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LAMPIRAN

Lampiran 1. Analisis Biokimia

Glukosa darah. Penentuan kadar glukosa dalam darah dianalisis dengan menggunakan acuan prosedur kimia klinik Diasys dengan menggunakan metode *glucose oxidase-phenol 4-aminoantipirin* (GOD-PAP). Pengukuran kadar glukosa dilakukan dengan cara serum dicampur dengan reagen *glucose liquiqolor* (Diasys, Amerika) dan diinkubasi selama 5 menit pada suhu 37 °C. Warna yang terbentuk kemudian dibaca absorbansinya dengan menggunakan spektrofotometer dengan panjang gelombang 500 nm. Kadar glukosa dihitung dengan rumus sebagai berikut :

$$\left| \text{Kadar glukosa} = \frac{\Delta \text{Absorbansi Sampel}}{\Delta \text{Absorbansi Standar}} \times \text{Konsentrasi standar} \left(\frac{\text{mg}}{\text{dL}} \right) \right|$$

Kolesterol darah. Penentuan kadar kolesterol dalam darah dianalisis berdasarkan acuan prosedur kimia klinik Diasys. Metode yang digunakan adalah dengan *cholesterol oxidase phenol aminophenazone* (CHOD-PAP). Prinsip dari metode ini menggunakan prinsip oksidasi dan hidrolisis enzimatis dengan indikator warna yaitu *quinelmine* yang berasal dari reaksi *4-aminoantipyrine*, *phenol* dan *hydrogen peroxidase* yang dikatalis oleh *peroxidase*. Sampel terdiri dari blanko, standar, dan sampel yang dilakukan pembacaan absorbansi dengan spektrofotometer Microlab 300 secara berurutan metode *end poin* pada panjang gelombang 540 nm. kadar kolesterol dalam darah dihitung sebagai berikut :

$$\left| \text{Kadar kolesterol} = \frac{\Delta \text{Absorbansi Sampel}}{\Delta \text{Absorbansi Standar}} \times \text{Konsentrasi standar} \left(\frac{\text{mg}}{\text{dL}} \right) \right|$$

Protein darah. Penentuan kadar protein dalam darah dianalisis dengan menggunakan acuan prosedur kimia klinik Diasys dengan menggunakan metode

photometrics bromcresol green. Prinsip dari metode ini adalah serum protein dengan adanya *bromcresol green* pada pH agak asam akan menghasilkan perubahan warna sebagai indikator kuning-hijau sampai hijau-biru. Pengukuran dilakukan dengan cara 10 µl serum dicampur dengan reagen kit albumin menggunakan *vortex mixer* kemudian diinkubasikan selama kurang lebih 10 menit pada suhu 37°C. Pembacaan absorbansi dilakukan dengan spektrofotometer Microlab 300 secara berurutan yaitu blanko, standar, sampel dengan metode *end poin* pada panjang gelombang 540 nm. Kadar albumin dalam darah dihitung dengan rumus sebagai berikut :

$$\text{Kadar protein} = \frac{\Delta \text{Absorbansi Sampel}}{\Delta \text{Absorbansi Standar}} \times \text{Konsentrasi standar} \left(\frac{\text{mg}}{\text{dL}} \right)$$

Urea darah. Penentuan kadar urea dalam darah dianalisis dengan menggunakan acuan prosedur kimia klinik Diasys dengan menggunakan metode *urease* dan *glutamate dehydrogenase* (GLDH). Reagen mix dihasilkan dari campuran reagen I dan reagen II dengan perbandingan 4:1 yang dicampurkan pada botol gelap dan dibiarkan selama 30 menit pada suhu 15 hingga 25°C. Reagen I berisi *buffer TRIS*, *2-oxoglutarate*, *ADP*, *glutamate dehydrogenase* (GLDH), dan *urease*. Reagen II berisi *NADH*. Pembacaan absorbansi dilakukan dengan spektrofotometer Microlab 300 secara berurutan yaitu blanko, standar, sampel dengan metode *end poin* pada panjang gelombang 540 nm. Kadar albumin dalam darah dihitung dengan rumus sebagai berikut :

$$\text{Kadar urea} = \frac{\Delta \text{Absorbansi Sampel}}{\Delta \text{Absorbansi Standar}} \times \text{Konsentrasi standar} \left(\frac{\text{mg}}{\text{dL}} \right)$$

