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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 lapiasi 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 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.



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 *gouch 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 *gouch crusible*. 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:

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

X

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
Odot	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
- Odot	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