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羔羊断奶前后血清生化指标、抗氧化和免疫性能的变化

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摘要:本研究旨在探究断奶前后血清生化指标、抗氧化和免疫性能的变化,分析羔羊早期断奶应激发生规律,为营养调控羔羊断奶应激提供理论依据。选取12只出生时间相同,体重相近的健康湖羊羔羊于30日龄进行断奶,羔羊断奶前(断奶0 d)和断奶后3、6和15 d采集血清用于生化指标以及激素浓度、抗氧化和免疫性能等指标的测定。结果表明:1)与断奶0 d相比,断奶15 d羔羊血清谷丙转氨酶(ALT)活性显著降低($P < 0.05$),断奶6 d羔羊血清碱性磷酸酶(ALP)活性和总蛋白(TP)含量显著降低($P < 0.05$)。断奶6 d羔羊血清总胆固醇(TC)含量显著高于其他各组($P < 0.05$),血清尿素氮(BUN)含量随着断奶时间的延长而逐渐升高,而各组间葡萄糖(GLU)含量无显著差异($P > 0.05$)。2)与断奶3 d相比,断奶15 d羔羊血清三碘甲状腺原氨酸(T_3)浓度和断奶6 d羔羊血清四碘甲状腺原氨酸(T_4)浓度均显著升高($P < 0.05$)。断奶6 d羔羊血清胰岛素样生长因子1(IGF-1)浓度显著低于断奶0和3 d($P < 0.05$),而各组间肾上腺素(EPI)浓度无显著差异($P > 0.05$)。3)与断奶0 d相比,断奶3 d羔羊血清总抗氧化能力(T-AOC)、谷胱甘肽过氧化物酶(GSH-Px)和过氧化氢酶(CAT)活性显著降低($P < 0.05$),而断奶15 d羔羊超氧化物歧化酶(SOD)和CAT活性则显著升高($P < 0.05$)。与断奶0 d相比,断奶3 d羔羊血清丙二醛(MDA)浓度显著升高($P < 0.05$),而断奶6和15 d核因子E2相关因子2(Nrf2)浓度则显著降低($P < 0.05$)。断奶0 d羔羊血清血红素加氧酶-1(HO-1)浓度显著低于其他各组($P < 0.05$)。4)与断奶3 d相比,断奶15 d羔羊免疫球蛋白A(IgA)浓度显著升高($P < 0.05$),断奶6和15 d羔羊血清免疫球蛋白G(IgG)浓度显著升高($P < 0.05$),而各组间免疫球蛋白M(IgM)浓度则无显著差异($P > 0.05$)。断奶0 d血清CD4⁺T淋巴细胞(CD4)浓度和CD8⁺T淋巴细胞(CD8)浓度的比值显著低于其他各组($P < 0.05$),其中二者均在断奶6 d最高,而各组间CD8浓度则无显著差异($P > 0.05$)。5)断奶3 d羔羊血清白细胞介素-1 β (IL-1 β)浓度显著高于其他各组($P < 0.05$),而干扰素- γ (IFN- γ)浓度则相反。与断奶3 d相比,断奶6和15 d羔羊血清白细胞介素-2(IL-2)、白细胞介素-6(IL-6)和肿瘤坏死因子- α (TNF- α)浓度显著下降($P < 0.05$)。与断奶0 d相比,断奶3、6和15 d羔羊血清白细胞介素-8(IL-8)和白细胞介素-22(IL-22)浓度均显著升高($P < 0.05$),而血清核因子 κ B(NF- κ B)浓度则相反。与断奶0 d相比,断奶6和15 d羔羊血清半胱氨酸蛋白酶3(Caspase3)浓度显著下降($P < 0.05$)。综上所述,羔羊早期断奶后会造成机体抗氧化能力和免疫力下降,增强炎症反应。随着断奶后时间的延长,羔羊抗氧化、抗炎能力和免疫力有所增强。

关键词:羔羊;早期断奶;抗氧化;血液生化指标;免疫性能

The changes of serum biochemical indexes, oxidation resistance and immune property in pre- and post-weaning lambs