Lampiran 2. Analisis Hormon Steroid

Progesteron. Pada prinsipnya pengujian konsentrasi progesteron dengan menggunakan DRG ELISA KIT adalah *solid phase enzyme-linked immunosorbent assay* (ELISA) bergantung pada prinsip ikatan kompetitif. Permukaan sumuran KIT telah di lapisi dengan sebuah *polyclonal antibody* yang bersifat langsung berikatan dengan sisi antigen dari hormon progesteron. Progesteron *endogenous* dari sampel darah akan berkompetisi dengan *progesterone horseradish peroxidase conjugate* yang akan berikatan dengan antibodi yang di tempelkan di sumuran KIT. Setelah masa inkubasi *conjugate* yang tidak berikatan akan tercuci dengan *wash solution*. Jumlah ikatan dari *peroxidase conjugate* merupakan kebalikan dari proporsi konsentrasi hormon progesteron dalam sampel darah. Setelah pemberian larutan substrat, intensitas dari warna di sumuran akan terbentuk, semakin pekat warna yang di timbulkan maka konsentrasi progesteron semakin kecil.

Metode dari analisis ini adalah pertama siapkan sumuran KIT, kemudian masukan 25 μ L larutan standar, control, dan plasma sampel, diamkan selama 5 menit pada suhu ruangan. Kemudian masukan 200 μ L *enzyme conjugate* dan di inkubasi selama 60 menit, kemudian cuci sumuran menggunakan *wash solution* sebanyak 400 μ L sebanyak 3 kali. Setelah itu masukan 200 μ L *substrate solution* dan di inkubasi selama 15 menit, kemudian masukan 100 μ L *stop solution*. Terakhir baca absorbansi (OD) menggunakan ELISA *reader* pada panjang cahaya 450nm.

Pembacaan data dilakukan dengan mengitung angka rataan absorbansi pada setiap standar, control dan sampel. Kemudian membuat kurva dengan memasukan rata-rata absorbansi di tiap nilai standart, dimana garis vertical (Y

axis) adalah nilai absorbansi dan garis horizontal (X axis) merupakan nilai konsentrasi yang didapatkan. Dengan menggunakan formula yang didapatkan kemudian dimasukan hasil dari OD sampel untuk menentukan nilai konsentrasi sampel.

Estrogen. Pada prinsipnya pengujian konsentrasi estrogen dengan menggunakan DRG ELISA KIT adalah *solid phase enzyme-linked immunosorbent assay* (ELISA) bergantung pada prinsip ikatan kompetitif. Permukaan sumuran KIT telah di lapisi dengan sebuah *polyclonal antibody* yang bersifat langsung berikatan dengan sisi antigen dari hormon estrogen. Estrogen *endogenous* dari sampel darah akan berkompetisi dengan *estrogen horseradish peroxidase conjugate* yang akan berikatan dengan antibodi yang di tempelkan di sumuran KIT. Setelah masa inkubasi *conjugate* yang tidak berikatan akan tercuci dengan *wash solution*. Jumlah ikatan dari *peroxidase conjugate* merupakan kebalikan dari proporsi konsentrasi hormon estrogen dalam sampel darah. Setelah pemberian larutan substrat, intensitas dari warna di sumuran akan terbentuk, semakin pekat warna yang di timbulkan maka konsentrasi progesteron semakin kecil.

Metode dari analisis ini adalah pertama siapkan sumuran KIT, kemudian masukan 25 μ L larutan standar, control, dan plasma sampel, diamkan selama 5 menit pada suhu ruangan. Kemudian masukan 100 μ L *enzyme conjugate* dan di inkubasi selama 90 menit, kemudian cuci sumuran menggunakan *wash solution* sebanyak 400 μ L sebanyak 3 kali. Setelah itu masukan 100 μ L *substrate solution* dan di inkubasi selama 30 menit, kemudian masukan 50 μ L *stop solution*. Terakhir baca absorbansi (OD) menggunakan ELISA *reader* pada panjang cahaya 450nm.

