater suckling drive produced more colostrum than ewes with less vigorous lambs. These are important questions because lambs from multiple litters experience higher levels of mortality than lambs born as singles. It will be multiples that benefit most by having a mother that produces a greater quantity of better quality colostrum.

CONCLUSIONS

The differences in Ig concentration between calm and nervous ewes are in many respects similar to other differences in characteristics that define the quality in colostrum. Just as calm ewes have been shown to have a greater lactose concentration and lower viscosity than nervous ewes, calm ewes also have greater Ig concentrations. This suggests that the lambs of calm ewes have an improved chance of survival during the critical neonatal period compared to the lambs of nervous ewes, because they have access to colostrum that has more energy and is easier to suck . By also having greater concentrations of colostral Ig calm ewes improve their lambs' chances of survival beyond the neonatal period by providing an increased level of immunity to the lamb until it begins to develop its own immune system. There are two studies needed to follow up this research. The first is to determine whether there is a difference in the response to nutritional supplements given during late pregnancy between calm and nervous ewes. The second is to verify that these differences also apply to ewes that rear multiple lambs. This is because these factors are even more important to the survival of multiple lambs as each lamb in the litter has a reduced amount of colostrum available to it when compared to singles.

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