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Abstract: This study investigated the changes in serum biochemical indexes, and antioxidant and immune properties in pre- and post-weaning lambs, analyzed the incidence of early weaning stress in lambs, with the aim providing a theoretical basis for nutritional regulation of weaning stress in lambs. Twelve healthy Hu lambs with the same birth date and similar body weight were chosen and weaned at 30 days of age. The serum of lambs was collected prior to weaning (Day 0) and at 3, 6 and 15 days post-weaning for measurement of biochemical parameters, hormone concentrations, and antioxidant and immune properties. The following results were obtained: 1) Compared with Day 0, serum alanine aminotransferase activity of lambs at Day 15 after weaning was significantly decreased ($P < 0.05$) and serum alkaline phosphatase (ALP) activity and total protein content of lambs at Day 6 were significantly decreased ($P < 0.05$). Serum total cholesterol content of lambs at Day 6 was significantly higher than that of other groups ($P < 0.05$). Serum urea nitrogen content increased gradually with post-weaning time, but blood glucose did not differ among groups ($P > 0.05$). 2) Compared with Day 3 post-weaning, serum triiodothyronine of lambs at Day 15 was significantly increased ($P < 0.05$). Similarly, Day 15 tetraiodothyronine concentration of lambs was increased relative to Day 6. Serum insulin-like growth factor-1 was significantly lower at Day 6 than at Days 0 or 3 ($P < 0.05$), but epinephrine concentration did not differ among groups ($P > 0.05$). 3) Compared with Day 0, serum total antioxidant capacity, glutathione peroxidase and catalase (CAT) activities of lambs at Day 3 was significantly decreased ($P < 0.05$), but superoxide dismutase and CAT activities of lambs at Day 15 was significantly increased ($P < 0.05$). Compared with Day 0, serum malondialdehyde concentration of lambs at Day 3 was significantly increased ($P < 0.05$), but nuclear factor-erythroid 2-related factor-2 concentration at Days 6 and 15 was significantly decreased ($P < 0.05$). Serum heme oxygenase-1 concentration of lambs at Day 0 was significantly lower than that of other groups ($P < 0.05$). 4) Compared with Day 3, serum immunoglobulin A concentration of lambs at Day 15 was significantly increased ($P < 0.05$), and immunoglobulin G concentrations of lambs at Days 6 and 15 were also significantly increased ($P < 0.05$), while immunoglobulin M did not differ between groups ($P > 0.05$). The immune cell CD 4⁺ T-lymphocyte concentration and CD 4⁺ T-lymphocyte: CD 8⁺ at Day 0 were significantly lower ($P < 0.05$) than those at other times, with the highest values observed at Day 6. The CD 8⁺ T-lymphocyte concentration did not differ among groups ($P > 0.05$). 5) Serum interleukin-1 β concentration of lambs at Day 3 was significantly higher than at other times, but interferon gamma concentration was lowest at Day 3. Compared with Day 3, the concentrations of interleukin-2, interleukin-6 and tumor necrosis factor- α at Days 6 and 15 were significantly decreased ($P < 0.05$). Compared with Day 0, the concentrations of interleukin-8 and interleukin-22 at Days 3, 6 and 15 were significantly increased ($P < 0.05$), but the serum nuclear factor kappa-B concentrations were decreased. Compared with Day 0, the concentration of cysteinyl aspartate specific proteinase 3 at Days 6 and 15 was significantly decreased ($P < 0.05$). In summary, early weaning decreased the body antioxidant and immune capacities and enhanced the inflammatory responses of lambs. The antioxidant, anti-inflammatory and immune capacities of the lambs were recovered with the passage of time after weaning.

Key words: lambs; early weaning; oxidation resistance; blood biochemical indexes; immune property