Pembacaan data dilakukan dengan mengitung angka rata-rata absorbansi pada setiap standar, control dan sampel. Kemudian membuat kurva dengan memasukan rata-rata absorbansi di tiap nilai standart, dimana garis vertical (Y axis) adalah nilai absorbansi dan garis horizontal (X axis) merupakan nilai konsentrasi yang didapatkan. Dengan menggunakan formula yang didapatkan kemudian dimasukan hasil dari OD sampel untuk menentukan nilai konsentrasi sampel.

Kortisol. Pada prinsipnya pengujian konsentrasi kortisol dengan menggunakan DRG ELISA KIT adalah *solid phase enzyme-linked immunosorbent assay* (ELISA) bergantung pada prinsip ikatan kompetitif. Permukaan sumuran KIT telah di lapisi dengan sebuah *polyclonal antibody* yang bersifat langsung berikatan dengan sisi antigen dari hormon kortisol. Kortisol *endogenous* dari sampel darah akan berkompetisi dengan *progesterone horseradish peroxidase conjugate* yang akan berikatan dengan antibodi yang di tempelkan di sumuran KIT. Setelah masa inkubasi *conjugate* yang tidak berikatan akan tercuci dengan *wash solution*. Jumlah ikatan dari *peroxidase conjugate* merupakan kebalikan dari proporsi konsentrasi hormon kortisol dalam sampel darah. Setelah pemberian larutan substrat, intensitas dari warna di sumuran akan terbentuk, semakin pekat warna yang di timbulkan maka konsentrasi kortisol semakin kecil.

Metode dari analisis ini adalah pertama siapkan sumuran KIT, kemudian masukan 25 μ L larutan standar, control, dan plasma sampel, diamkan selama 5 menit pada suhu ruangan. Kemudian masukan 200 μ L *enzyme conjugate* dan di inkubasi selama 60 menit, kemudian cuci sumuran menggunakan *wash solution* sebanyak 400 μ L sebanyak 3 kali. Setelah itu masukan 200 μ L *substrate solution*

dan di inkubasi selama 15 menit, kemudian masukan 100 μ L *stop solution*. Terakhir baca absorbansi (OD) menggunakan ELISA *reader* pada panjang cahaya 450nm.

Pembacaan data dilakukan dengan mengitung angka rataan absorbansi pada setiap standar, control dan sampel. Kemudian membuat kurva dengan memasukan rata-rata absorbansi di tiap nilai standart, dimana garis vertical (Y axis) adalah nilai absorbansi dan garis horizontal (X axis) merupakan nilai konsentrasi yang didapatkan. Dengan menggunakan formula yang didapatkan kemudian dimasukan hasil dari OD sampel untuk menentukan nilai konsentrasi sampel.

Lampiran 3. Analisis Proksimat pakan.

Penetapan bahan kering. *Silica disk* dikeringkan dalam oven 105°C selama satu jam. Selanjutnya dimasukkan ke dalam desikator selama satu jam, kemudian ditimbang (X g). Sampel ditimbang (Y g) dan dimasukkan ke dalam *silica disk*, kemudian dimasukkan ke dalam oven 105°C sampai beratnya tetap. Selanjutnya dimasukkan ke dalam desikator selama satu jam, kemudian ditimbang (Z g).

Kadar bahan kering dihitung dengan rumus :

$$\text{Kadar bahan kering} = \frac{Z - X}{Y} \times 100\%$$

Keterangan : Z = berat *silica disk* dan sampel setelah oven 105°C

X = berat *silica disk*

Y = berat sampel

Penetapan bahan organik. *Silica disk* dikeringkan ke dalam oven 105°C selama satu jam, kemudian dimasukkan ke dalam desikator selama satu jam dan ditimbang (X g). Sampel ditimbang (Y g) dan dimasukkan ke dalam *silica disk*, kemudian diabukan pada tanur suhu 550 - 600°C sampai berwarna putih. Selanjutnya *silica disk* yang berisi abu diambil dan dimasukkan ke dalam desikator selama satu jam kemudian ditimbang sebagai (Z g).