羔羊早期断奶能够提高母羊利用率,缩短繁殖周期,增加羔羊生产效益^[1]。然而,羔羊早期断奶会因与母羊隔离、日粮与饲喂方式的改变产生一系列的心理和生理应激反应,导致运动增加,减少休息和进食行为,降低体重^[2-3]。此外,由于羔羊的免疫系统正在发育,早期断奶应激对羔羊肠道健康造成负面影响,引起腹泻和死亡,进而影响生长性能^[4]。柴建民等^[5]研究发现,羔羊10和30日龄断奶后10 d,羔羊体重、日增重以及氮、粗脂肪、钙、磷等营养物质表观消化率均显著低于对照组(随母哺乳组)。沈露等^[6]研究发现,25日龄断奶的羔羊平均日采食量、沉积氮显著高于35和45日龄断奶羔羊,但粗蛋白表观消化率则相反。此外,与常规断奶相比,早期断奶犊牛的末重、平均日增重均显著降低^[7]。然而,也有研究发现,30、75和180 d断奶的犊牛月增重和平均日增重均无显著差异^[8]。结果表明,早期断奶会影响机体对营养物质消化吸收,进而影响幼龄反刍动物的生长、发育,但不同品种存在差异。血清生化指标的变化在一定程度上能够反映机体的健康状况。范超等^[9]研究奶水牛犊牛断奶前1 d和断奶后1、14和21 d血清生化指标的变化,发现断奶后犊牛血清乳酸脱氢酶(lactic dehydrogenase, LDH)、谷丙转氨酶(alanine aminotransferase, ALT)活性、血清尿素氮(urea nitrogen, BUN)含量显著高于断奶前,断奶后21 d犊牛中性粒细胞数和血红蛋白含量均高于断奶前。在探究中国荷斯坦母犊牛断奶前后血液生化指标变化规律的研究中发现,断奶前2 d犊牛的血清BUN、总胆固醇(total cholesterol, TC)、甘油三酯(triglyceride, TG)含量高于断奶后1、2和3周,而总蛋白(total protein, TP)、球蛋白(globulin, GLB)、谷草转氨酶(aspartate aminotransferase, AST)、白细胞介素-1 β (interleukin-1 β , IL-1 β)和结合珠蛋白(haptoglobin, HP)含量则相反;断奶后3周血液性磷酸酶(alkaline phosphatase, ALP)和二胺氧化酶(diamine oxidase, DAO)含量高于断奶前2 d^[10]。此外,研究发现,在进行10 d的驯化后断奶的羔羊血清TP、BUN、GLB、白蛋白和皮质醇(cortisol, COR)浓度与未断奶羔羊无显著差异,但TC的浓度则显著降低^[11];30 d断奶犊牛在第31、32、180和181天的COR水平高于75和180 d断奶犊牛,75 d断奶犊牛在第77和82天的COR水平高于180 d断奶犊牛^[12]。以上结果表明,可以通过测定血液生化指标了解幼龄反刍动物断奶应激反应和生长发育的情况。目前,有关羔羊早期断奶前后血液生化指标变化规律的研究鲜见报道。为此,本试验旨在通过研究湖羊羔羊30日龄断奶前后血液生理生化指标的变化,探究羔羊早期断奶应激反应变化规律,为下一步营养调控早期断奶应激提供理论依据。

1 材料与方法

1.1 试验设计与饲养管理

本试验于2022年6—7月在江西省赣州绿林湾农牧有限公司湖羊养殖场进行。试验选取12只出生时间相同、体重相近且健康的湖羊公羔羊,出生至29日龄随母哺乳,15日龄开始补饲,30日龄进行断奶。在断奶前(0 d)、断奶后3、6和15 d晨饲前进行颈静脉采血,然后收集血清,−20℃保存待测。羔羊舍饲,自由采食,自由饮水,其他饲养管理按照养殖场的规定执行。羔羊补饲日粮为市购羔羊料,其组成为玉米(*Zea mays*) 63.50%,豆粕16.50%,棉籽粕6.00%,菜籽粕5.00%,玉米干酒糟1.20%,玉米皮4.32%,石粉1.18%,磷酸氢钙1.00%,氯化钠0.30%,预混料1.00%;营养水平实测值为消化能12.29 MJ·kg⁻¹,粗蛋白18.85%,粗灰分7.35%,粗脂肪3.44%,中性洗涤纤维28.24%,酸性洗涤纤维12.54%,钙1.49%,磷0.73%。

1.2 血清相关指标的测定

1.2.1 生化指标和抗氧化指标的测定 血清生化指标和抗氧化指标均采用购买的南京建成生物工程研究所生产的试剂盒进行检测,测定步骤按试剂盒说明书进行操作,最后在酶标仪(Rayto RT-6100,深圳雷杜生命科学股份有限公司)上检测。所测血清生化指标有总蛋白(TP)、总胆固醇(TC)、葡萄糖(glucose, GLU)、尿素氮(BUN)含量、谷丙转氨酶(ALT)、谷草转氨酶(AST)和碱性磷酸酶(ALP)活性;抗氧化指标有谷胱甘肽过氧化物酶(glutathione peroxidase, GSH-Px)、过氧化氢酶(catalase, CAT)、超氧化物歧化酶(superoxide dismutase, SOD)活性、总抗氧化能力(total antioxidant capacity, T-AOC)和丙二醛(malondialdehyde, MDA)含量。

1.2.2 激素和免疫性能指标的测定 血清三碘甲状腺原氨酸(triiodothyronine, T₃)、四碘甲状腺原氨酸(tetraiodothyronine, T₄)、胰岛素样生长因子-1(insulin-like growth factor-1, IGF-1)和肾上腺素(epinephrine, EPI)以及免疫球蛋白G(immunoglobulin G, IgG)、免疫球蛋白A(immunoglobulin A, IgA)、免疫球蛋白M

(immunoglobulin M, IgM)和细胞因子白细胞介素-1 β (interleukin-1 β , IL-1 β)、肿瘤坏死因子- α (tumor necrosis factor- α , TNF- α)、白细胞介素-2(interleukin-2, IL-2)、白细胞介素-6(interleukin-6, IL-6)和干扰素- γ (interferon- γ , IFN- γ)均采用上海科艾博生物技术有限公司生产的酶联免疫分析试剂盒检测;白细胞介素-8(interleukin-8, IL-8)、白细胞介素-22(interleukin-22, IL-22)、CD 4⁺ T淋巴细胞(CD 4⁺ T-lymphocyte, CD 4)、CD 8⁺ T淋巴细胞(CD 8⁺ T-lymphocyte, CD 8)、半胱氨酸蛋白酶3(cysteiny l aspartate specific proteinase 3, Caspase3)、核因子 κ B(nuclear factor kappa-B, NF- κ B)、核因子E2相关因子2(nuclear factor-erythroid 2-related factor-2, Nrf2)以及血红素加氧酶-1(heme oxygenase-1, HO-1)均采用上海科兴商贸有限公司提供的酶联免疫分析试剂盒检测。所有指标的检测方法参照试剂盒说明书进行。

1.3 数据处理与分析

采用WPS Office 2019进行数据整理,再用IBM SPSS 21.0软件进行单因素方差分析(ANOVA),Duncan氏法进行多重比较,结果以“平均值 \pm 标准误”来表示, $P < 0.05$ 表示差异显著, $P > 0.05$ 表示差异不显著。

2 结果与分析

2.1 断奶前后羔羊血清生化指标的变化

与断奶0 d相比,断奶15 d羔羊血清ALT活性显著降低($P < 0.05$,表1);断奶6 d羔羊血清TP含量和ALP活性显著降低($P < 0.05$),而TC含量则显著升高($P < 0.05$)。羔羊血清BUN含量随着断奶时间的延长而逐渐升高,而各组间血清GLU无显著差异($P > 0.05$)。从本试验结果来看,断奶3 d后会影响机体对蛋白质和脂肪的吸收与代谢。

表1 断奶前后羔羊血液生化指标变化

Table 1 The change of serum biochemistry indices in pre- and post-weaning lambs

项目 Item	断奶天数 Weaning days (d)			
	0	3	6	15
谷丙转氨酶 Alanine aminotransferase (ALT, U \cdot L ⁻¹)	11.32 \pm 1.46a	8.87 \pm 2.10abc	9.36 \pm 2.13ab	3.99 \pm 0.50c
谷草转氨酶 Aspartate aminotransferase (AST, U \cdot L ⁻¹)	31.72 \pm 4.98a	18.27 \pm 3.63a	18.22 \pm 9.18a	31.63 \pm 7.72a
碱性磷酸酶 Alkaline phosphatase (ALP, KU \cdot 100 mL ⁻¹)	11.38 \pm 3.21a	7.26 \pm 0.56ab	3.27 \pm 0.60b	6.15 \pm 2.56ab
葡萄糖 Glucose (GLU, mmol \cdot L ⁻¹)	6.94 \pm 0.28a	5.80 \pm 0.51a	9.32 \pm 2.71a	6.46 \pm 0.26a
总蛋白 Total protein (TP, mg \cdot mL ⁻¹)	91.27 \pm 8.92a	94.69 \pm 8.82a	47.83 \pm 6.84b	71.50 \pm 7.70ab
总胆固醇 Total cholesterol (TC, mmol \cdot L ⁻¹)	2.86 \pm 0.37b	3.54 \pm 0.27b	12.33 \pm 2.64a	6.13 \pm 0.61b
尿素氮 Urea nitrogen (BUN, mmol \cdot L ⁻¹)	2.20 \pm 0.19c	2.39 \pm 0.21c	4.42 \pm 0.33b	7.20 \pm 0.95a

同行不同字母表示各处理间差异显著($P < 0.05$)。下同。Different lowercase letters in the same row mean significant difference among treatments ($P < 0.05$). The same below.