Kadar bahan organik dihitung dengan rumus :

$$\text{Kadar bahan organik} = \frac{(X+Y-Z)}{Y} \times 100\%$$

Keterangan :

Z = Berat *silica disk* dan sampel setelah diabukan

X = Berat *silica disk*

Y = Berat sampel

Penetapan serat kasar. Sampel ditimbang sebanyak 2 g (X g) kemudian dimasukkan ke dalam *beaker glass* 600 mL dan ditambah 200 mL H₂SO₄ 1,25% kemudian dipasang pada pemanas yang mempunyai aliran pendingin dan dididihkan selama 30 menit. Kemudian disaring menggunakan *gooch crucible* yang telah berisi *glass wool*. Selanjutnya hasil saringan dimasukkan dalam *beaker glass* 600 mL dan ditambah dengan 200 mL NaOH 1,25% kemudian dididihkan selama 30 menit. Setelah itu disaring dengan *glass wool* menggunakan *gooch crucible*. Dicuci dengan beberapa air panas dan dengan etil alkohol 95%. Hasil saringan termasuk *glass wool* dimasukkan pada oven 105°C selama semalam, kemudian dimasukkan ke dalam desikator selama satu jam, kemudian ditimbang (Y g). Selanjutnya dimasukkan ke dalam tanur 600°C selama semalam hingga abu berwarna putih seluruhnya. Sampel dikeluarkan dan dimasukkan ke dalam desikator selama satu jam, kemudian ditimbang (Z g).

Kadar serat kasar dihitung dengan rumus:

$$\text{Kadar serat kasar} = \frac{Y - Z}{X} \times 100\%$$

Keterangan:

Y = Berat sampel setelah oven 105°C

Z = Berat setelah diabukan

X = Berat sampel awal

Penentuan kadar lemak kasar. Sampel bahan ditimbang 0,5 - 1 g (X g) dan dibungkus dengan kertas saring bebas lemak sebanyak 3 bungkus, masing-masing bungkus dimasukkan ke dalam oven pengering 105°C selama

semalam. Kemudian sampel ditimbang dalam keadaan panas satu persatu (Y g).

Bungkusan dimasukkan ke dalam soxhlet dan diekstraksi dengan petroleum benzen selama + 16 jam (sampai petroleum benzen dalam soxhlet jernih).

Bungkusan dikeluarkan dan di masukkan dalam oven 105°C selama semalam.

Kemudian ditimbang dalam keadaan panas bungkusan tersebut satu persatu (Z g).

Kadar lemak kasar dihitung dengan rumus:

$$\text{Kadar lemak kasar} = \frac{Y - Z}{X} \times 100\%$$

Keterangan :

X = berat sampel awal

Y = berat sampel setelah oven 105 °C

Z = berat sampel setelah diekstraksi

Lampiran 4. Komposisi pakan Tahap I

Tabel. Komposisi Pakan Konsentrat

| Bahan | Proporsi (%) | (% dalam BK) | | | | |
|-----------------|---------------|--------------|--------------|-------------|--------------|--------------|
| | | BK | PK | LK | SK | TDN |
| Bungkil Kedelai | 11,00 | 9,79 | 5,28 | 0,25 | 0,57 | 9,70 |
| CGF | 15,00 | 13,37 | 3,15 | 0,20 | 1,50 | 11,40 |
| Bungkil Kelapa | 22,00 | 19,84 | 4,76 | 2,49 | 3,45 | 17,00 |
| Mineral | 2,00 | 1,98 | 0,00 | 0,01 | 0,01 | 0,00 |
| Onggok | 20,00 | 17,03 | 0,28 | 0,06 | 1,39 | 15,00 |
| CGM | 3,00 | 2,73 | 1,42 | 0,07 | 0,15 | 2,65 |
| Polard | 27,00 | 23,63 | 3,73 | 1,26 | 3,57 | 20,64 |
| Total | 100,00 | 88,38 | 18,61 | 4,33 | 10,65 | 76,39 |