2.2 断奶前后羔羊血清激素水平的变化

与断奶3 d相比,断奶15 d羔羊血清T₃浓度显著升高($P < 0.05$,表2),断奶6 d羔羊血清T₄浓度显著升高($P < 0.05$)。断奶6 d羔羊血清IGF-1浓度显著低于断奶0和3 d($P < 0.05$),而各组间EPI浓度无显著差异($P > 0.05$)。从本试验结果来看,断奶后会显著影响羔羊甲状腺激素和胰岛素样生长因子1的分泌。

2.3 断奶前后羔羊血清抗氧化能力的变化

与断奶0 d相比,断奶3 d羔羊血清T-AOC、GSH-Px和CAT活性显著降低($P < 0.05$,表3),而断奶15 d羔羊CAT活性则显著升高($P < 0.05$)。与断奶0 d相比,断奶15 d羔羊血清SOD活性和断奶3 d羔羊血清MDA浓度均显著升高($P < 0.05$);断奶6和15 d羔羊血清Nrf2浓度显著降低($P < 0.05$);断奶3、6和15 d羔羊血清HO-1浓度均显著升高($P < 0.05$),其中断奶3 d羔羊血清HO-1浓度最高。从本试验结果来看,断奶3 d会降低羔羊机体抗氧化能力,随着断奶时间的延长机体抗氧化能力有所升高。

表2 断奶前后羔羊血清激素浓度变化

Table 2 The change of serum hormone concentration in pre- and post-weaning lambs

项目 Item	断奶天数 Weaning days (d)			
	0	3	6	15
三碘甲状腺原氨酸 Triiodothyronine (T_3 , nmol·L ⁻¹)	3.66±0.28ab	3.14±0.29b	3.63±0.40ab	4.48±0.27a
四碘甲状腺原氨酸 Tetraiodothyronine (T_4 , nmol·L ⁻¹)	130.51±10.11ab	116.42±9.17b	150.53±9.15a	144.98±10.91ab
胰岛素样生长因子-1 Insulin-like growth factor-1 (IGF-1, μg·mL ⁻¹)	1.45±0.07a	1.49±0.11a	1.18±0.08b	1.26±0.08ab
肾上腺素 Epinephrine (EPI, ng·mL ⁻¹)	10.34±0.79a	8.04±0.88a	8.98±0.77a	9.89±0.64a

表3 断奶前后羔羊抗氧化能力的变化

Table 3 The change of antioxidant capacity in pre- and post-weaning lambs

项目 Item	断奶天数 Weaning days (d)			
	0	3	6	15
总抗氧化能力 Total antioxidant capacity (T-AOC, U·mL ⁻¹)	16.36±0.92a	12.89±1.08b	17.43±0.84a	18.94±1.18a
过氧化氢酶 Catalase (CAT, U·mL ⁻¹)	34.60±2.65b	26.20±2.44c	40.51±2.09ab	42.35±2.03a
谷胱甘肽过氧化物酶 Glutathione peroxidase (GSH-Px, U·mL ⁻¹)	326.23±16.33a	277.71±11.82b	327.14±17.86a	309.98±13.68a
超氧化物歧化酶 Superoxide dismutase (SOD, U·mL ⁻¹)	103.31±6.16b	98.37±5.68b	113.44±6.65b	135.25±7.30a
丙二醛 Malondialdehyde (MDA, nmol·mL ⁻¹)	5.52±0.26b	6.53±0.22a	5.71±0.34b	5.44±0.25b
核因子E2相关因子2 Nuclear factor-erythroid 2-related factor-2 (Nrf2, pg·L ⁻¹)	291.60±1.30a	289.61±0.83ab	288.27±0.63b	288.11±0.60b
血红素加氧酶-1 Heme oxygenase-1 (HO-1, pg·L ⁻¹)	24.91±0.06c	33.38±0.11a	33.07±0.08b	33.17±0.06ab

2.4 断奶前后羔羊血清免疫性能的变化

断奶3 d羔羊血清IgA浓度显著低于断奶15 d ($P < 0.05$, 表4), 其他各组间无显著差异 ($P > 0.05$)。与断奶0 d相比, 断奶15 d羔羊血清IgG浓度显著升高 ($P < 0.05$)。断奶6和15 d羔羊血清IgG浓度显著高于断奶3 d ($P < 0.05$)。与断奶0 d相比, 断奶3、6和15 d羔羊血清CD4浓度和CD4/CD8均显著升高 ($P < 0.05$)。断奶6 d羔羊血清CD4浓度显著高于断奶15 d ($P < 0.05$), 而各组间IgM和CD8浓度则无显著差异 ($P > 0.05$)。从本试验结果来看, 羔羊免疫性能随断奶后时间的延长而有所改善。

表4 断奶前后羔羊血清免疫性能的变化

Table 4 The change of immune indices of serum in pre- and post-weaning lambs

项目 Item	断奶天数 Weaning days (d)			
	0	3	6	15
免疫球蛋白A Immunoglobulin A (IgA, μg·mL ⁻¹)	177.62±14.73ab	139.73±13.40b	166.60±10.43ab	190.62±10.89a
免疫球蛋白M Immunoglobulin M (IgM, μg·mL ⁻¹)	1522.15±99.55a	1303.41±99.14a	1526.23±79.49a	1549.32±65.07a
免疫球蛋白G Immunoglobulin G (IgG, μg·mL ⁻¹)	76.22±3.46bc	67.97±4.05c	80.62±3.22ab	86.31±2.23a
CD4 ⁺ T淋巴细胞 CD4 ⁺ T-lymphocyte (CD4, ng·L ⁻¹)	122.79±3.73c	256.21±3.26ab	263.61±5.29a	248.12±2.13b
CD8 ⁺ T淋巴细胞 CD8 ⁺ T-lymphocyte (CD8, ng·L ⁻¹)	63.81±4.84a	55.32±4.70a	53.80±2.59a	56.64±3.84a
CD4/CD8	1.99±0.10b	4.97±0.38a	5.03±0.26a	4.57±0.26a

2.5 断奶前后羔羊抗炎能力的变化

与断奶0 d相比, 断奶3 d羔羊血清IL-1β浓度显著升高 ($P < 0.05$, 表5), 而断奶6和15 d则显著降低 ($P < 0.05$)。断奶3 d羔羊血清IL-1β浓度显著高于断奶6和15 d ($P < 0.05$)。与断奶3 d相比, 断奶6和15 d羔羊血清

TNF- α 、IL-2和IL-6浓度均显著下降($P<0.05$),而断奶0d则无显著差异($P>0.05$)。与断奶0d相比,断奶3、6和15d羔羊血清IL-8和IL-22浓度均显著升高($P<0.05$),而血清NF- κ B浓度则相反。与断奶0d相比,断奶3d羔羊血清IFN- γ 显著降低($P<0.05$),其他两组则无显著差异($P>0.05$)。与断奶0d相比,断奶6和15d羔羊血清Caspase3浓度显著降低($P<0.05$),断奶3d则无显著差异($P>0.05$)。从本试验结果来看,断奶3d后羔羊抗炎能力随着时间的延长而提高。

表5 断奶前后羔羊抗炎能力的变化

Table 5 The change of anti-inflammatory ability in pre- and post-weaning lambs

项目 Item	断奶天数 Weaning days (d)			
	0	3	6	15
白细胞介素-1 β Interleukin-1 β (IL-1 β , pg·mL $^{-1}$)	637.73 \pm 36.68b	790.81 \pm 23.56a	528.88 \pm 32.78c	533.81 \pm 39.37c
白细胞介素-2 Interleukin-2 (IL-2, pg·mL $^{-1}$)	634.57 \pm 29.84ab	706.05 \pm 49.07a	533.80 \pm 36.14b	513.45 \pm 43.29b
白细胞介素-6 Interleukin-6 (IL-6, pg·mL $^{-1}$)	140.61 \pm 6.07a	145.54 \pm 4.33a	117.19 \pm 5.93b	112.38 \pm 6.31b
白细胞介素-8 Interleukin-8 (IL-8, pg·L $^{-1}$)	174.01 \pm 2.28b	407.17 \pm 1.22a	408.72 \pm 1.30a	406.96 \pm 0.90a
白细胞介素-22 Interleukin-22 (IL-22, pg·L $^{-1}$)	251.74 \pm 0.30b	255.53 \pm 0.37a	256.21 \pm 0.33a	255.50 \pm 0.24a
肿瘤坏死因子- α Tumor necrosis factor- α (TNF- α , pg·L $^{-1}$)	172.53 \pm 8.22ab	179.74 \pm 7.62a	152.13 \pm 8.53bc	141.60 \pm 5.47c
干扰素- γ Interferon- γ (IFN- γ , pg·mL $^{-1}$)	515.75 \pm 40.51a	406.41 \pm 30.83b	506.69 \pm 27.94a	569.00 \pm 27.12a
半胱氨酸蛋白酶3 Cysteinyl aspartate specific proteinase 3 (Caspase3, nmol·mL $^{-1}$)	102.87 \pm 6.15a	95.99 \pm 3.45ab	86.67 \pm 4.28b	84.66 \pm 3.01b
核因子 κ B Nuclear factor kappa-B (NF- κ B, pg·L $^{-1}$)	120.95 \pm 0.10a	23.58 \pm 0.09b	23.52 \pm 0.11b	23.46 \pm 0.06b