Tabel. Komposisi Pakan Hijauan

| Bahan | Proporsi (%) | Komposisi Nutrien (gr) | | | | |
|--------------|--------------|------------------------|--------------|-------------|--------------|--------------|
| | | BK | PK | LK | SK | TDN |
| Jagung | 46 | 12,03 | 3,69 | 1,29 | 11,96 | 31,28 |
| Odor | 23 | 3,05 | 2,30 | 0,99 | 6,84 | 15,54 |
| Kaliandra | 31 | 9,69 | 5,95 | 0,88 | 6,28 | 25,49 |
| Total | 100 | 24,77 | 11,94 | 3,16 | 25,09 | 72,31 |

Tabel . Jumlah pemberian pakan (g BK/hari)

| Bahan pakan | Jumlah pemberian (gr BK) | Jumlah pemberian (BK %) |
|-------------------|--------------------------|-------------------------|
| Konsentrat | 309,31 | 29,39 |
| - Bungkil kedelai | 34,02 | 3,23 |
| - CGF | 46,40 | 4,41 |
| - Bungkil kelapa | 68,05 | 6,47 |
| - Mineral | 6,19 | 0,59 |
| - Onggok | 61,86 | 5,88 |
| - CGM | 9,28 | 0,88 |
| - Polard | 83,51 | 7,94 |
| Hijauan | 742,99 | 70,61 |
| - Jagung | 360,87 | 32,48 |
| - Odor | 91,49 | 16,24 |
| - kaliandra | 290,63 | 21,89 |
| Kandungan nutrisi | | |
| - PK | 93,89 | 14,39 |
| - LK | 22,31 | 3,39 |
| - SK | 180,00 | 20,23 |
| - TDN | 546,15 | 74,35 |

Tabel. Koreksi konsumsi pakan dengan tabel kebutuhan nutrisi pakan NRC 1981 dengan berat rata-rata kambing 45kg

| | TDN | PK |
|----------------|----------------|---------------|
| Pemberian (gr) | 782,42 | 151,46 |
| Kebutuhan (gr) | 611,00 | 84,00 |
| Koreksi (gr) | +171,42 | +67,46 |
| Koreksi (%) | +28,05 | +80,31 |

Lampiran 5. Komposisi Pakan Tahap II

Tabel. Komposisi Pakan Konsentrat yang diberikan

| Bahan | Proporsi | BK | PK | LK | SK | TDN |
|-----------------|----------|--------|--------|-------|-------|--------|
| Konsentrat (%) | 100 | 91.52 | 16.38 | 5.35 | 9.08 | 77.47 |
| Konsentrat (gr) | 1000 | 915.52 | 149.83 | 49.98 | 83.08 | 709.21 |

Tabel. Komposisi Pakan Hijauan yang diberikan

| Bahan | Proporsi | BK | PK | LK | SK | TDN |
|-----------------|----------|--------|-------|-------|-------|-------|
| Konsentrat (%) | 100 | 59.67 | 9.07 | 3.09 | 6.67 | 39.66 |
| Konsentrat (gr) | 500 | 201.68 | 18.29 | 13.44 | 13.44 | 79.99 |

Tabel. Jumlah Pemberian Pakan (gram BK/hari)

| Bahan pakan | Jumlah pemberian (BK gr) | Jumlah pemberian (%) |
|------------------------------|--------------------------|----------------------|
| Konsentrat | 915,52 | 81,95 |
| Hijauan | 201,68 | 18,06 |
| Kandungan nutrisi(ekor/hari) | | |
| PK | 789,21 | 15,05 |
| LK | 55,22 | 4,94 |
| SK | 96,53 | 8,64 |
| TDN | 789,21 | 86,20 |

Tabel. Koreksi konsumsi pakan dengan tabel kebutuhan nutrisi pakan NRC 1981 dengan berat rata-rata kambing 40kg

| | TDN | PK |
|----------------|----------------|----------------|
| Pemberian (gr) | 789,21 | 168,13 |
| Kebutuhan (gr) | 560,00 | 77,00 |
| Koreksi (gr) | +229,21 | +91,13 |
| Koreksi (%) | +40,93 | +118,34 |

Lampiran 6. Komposisi Pakan Tahap III

Tabel. Komposisi Proksimat Pakan Konsentrat

| Bahan | Komposisi Nutrien (gr) | | | | |
|------------|------------------------|-------|------|-------|-----|
| | BK | PK | LK | SK | TDN |
| Konsentrat | 88,94 | 12,39 | 6,49 | 15,91 | 68 |