3 讨论

TC是反映机体脂类的吸收利用和代谢情况的一个重要指标,其含量越低表明体内脂肪的利用率越高^[13]。从本试验结果来看,早期断奶后第6天会严重影响羔羊对脂类的吸收和代谢,随后有所恢复。当体内蛋白质合成发生障碍或流失过多会导致血清TP浓度降低,BUN含量降低时表明机体氨基酸代谢正常,蛋白质合成率较高,而升高时则说明体内蛋白质转化出现问题^[14]。ALT和AST是一组参与机体蛋白质代谢和氨基酸转运等生理过程的酶类,营养不良可能会引起ALT和AST活性降低,而肝脏有不同程度的损伤会引起两者活性升高^[15]。在本试验结果中,断奶6、15d羔羊血清ALT和AST活性、TP含量均低于断奶0d,而BUN含量则相反,说明早期断奶后第6天开始会严重影响羔羊对蛋白质的吸收和代谢。当肠道吸收发生障碍时ALP活性会降低^[16]。对仔猪的研究中,与断奶前相比,断奶后第2天血清ALP活性显著降低,在断奶后第8天极显著降低^[17],本试验结果与其相似。表明早期断奶会影响肠道吸收,在断奶后第6天影响最大。陈治徽等^[10]研究发现,断奶后1、2、3周犊牛血清TP含量、AST活性显著高于断奶前,而TC和GLU含量则相反,本试验结果与其不同,可能与物种不同有关。

甲状腺激素包括T₃和T₄,能促进动物机体蛋白质和DNA合成,提高机体的基础代谢率,增强糖原和脂肪的分解等^[18]。据报道,与哺乳仔猪相比,仔猪早期断奶后4d,血清T₃和T₄浓度下降,但在断奶9d后T₄含量上升^[19]。此外,断奶后犊牛血清中T₃的含量逐渐下降,7d达到最低;断奶后1、3、5、7d血清中T₄水平显著低于断奶前,于14d恢复到断奶前水平^[20]。本试验结果与上述研究结果相似。IGF-1能够提高氨基酸的转化率,增加蛋白质合成率,调节糖代谢,可介导生长激素激发生物活性作用于靶细胞来促进动物生长^[21]。据报道,断奶早期产生的应激会抑制IGF-1激素的分泌^[22],本研究也发现,早期断奶6d羔羊血清IGF-1激素分泌显著降低,然而断奶15d有所增加。因此,从本试验结果来看,羔羊断奶后15d可能会通过提高机体T₃、T₄和IGF-1激素分泌来提高机体营养物质的吸收和代谢。

T-AOC是衡量动物机体抵抗氧化损伤的重要指标之一,GSH-Px、SOD和CAT是反映机体抗氧化能力的重要酶类,MDA是机体内自由基与脂质发生过氧化反应的终产物,它们的水平高低反映机体抗氧化能力的强