Tabel. Komposisi Proksimat Bahan Pakan Hijauan

| Bahan | Komposisi Nutrien (gr) | | | | |
|--------------|------------------------|-------|------|------|-------|
| | BK | PK | LK | SK | TDN |
| Kaliandra | 30,31 | 15,14 | 0,68 | 7,22 | 60,37 |
| Rumput Gajah | 23,28 | 9,92 | 0,40 | 7,67 | 62,30 |

Tabel. Jumlah pemberian pakan (g BK/hari) Pakan Kontrol Nararya

| Bahan pakan | Jumlah pemberian | |
|-------------------|------------------|--------|
| | (BK gr) | (% BK) |
| Konsentrat | 444,70 | 67,47 |
| Hijauan | 214,36 | 32,53 |
| - Kaliandra | 121,24 | 18,39 |
| - Rumput gajah | 93,12 | 14,13 |
| Kandungan nutrisi | | |
| - PK | 82,69 | 12,54 |
| - LK | 30,06 | 4,56 |
| - SK | 86,65 | 13,15 |
| - TDN | 433,60 | 65,79 |

Tabel. Koreksi konsumsi pakan dengan tabel kebutuhan nutrisi pakan NRC 1981 dengan berat rata-rata kambing 45kg

| | TDN | PK |
|----------------|---------------|--------------|
| Pemberian (gr) | 433,60 | 82,69 |
| Kebutuhan (gr) | 611,00 | 84,00 |
| Koreksi (gr) | -29,03 | -1,31 |
| Koreksi (%) | -29,04 | -1,56 |

Tabel. Jumlah pemberian pakan (g BK/hari) Pakan I (TDN dan PK ideal \pm 5% dari kebutuhan NRC)

| Bahan pakan | Jumlah pemberian (BK gr) | Jumlah pemberian (% BK) |
|-------------------|-----------------------------|----------------------------|
| Konsentrat | 711,52 | 71,96 |
| Hijauan | 288,17 | 28,03 |
| - Kaliandra | 90,93 | 9,19 |
| - Rumput gajah | 186,24 | 18,84 |
| Kandungan nutrisi | | |
| - PK | 87,34 | 8,83 |
| - LK | 47,54 | 4,81 |
| - SK | 134,05 | 13,56 |
| - TDN | 654,76 | 66,22 |

Tabel. Koreksi konsumsi pakan dengan tabel kebutuhan nutrisi pakan NRC 1981 dengan berat rata-rata kambing 40kg

| | TDN | PK |
|----------------|--------------|--------------|
| Pemberian (gr) | 654,76 | 87,34 |
| Kebutuhan (gr) | 611,00 | 84,00 |
| Koreksi (gr) | 43,76 | 3,98 |
| Koreksi (%) | +7,16 | +3,98 |

Tabel. Jumlah pemberian pakan (g BK/hari) Pakan II (TDN dan PK lebih dari kebutuhan \pm 20% dari kebutuhan NRC)

| Bahan pakan | Jumlah pemberian (BK gr) | Jumlah pemberian (% BK) |
|-------------------|-----------------------------|----------------------------|
| Konsentrat | 800,46 | 67,56 |
| Hijauan | 384,35 | 32,44 |
| - Kaliandra | 151,55 | 12,79 |
| - Rumput gajah | 232,80 | 19,65 |
| Kandungan nutrisi | | |
| - PK | 101,14 | 8,53 |
| - LK | 53,91 | 4,55 |
| - SK | 156,15 | 13,18 |
| - TDN | 780,84 | 65,90 |

Tabel. Koreksi konsumsi pakan dengan tabel kebutuhan nutrisi pakan NRC 1981 dengan berat rata-rata kambing 40kg

| | TDN | PK |
|----------------|---------------|---------------|
| Pemberian (gr) | 780,84 | 101,13 |
| Kebutuhan (gr) | 611,00 | 84,00 |
| Koreksi (gr) | 169,83 | 17,13 |
| Koreksi (%) | +27,79 | +20,40 |