弱^[23-24]。廖荣荣等^[25]研究发现,与60日龄断奶羔羊相比,30日龄断奶羔羊血清SOD和GSH-Px活性均显著降低,而30和60日龄断奶后4d羔羊血清SOD和GSH-Px活性显著低于断奶前,本试验结果与其相似。这表明,早期断奶和断奶后应激均会造成羔羊机体抗氧化能力降低。在本试验中,早期断奶后3d,羔羊血清T-AOC、GSH-Px和CAT活性降低,MDA含量升高,而随着断奶后时间的延长羔羊血清T-AOC、GSH-Px和CAT活性升高,MDA含量降低。表明随着羔羊断奶后时间的延长,机体抗氧化能力有所增强。Nrf2是一种细胞抗氧化应激的转录调节因子,正常情况下,Nrf2存在于细胞质,会被Keap1复合体泛素化修饰降解^[26]。在大量自由基、活性氧簇(reactive oxygen species, ROS)刺激下,Nrf2聚集在细胞核中与Keap1解偶联并激活磷酸化,结合靶基因启动子区的抗氧化反应元件(antioxidant response element, ARE),诱导启动下游HO-1、SOD、GSH-Px和CAT等多个抗氧化酶系的表达^[27]。在本试验中,早期断奶后羔羊血清Nrf2含量降低可能是由于其大量进入细胞核内发挥作用,导致较少分泌到胞外。HO-1是一种Ⅱ相解毒酶,可在多种不同的应激刺激下上调,包括各种促氧化剂和促炎症介质^[28]。从本试验结果来看,羔羊早期断奶3d后血清HO-1水平显著升高可能与其促炎症介质水平升高有关。

免疫球蛋白能够反映动物的免疫能力,含量增加则免疫功能增强^[29]。研究发现,与断奶前相比,荷斯坦犊牛断奶后1d血清IgA含量显著降低,随后上升到断奶前水平,但对断奶后1~14d血清IgM含量无显著影响^[20],本试验结果与其相似。然而,仔猪断奶2、4和8d血清IgG含量显著低于断奶前,但IgA含量无显著差异^[17];奶牛水牛犊牛断奶前和断奶后1~21d血清IgG含量无显著差异^[9],本试验结果与上述不同,可能与动物品种不同有关。CD4和CD8细胞能够反映T淋巴细胞的激活和免疫应答水平,当CD4/CD8升高表明机体免疫性能增强^[30]。从本试验可知,羔羊早期断奶后,机体会通过激活CD4免疫细胞来增强免疫性能。结果表明,随着羔羊早期断奶后时间的延长,机体可能会通过增强体液免疫和细胞免疫来提高机体的免疫功能。

IL-1 β 作为促炎反应的核心中介者,可激活炎症介质IL-6和TNF- α ,从而介导炎症反应^[31]。断奶应激会引起IL-1、IL-8和TNF- α 等细胞因子分泌,上调相关细胞因子的基因表达^[20,32]。从本试验结果来看,断奶3d羔羊产生的应激较大,随着断奶时间的延长有所恢复。机体产生大量的ROS会上调IL-2的表达,而IL-2能够使T细胞活化,调节下游氧化应激水平^[33]。在本试验中,随着羔羊断奶后时间的延长,IL-2浓度逐渐降低,可能与机体清除ROS能力增强有关。在炎症或感染反应中,IL-22能够通过降低ROS含量来减轻炎症小体活化,上调Nrf2/HO-1抗氧化通路来改善组织功能,进而促进各种组织和器官的增殖、重塑和修复^[34-35]。从本试验结果来看,羔羊断奶后机体可能会通过激活IL-22调控Nrf2/HO-1通路来缓解氧化应激。IFN- γ 具有抗病毒、抗肿瘤和免疫调节等作用,当机体的细胞免疫功能低下时,T细胞活化发生障碍会使IFN- γ 的产生减少^[36]。因此,本试验中早期断奶后3d羔羊血清IFN- γ 浓度最低,可能与机体免疫功能降低有关。Caspase 3是细胞凋亡的参与者,正常情况下以无活性的酶原形式存在于胞质中,但TNF介导死亡受体等途径可激活Caspase 3诱导细胞死亡^[37]。本试验中,羔羊血清Caspase 3和TNF- α 含量随断奶后时间的延长呈下降趋势。表明随着断奶后时间的延长,机体可能通过降低TNF- α 来抑制Caspase 3的激活,进而抑制细胞凋亡。而断奶0d羔羊血清Caspase 3含量最高,可能其主要是以无活性的酶原形式存在。活化的核转录因子NF- κ B能够诱导炎症因子TNF- α 和IL-1 β 等的表达,引起机体氧化应激异常,而HO-1能够阻断NF- κ B依赖的转录机制,进而实现细胞免受炎症和氧化损伤^[37]。从本试验来看,羔羊断奶后血清NF- κ B含量降低可能与机体HO-1水平高有关。

4 结论

羔羊断奶后会促进炎症反应,降低机体抗氧化能力,引起免疫力下降,但随着断奶后时间的延长,机体抗氧化能力、抗炎能力和免疫力有所增强。

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